Effects of Acremonium terricola Culture on Growth Performance, Serum and Liver Antioxidant Capacity, and Immune Indices in Rats: Postprint

Authors: Li Yang, Lin Cong, Xu Wenbin, Zhang Xingyi, Wang Yizhen, Gao Hong, Dou Xiujing, Hao Xiaoyan, Zhang Yonggen

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Abstract

This experiment aimed to investigate the effects of Acremonium terricola culture on growth performance, serum and hepatic antioxidant capacity, and immune indices in rats. Forty SD rats with similar body weight were selected and randomly divided into 5 groups, with 8 replicates per group and 1 rat per replicate. The five groups consisted of a control group and four different dose groups of Acremonium terricola culture (10, 50, 250, and 1,250 mg/kg BW), administered by gavage for 21 consecutive days. The results showed that Acremonium terricola culture extremely significantly increased the average daily gain of rats ($P<0.01$); increased the liver index and thymus index; elevated the contents of total protein (TP), albumin (ALB), globulin (GLB), and glucose (GLU) in rat serum, while decreasing the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and the content of low-density lipoprotein (LDL); enhanced the activity of glutathione peroxidase (GSH-Px) and total antioxidant capacity (T-AOC) in serum and liver, reduced malondialdehyde (MDA) content, and concurrently increased total superoxide dismutase (T-SOD) content in the liver; increased the contents of immunoglobulin A, immunoglobulin G, and immunoglobulin M in serum, while elevating interleukin-4 (IL-4) content in serum and liver, and reducing interleukin-1 (IL-1) and interleukin-17 (IL-17) contents. Based on comprehensive analysis of all indices, Acremonium terricola culture can increase the average daily gain of SD rats, improve serum biochemical indices, enhance the antioxidant capacity and immune function of rats, with the optimal supplementation level being 250 mg/kg BW.

Full Text

Effects of *Acremonium terricola* Culture on Growth Performance, Antioxidant and Immune Indexes of Serum and Liver in Sprague Dawley Rats

LI Yang, LIN Cong, XU Wenbin, ZHANG Xingyi, WANG Yizhen, GAO Hong, DOU Xiujing, HAO Xiaoyan, ZHANG Yonggen*

College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, China

Abstract

This study investigated the effects of *Acremonium terricola* culture on growth performance, and antioxidant and immune indexes in serum and liver of Sprague Dawley rats. Forty SD rats with similar body weight were randomly divided into 5 groups with 8 replicates per group and 1 rat per replicate. The five groups consisted of a control group and four experimental groups receiving different doses of *A. terricola* culture (10, 50, 250, and 1,250 mg/kg BW) via continuous gavage for 21 days. The results showed that *A. terricola* culture significantly improved average daily gain ($P<0.01$), increased liver and thymus indexes, and elevated serum contents of total protein (TP), albumin (ALB), globulin (GLB), and glucose (GLU) while decreasing activities of alanine transaminase (ALT) and aspartate transaminase (AST) and serum low-density lipoprotein (LDL) content. The treatment also enhanced glutathione peroxidase (GSH-Px) activity and total antioxidant capacity (T-AOC) in both serum and liver, reduced malondialdehyde (MDA) content, and increased total superoxide dismutase (T-SOD) activity in liver. Furthermore, it elevated serum immunoglobulin A, G, and M contents, increased interleukin-4 (IL-4) levels in serum and liver, and decreased interleukin-1 (IL-1) and interleukin-17 (IL-17) contents. Based on comprehensive evaluation of all indicators, *A. terricola* culture can improve average daily gain, serum biochemical parameters, and antioxidant and immune capacity in SD rats, with 250 mg/kg BW being the optimal supplementation level.

Keywords: *Acremonium terricola* culture; Sprague Dawley rats; growth performance; antioxidant; immunity

Introduction

Cordyceps gunnii is a rare medicinal fungus in China containing pharmacological components similar to *Cordyceps sinensis*, with demonstrated effects on improving sleep, regulating immunity, enhancing memory, and providing sedation and analgesia [1-2]. This fungus produces important secondary metabolites including cordycepic acid, cordycepin, cordyceps polysaccharides, and anti-ultraviolet components [3]. Despite its excellent pharmacological functions, the high cost

of *C. gunnii* limits its application in animal production. Consequently, artificial culture products have been developed as alternatives.

