Effects of *Macleaya cordata* Extracts on Growth Performance and Immune Function of Immunosuppressed Laying Chicks

Wang Man[1,2], Li Min[1], He Yuanqing[1], Xiong Zhaolong[1], He Jian[1*]

1. College of Life Science and Engineering, Southwest University of Science and Technology, Mianyang 621000, China; 2. Feng Guangde Laboratory of Sichuan Tie Qi Li Shi Group, Mianyang 621000, China

Abstract **[Objective]** The paper was to study the effects of *Macleaya cordata* extracts on growth performance and immune function of immunosuppressed laying chicks. **[Method]** A total of 120 1-day-old laying chicks with the body weight of (38.76±1.12) g were randomly divided into 3 groups with 40 replicates per treatment and 1 chick per replicate according to the principle of consistent body weight and half male and half female, namely control group, cyclophosphamide (CTX) group and *M. cordata* group. The trial lasted 21 d. The chicks in control group and CTX group were fed with basal diet, and those in *M. cordata* group were fed with the basal diet supplemented with 40 mg/kg *M. cordata* extract. On the 8th, 9th, 10th and 17th day of the trial, the chicks in CTX group and *M. cordata* group were intraperitoneally injected with 80 mg/kg BW CTX, and those in control group were intraperitoneally injected with equal dose of normal saline. **[Result]** ① Compared with control group, the average daily gain (ADG) and average daily feed intake (ADFI) of chicks in CTX group reduced significantly; the feed gain ratio (F/G) increased significantly; the white blood cell count (WBC) decreased significantly; the alanine transaminase (ALT) and aspertate aminotransferase (AST) levels in the blood increased significantly; the total protein (TP) and globulin (GLOB) content in the blood decreased significantly; the serum malondialdehyde (MDA) content increased significantly; and the contents of immunoglobulin G (IgG), immunoglobulin M (IgM), interleukin-1 β (IL-1β) and interleukin-10 (IL-10) in the serum decreased significantly. ②Compared with CTX group, the ADG and ADFI of chicks in *M. cordata* group increased significantly; the F/G decreased significantly; the WBC, red blood cell count (RBC), hemoglobin (HGB), hematokrit (HCT) and mean corpuscular hemoglobin (MCH) increased significantly; the contents of ALT, AST and MDA in the serum decreased significantly; the contents of TP, GLOB, IgG, IgM, IL-1β, interleukin-6 (IL-6) and IL-10 in the serum increased significantly; and the bursa of fabricius index increased significantly. ③ Compared with control group, the ADG and ADFI of chicks in *M