Acremonium terricola culture is a product obtained through artificial fermentation of *A. terricola* isolated from *C. gunnii*, containing functional components similar to natural cordyceps such as cordycepin, cordycepic acid, polysaccharides, sterols, and amino acids. If *A. terricola* culture can be reasonably applied in animal production, transferring its active components or functions to livestock products like meat, eggs, and milk would have significant implications. Previous studies have demonstrated that *A. terricola* culture supplementation can increase weight gain in piglets [4], improve body weight and egg production rate in laying ducks, and enhance egg quality [5]. Additionally, *A. terricola* mycelium can significantly improve immune function in rats with hepatic fibrosis [6], and extracts from *Acremonium* fermentation broth exhibit free radical scavenging capacity superior to vitamin E [7]. Furthermore, research has shown that functional components in *A. terricola* culture can improve production performance and immune capacity in white shrimp [8], while cordyceps products with similar functional components can enhance antioxidant and immune capacity in mice [9]. These studies confirm the potential of *A. terricola* culture in improving animal immune function. However, current research on *A. terricola* culture has been limited to improvements in livestock production performance and antibody immune titer, lacking comprehensive studies on animals' antioxidant and immune capacities. Therefore, this experiment systematically investigated the effects of *A. terricola* culture on growth performance and antioxidant and immune indexes in serum and liver of rats to provide theoretical basis and data support for future production applications.

Materials and Methods

1.1 Experimental Animals and Materials A total of 40 male specific-pathogen-free SD rats aged 7 weeks and weighing approximately 160 g were selected. The rats and feed were purchased from the Experimental Animal Center of Jilin University. Rats were housed 4 per cage with ad libitum access to feed and water. The environment was maintained with a 12-hour light cycle, temperature of (25±0.5)°C, and relative humidity of (55±5)%.

Acremonium terricola culture (ATC) was provided by Hefei Micron Bioengineering Co., Ltd. as a deactivated cordyceps fungal feed additive obtained through artificial fermentation of *A. terricola* extracted from *C. gunnii*. The product contained 26.84% crude protein, 5.00% crude fiber, 3.06% crude fat, 4.04% crude ash, and 61.06% nitrogen-free extract (dry matter basis). Functional component contents were: cordycepic acid 84.50 g/kg, cordyceps polysaccharides 44.60 g/kg, cordycepin 0.432 g/kg, sterols 0.597 g/kg, and total amino acids 218.1 g/kg.

1.2 Experimental Design The 7-week-old SD rats were divided into 5 groups (n=8), with each rat serving as one replicate. Groups included a control group and *A. terricola* culture groups receiving 10, 50, 250, and 1,250 mg/kg

BW. The culture was administered via continuous gavage for 21 days, while the control group received physiological saline.

1.3 Sample Collection and Processing After gavage administration, rats were fasted for 24 hours, weighed, and average daily gain was calculated. Following blood collection via eyeball removal, rats were immediately euthanized by cervical dislocation. Heart, liver, spleen, lung, kidney, thymus, stomach, and testis were collected and weighed for organ index calculation using the formula: Organ index (%) = (organ weight/body weight) × 100.

Serum biochemical parameters were measured using an Italian Fully automatic biochemical analyzer: total protein (TP), albumin (ALB), globulin (GLB), alanine transaminase (ALT), aspartate transaminase (AST), triglycerides (TG), cholesterol (CHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), urea nitrogen (UN), and glucose (GLU). All kits were purchased from Beijing Zhongsheng Beikong Biotechnology Co., Ltd., with specific procedures following kit instructions.

Liver samples were homogenized in physiological saline at a 1:9 ratio (m/V) to prepare 10% homogenate. Activities of catalase (CAT), total superoxide dismutase (T-SOD), and glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) content were determined in serum and liver homogenate using kits from Nanjing Jiancheng Bioengineering Institute according to manufacturer instructions.

Liver and serum immune indexes were detected by enzyme-linked immunosorbent assay (ELISA). Parameters included interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-17 (IL-17), tumor necrosis factor- (TNF-), and immunoglobulins A (IgA), G (IgG), and M (IgM). All kits were purchased from Wuhan Boster Biological Technology Co., Ltd., with procedures following kit instructions.

1.4 Data Processing and Analysis Data were analyzed using SAS 9.1.2 software with Duncan's multiple comparison test. Results are expressed as mean ± standard error. $P<0.05$ indicated significant difference, $P<0.01$ indicated extremely significant difference, and $0.05 P<0.10$ indicated a trend.

Results

2.1 Effects of *A. terricola* Culture on Growth Performance in SD Rats As shown in Table 1 , initial body weight showed no significant difference among groups ($P>0.05$). After 21 days of gavage, final body weight in treatment groups was significantly higher than in the control group ($P<0.01$). Among treatment groups, the 1,250 mg/kg BW group was significantly higher than the 10 mg/kg BW group ($P<0.01$), with no significant differences from the other two groups ($P>0.05$). The 10 mg/kg BW group showed no significant differences from the 50 mg/kg BW and 250 mg/kg BW groups ($P>0.05$). Compared with the control

group, average daily gain in treatment groups increased significantly (P<0.01), with no significant differences among treatment groups (P>0.05).

Table 1 Effects of *Acremonium terricola* culture on growth performance in Sprague Dawley rats (g)

Items	Control	10 mg/kg	50 mg/kg	250 mg/kg	1,250 mg/kg	P-value
Initial body weight	160.00	160.00	160.00	160.00	160.00	>0.05
Final body weight	329.00Cc	343.00Bb	347.00ABab	351.50ABab	357.25Aa	<0.01
Average daily gain	7.75Bb	8.64Aa	8.90Aa	9.07Aa	9.25Aa	<0.01

In the same row, values with no letter or the same letter superscripts mean no significant difference (P>0.05), while different lowercase letters indicate significant difference (P<0.05), and different uppercase letters indicate extremely significant difference (P<0.01). The same as below.

2.2 Effects of *A. terricola* Culture on Organ Indexes in SD Rats As shown in Table 2 , liver index in treatment groups was significantly higher than in the control group (P<0.01), with no significant differences among treatment groups (P>0.05). Compared with the control and 10 mg/kg BW groups, thymus index in the 250 mg/kg BW and 1,250 mg/kg BW groups increased significantly (P<0.01), with no significant differences among the 50, 250, and 1,250 mg/kg BW groups (P>0.05). No significant difference was observed between the control and 10 mg/kg BW groups (P>0.05). *A. terricola* culture had no significant effect on other organ indexes (P>0.05).

Table 2 Effects of *Acremonium terricola* culture on organ coefficient in Sprague Dawley rats (%)

Items	Control	10 mg/kg	50 mg/kg	250 mg/kg	1,250 mg/kg	P-value
Heart	0.31	0.32	0.31	0.32	0.32	>0.05
Liver	3.40Bb	3.60Aa	3.57Aa	3.67Aa	3.68Aa	<0.01
Spleen	0.23	0.24	0.25	0.25	0.25	>0.05
Lung	0.53	0.54	0.54	0.55	0.55	>0.05
Kidney	0.73	0.74	0.74	0.75	0.75	>0.05
Thymus	0.19Bb	0.20Bb	0.23ABab	0.25Aa	0.28Aa	<0.01

Items	Control	10 mg/kg	50 mg/kg	250 mg/kg	1,250 mg/kg	P-value
Stomach	0.52	0.53	0.53	0.54	0.54	>0.05
Testis	0.84	0.85	0.85	0.86	0.86	>0.05

2.3 Effects of *A. terricola* Culture on Serum Biochemical Indexes in SD Rats As shown in Table 3 , gavage administration of *A. terricola* culture increased serum TP, ALB, GLB, and GLU contents while decreasing ALT and AST activities and LDL content. Specifically, TP contents in the 10, 50, and 250 mg/kg BW groups showed no significant differences among them (P>0.05) but were significantly higher than the control group (P<0.01). The 1,250 mg/kg BW group showed no significant difference from the control group (P>0.05). Serum ALB content in the 250 mg/kg BW group was significantly higher than the control group (P<0.01), while the 1,250 mg/kg BW group was significantly lower than the 10, 50, and 250 mg/kg BW groups (P<0.01). No significant differences were observed among other groups (P>0.05). Serum GLB and GLU contents in the control group were significantly lower than in treatment groups (P<0.01), with no significant differences in GLB among treatment groups (P>0.05). The 250 mg/kg BW group showed the highest GLU content, significantly higher than other treatment groups (P<0.01). Serum ALT, AST activities, and LDL content decreased with increasing gavage dosage. No significant differences in ALT activity were observed among the 50, 250, and 1,250 mg/kg BW groups (P>0.05), but all were significantly lower than the control and 10 mg/kg BW groups (P<0.01). The control group showed no significant difference from the 10 mg/kg BW group (P>0.05). Serum AST activity in the control group was significantly higher than in the 50 and 250 mg/kg BW groups (P<0.01), while the 1,250 mg/kg BW group was significantly lower than both the control and 10 mg/kg BW groups (P<0.01). The 1,250 mg/kg BW group showed the lowest serum LDL content, significantly lower than all other groups (P<0.01). The 250 mg/kg BW group was significantly lower than the control and 10 mg/kg BW groups (P<0.01), with no significant differences among the control, 10 mg/kg BW, and 50 mg/kg BW groups (P>0.05). With increasing dosage, HDL content in the 50 mg/kg BW group showed an increasing trend (P=0.05), while other serum biochemical indexes were unaffected.

Table 3 Effects of *Acremonium terricola* culture on serum biochemical indexes in Sprague Dawley rats

Items	Control	10 mg/kg	50 mg/kg	250 mg/kg	1,250 mg/kg	P-value
TP (g/L)	64.00Cc	68.25ABab	68.50ABab	70.25Aa	65.75BCbc	<0.01
ALB (g/L)	44.75BCbc	45.25ABab	45.75ABab	46.75Aa	43.75Cc	<0.01

Items	Control	10 mg/kg	50 mg/kg	250 mg/kg	1,250 mg/kg	P-value
GLB (g/L)	19.25Bb	21.00Aa	22.00Aa	21.00Aa	22.00Aa	<0.01
ALT (U/L)	54.25Aa	54.00Aa	44.75Bb	43.88Bb	34.00Bb	<0.01
AST (U/L)	185.25Aa	176.75ABab	168.25BCbc	165.25BCbc	155.75Cc	<0.01
TG (mmol/L)	0.39	0.40	0.34	0.33	0.27	>0.05
CHOL (mmol/L)	1.51Cc	1.55Cc	1.59BCbc	1.61Bb	1.69Aa	<0.01
HDL (mmol/L)	0.40	0.40	0.41	0.42	0.42	0.05
LDL (mmol/L)	0.35Bb	0.35Bb	0.34ABab	0.33Bb	0.27Cc	<0.01
UN (mmol/L)	8.35	8.19	8.15	7.86	7.69	>0.05
GLU (mmol/L)	5.12Dd	7.92Aa	5.95Cc	7.82ABab	6.97Bc	<0.01

2.4 Effects of *A. terricola* Culture on Serum Antioxidant Indexes in SD Rats As shown in Table 4 , serum GSH-Px activity and T-AOC in treatment groups were significantly higher than in the control group (P<0.01). Among treatment groups, the 1,250 mg/kg BW group showed significantly higher GSH-Px activity than all other groups (P<0.01), with no significant difference between the 50 and 250 mg/kg BW groups (P>0.05) and the lowest activity in the 10 mg/kg BW group. No significant difference in T-AOC was observed between the 1,250 and 250 mg/kg BW groups (P>0.05), but both were significantly higher than the other three groups (P<0.01). The 10 and 50 mg/kg BW groups showed no significant difference between them (P>0.05) but were significantly higher than the control group (P<0.01). Serum MDA content in the control group was significantly higher than in the 50, 250, and 1,250 mg/kg BW groups (P<0.01), with no significant difference from the 10 mg/kg BW group (P>0.05). The 250 mg/kg BW group showed the lowest MDA content. *A. terricola* culture had no significant effect on serum CAT and T-SOD activities (P>0.05).

Table 4 Effects of *Acremonium terricola* culture on serum antioxidant indexes in Sprague Dawley rats

Items	Control	10 mg/kg	50 mg/kg	250 mg/kg	1,250 mg/kg	P-value
CAT (U/mL)	2.09	2.11	2.13	2.15	2.16	>0.05
GSH-Px (U/mL)	609.65Dd	568.73Cc	1,004.83Bb	1,027.41Bb	1,447.88Aa	<0.01
T-SOD (U/mL)	75.12	75.35	75.68	75.89	76.12	>0.05
T-AOC (U/mL)	5.12Dd	7.92Aa	5.95Cc	7.82ABab	7.50Bb	<0.01
MDA (nmol/mL)	7.31ABab	7.96Aa	5.74Cc	4.21Dd	5.74Cc	<0.01

2.5 Effects of *A. terricola* Culture on Serum Immune Indexes in SD Rats As shown in Table 5 , serum IL-1 content in the 250 mg/kg BW group was significantly lower than in all other groups (P<0.01), while the control group was significantly higher than all other groups (P<0.01) with no significant differences among the remaining three groups (P>0.05). Compared with the control and 10 mg/kg BW groups, serum IL-4 content in the 50, 250, and 1,250 mg/kg BW groups increased significantly (P<0.01), with no significant difference between the control and 10 mg/kg BW groups (P>0.05) or among the other groups (P>0.05). Serum IL-17 content in the 250 mg/kg BW group was significantly lower than in all other groups (P<0.01), with no significant differences among treatment groups (P>0.05). The control group showed no significant difference from the 10 and 1,250 mg/kg BW groups (P>0.05) but was significantly lower than the 50 mg/kg BW group (P<0.01). Serum IgA, IgG, and IgM contents increased with dosage. The 1,250 mg/kg BW group showed the highest IgA content, significantly higher than all other groups (P<0.01), while the 250 mg/kg BW group was significantly higher than the control, 10, and 50 mg/kg BW groups (P<0.01). No significant difference was observed between the control and 10 mg/kg BW groups (P>0.05). No significant difference in IgG content was found between the 250 and 1,250 mg/kg BW groups (P>0.05), but both were significantly higher than the other three groups (P<0.01). The 50 mg/kg BW group was significantly higher than the control and 10 mg/kg BW groups (P<0.01), with no significant difference between these two groups (P>0.05). Serum IgM content in the 50, 250, and 1,250 mg/kg BW groups was significantly higher than in the control group (P<0.01), with no significant difference between the 10 mg/kg BW group and control group (P>0.05) or between the 10 mg/kg BW and 50 mg/kg BW groups (P>0.05). No significant difference was observed between the 50 and 1,250 mg/kg BW groups (P>0.05). *A. terricola* culture had no significant effect on other inflammatory factors in

rat serum (P>0.05).

Table 5 Effects of *Acremonium terricola* culture on serum immune indexes in Sprague Dawley rats

Items	Control	10 mg/kg	50 mg/kg	250 mg/kg	1,250 mg/kg	P-value
IL-1 (ng/L)	41.13Aa	35.67Bb	36.37Bb	32.45Cc	37.32Bb	<0.01
IL-2 (ng/L)	125.87Bb	120.18Bb	135.95Aa	143.32Aa	135.94Aa	<0.01
IL-4 (ng/L)	23.34Aa	22.35ABab	21.61Bb	19.75Cc	22.54ABab	<0.01
IL-6 (ng/L)	27.40Dd	27.09Dd	30.93Cc	35.13Bb	37.95Aa	<0.01
IL-10 (ng/L)	287.36Cc	281.84Cc	311.34Bb	365.27Aa	368.88Aa	<0.01
IL-17 (ng/L)	7.64Dd	7.76CDcd	8.41BCbc	10.31Aa	8.77Bb	<0.01
TNF- (ng/L)	287.36	281.84	311.34	365.27	368.88	>0.05
IgA (g/L)	0.84Bb	0.85Bb	0.88ABab	0.92Aa	0.95Aa	<0.01
IgG (g/L)	5.12Dd	5.25Cc	5.68Bb	6.25Aa	6.31Aa	<0.01
IgM (g/L)	0.52Bb	0.53ABab	0.56Aa	0.58Aa	0.59Aa	<0.01

2.6 Effects of *A. terricola* Culture on Liver Antioxidant Indexes in SD Rats As shown in Table 6 , gavage administration of *A. terricola* culture had no effect on liver CAT activity but increased liver GSH-Px and T-SOD activities and T-AOC while decreasing MDA content. GSH-Px activity in the 1,250 mg/kg BW group was significantly higher than in the 250 mg/kg BW group (P<0.01), which in turn was significantly higher than all other groups (P<0.01) with no significant differences among the remaining groups (P>0.05). Compared with the control group, T-SOD activity in treatment groups increased significantly (P<0.01), with the 1,250 mg/kg BW group significantly higher than the other three treatment groups (P<0.01) and no significant differences among those three groups (P>0.05). MDA content in treatment groups was significantly lower than in the control group (P<0.01), with no significant differences among the 10, 250, and 1,250 mg/kg BW groups (P>0.05). Both the 10 and 1,250 mg/kg BW groups were significantly higher than the 50 mg/kg BW group (P<0.01).

Table 6 Effects of *Acremonium terricola* culture on liver antioxidant indexes in Sprague Dawley rats

Items	Control	10 mg/kg	50 mg/kg	250 mg/kg	1,250 mg/kg	P-value
CAT (U/mg)	246.26Cc	246.93Cc	268.31Bb	270.93Bb	281.58Bb	<0.01
GSH-Px (U/mg)	222.03Cc	221.67Cc	313.38Bb	423.74Aa	509.41Aa	<0.01
T-SOD (U/mg)	12.65Bb	11.64Bb	11.20Bb	18.95Aa	19.75Aa	<0.01
T-AOC (U/mg)	19.75Aa	16.74Bb	14.50Cc	14.85BCbc	16.92Bb	<0.01
MDA (nmol/mg)	7.31ABab	7.96Aa	5.74Cc	4.21Dd	5.74Cc	<0.01

2.7 Effects of *A. terricola* Culture on Liver Immune Indexes in SD Rats As shown in Table 7 , liver IL-1 content in the 250 and 1,250 mg/kg BW groups was significantly lower than in the control group (P<0.05), with no significant difference between these two groups (P>0.05). No significant differences were observed among the control, 10 mg/kg BW, and 50 mg/kg BW groups (P>0.05). Liver IL-4 content in the 50 mg/kg BW group was significantly higher than in all other groups (P<0.01), with no significant difference between the control and 10 mg/kg BW groups (P>0.05) or between the 250 and 1,250 mg/kg BW groups (P>0.05). Compared with the control group, liver IL-17 content in treatment groups decreased significantly (P<0.01). Among treatment groups, the 50 mg/kg BW group was significantly higher than the 10 and 1,250 mg/kg BW groups (P<0.01), while the 10 mg/kg BW group was significantly higher than the 250 and 1,250 mg/kg BW groups (P<0.01). *A. terricola* culture had no significant effect on other inflammatory factors in liver (P>0.05).

Table 7 Effects of *Acremonium terricola* culture on liver immune indexes in Sprague Dawley rats

Items	Control	10 mg/kg	50 mg/kg	250 mg/kg	1,250 mg/kg	P-value
IL-1 (ng/g)	37.87a	35.4ab	35.05ab	30.52b	31.15b	<0.01
IL-2 (ng/g)	4,857.01Aa	5,153.62Cc	5,150.32Cc	6,929.27Bb	7,082.37Bb	<0.01
IL-4 (ng/g)	4,072.05Bb	4,857.01Aa	8,999.71Aa	3,120.53Dd	3,208.79CDcd	<0.01
IL-6 (ng/g)	3,560.34Cc	3,680.45Bb	3,720.38Bb	4,150.25Aa	4,200.15Aa	<0.01

Items	Control	10 mg/kg	50 mg/kg	250 mg/kg	1,250 mg/kg	P-value
IL-10 (ng/g)	287.36	281.84	311.34	365.27	368.88	>0.05
IL-17 (ng/g)	7.64Dd	7.76CDcd	8.41BCbc	10.31Aa	8.77Bb	<0.01
TNF- (ng/g)	287.36	281.84	311.34	365.27	368.88	>0.05

Discussion

3.1 Effects of *A. terricola* Culture on Growth Performance in Rats Previous studies have demonstrated that cordyceps substances and their active components can improve production performance. The improvement in production performance of broilers and laying hens by *Cordyceps militaris* fermentation products [10] and cordyceps by-products [11], as well as the enhancement of weight gain and feed conversion ratio in white shrimp by *C. militaris* polysaccharides [8], confirm the feasibility of applying cordyceps substances and their active components in animal husbandry. Wei et al. [4] proved through feeding trials on nursery piglets that *A. terricola* culture can promote growth and improve immunity, while Sun et al. [5] also showed that *A. terricola* culture can increase body weight in laying ducks, consistent with our results. The improved production performance may be attributed to enhanced feed conversion efficiency [4,8]. The antibacterial activity of cordyceps active components may also contribute to increased body weight gain. Koh et al. [12] demonstrated that cordyceps extracts can improve intestinal microflora and control pathogenic bacteria in broilers, thereby enhancing growth performance. Healthy pigs have higher numbers of intestinal lactobacilli than *E. coli*, while diarrheic pigs show the opposite pattern [13]. Therefore, the antibacterial and probiotic effects of functional components in *A. terricola* culture are key to improving growth performance in rats.

3.2 Effects of *A. terricola* Culture on Organ Indexes in Rats Organ index is an important biological indicator that can reflect organ function to some extent [14], with thymus and spleen indexes reflecting immune function. In this study, *A. terricola* culture increased liver and thymus indexes in rats. Sun et al. [15] found that *C. militaris* substances can significantly increase thymus and spleen indexes in mice through continuous gavage. Cordyceps products [9] and cordyceps polysaccharides [16] also significantly increased spleen and thymus indexes in immunosuppressed mice, consistent with our findings. However, this study found no effect on spleen index, possibly related to differences in product administration methods and processing techniques. The increased liver index suggests that *A. terricola* culture affects liver function [14], possibly because the culture is metabolized by the liver before acting on the body. The increased thymus and liver indexes indicate positive effects on immune function and liver function in rats.

3.3 Effects of *A. terricola* Culture on Serum Biochemical Indexes in Rats Serum biochemical parameters are the most sensitive indicators of metabolic status. TP, ALB, and GLB contents reflect not only protein absorption, synthesis, and catabolism but also immune capacity [17]. In this study, *A. terricola* culture increased serum TP, ALB, and GLB contents in SD rats, indicating enhanced immune capacity. ALT and AST are two major transaminases normally present at high levels in liver; when liver is damaged, these enzymes are released into blood, increasing serum levels [18]. The decreased serum AST and ALT activities in this study demonstrate that *A. terricola* culture has no adverse effects on liver tissue. LDL is a cholesterol-rich lipoprotein that transports cholesterol from liver to peripheral tissues; elevated serum LDL can cause arteriosclerosis and coronary heart disease. Traditional Chinese medicines such as ginger, curcumin, and astragalus have been studied for LDL reduction [19-21]. The significant LDL-lowering effect of *A. terricola* culture in this study demonstrates its positive role in cholesterol metabolism. The increased serum GLU content in treatment groups proves the promoting effect of *A. terricola* culture on glucose metabolism, though the specific metabolic pathways require further investigation.

3.4 Effects of *A. terricola* Culture on Antioxidant Indexes in Serum and Liver This study demonstrated that *A. terricola* culture significantly increased liver index and markedly improved serum biochemical parameters, suggesting positive effects on liver antioxidant and immune capacity. We comprehensively evaluated the effects on antioxidant capacity by measuring antioxidant indexes in both serum and liver.

Reactive oxygen species (ROS) are products of cellular oxidative metabolism that can cause various oxidative stress-related diseases. T-AOC is a relatively independent indicator describing the dynamic balance between oxidation and antioxidation in blood. Lipid metabolism is also an important consequence of oxidative stress, with MDA being a product of lipid oxidation commonly used as an oxidative stress marker. SOD, CAT, and GSH-Px play crucial roles in maintaining oxidation-antioxidation balance and repairing oxidative damage [22-23], with their activities directly reflecting antioxidant processes. Our results prove that *A. terricola* culture can significantly increase T-AOC and T-SOD and GSH-Px activities while decreasing MDA content in both serum and liver, with all values within normal ranges. Based on these results, we hypothesize that *A. terricola* culture improves immune capacity by reducing oxidative stress and increasing antioxidant enzyme activities in serum and liver. Previous studies have also demonstrated that cordyceps substances and functional components possess potential antioxidant and anti-lipid peroxidation activities [24], increasing SOD and GSH-Px activities and significantly reducing MDA content in normal mice [15], immunosuppressed mice [9,16], and white shrimp [8], consistent with our findings.

3.5 Effects of *A. terricola* Culture on Immune Indexes in Serum and Liver Studies have reported that antioxidant intake can affect immune response and status [25-26]. Cordyceps substances can improve antioxidant capacity and regulate inflammatory factor levels, thereby modulating immune function [16,27]. Inflammatory factors are involved in various aspects of immunity and oxidation, including immune function development and functionalization, cell proliferation and differentiation, cell activation, and regulation of extracellular matrix protein proliferation [28]. IL-4 is a traditional anti-inflammatory factor [29], while IL-1 , IL-2, IL-6, IL-17, and TNF- are pro-inflammatory factors [30-33]; reducing pro-inflammatory factor content can protect tissues from excessive inflammatory responses [34]. This study demonstrated that gavage administration of *A. terricola* culture down-regulated pro-inflammatory factors IL-1 and IL-17 in rat serum and liver, proving its immunomodulatory function. Immunoglobulins can bind to foreign substances such as bacteria and viruses to help eliminate these antigens. The significant increase in serum IgA, IgG, and IgM contents in this study also demonstrates the immune-enhancing capacity of *A. terricola* culture. Piao et al. [9] proved that *C. militaris*-soybean can significantly increase IgG, IgM, and IL-4 contents in blood and liver of immunosuppressed mice, while Zhang et al. [28] demonstrated that *C. militaris* polysaccharides can improve immune capacity in immunosuppressed BALB/c mice by reducing oxidative damage and regulating inflammatory factors, both consistent with our results.

The immune-enhancing effect of *A. terricola* culture may be attributed to functional component intake. The culture is produced through artificial fermentation of *A. terricola* extracted from *C. gunnii*, with active components (sterols and adenosine) similar to natural cordyceps. Numerous studies have proven that these active components possess immunomodulatory and pharmacological effects [35-38]. Additionally, various active components have been reported in other cordyceps species, including cordycepin, mannitol, glycoproteins, peptides, and polysaccharides [39]. However, the mechanism by which *A. terricola* culture enhances immune capacity requires further investigation.

Conclusion

Based on the small dosage administered during the entire experimental period (40-100 mg per rat) and the physical characteristics of the product (solid powder), and referring to other cordyceps-related studies [15-16], we selected gavage administration. For purified extracts such as cordycepin, intraperitoneal injection has also been used [40]. When dosage is large, dietary administration can be adopted [16,28], which reduces stress from handling and gavage while decreasing workload. Similarly, in future production and application, *A. terricola* culture can be added to feed after premixing for convenient operation.

Acremonium terricola culture can improve average daily gain, serum biochemical parameters, and antioxidant and immune capacity in SD rats.

Based on comprehensive evaluation of experimental results and economic costs,

the optimal supplementation level of *A. terricola* culture for SD rats is 250 mg/kg BW.

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Note: Figure translations are in progress. See original paper for figures.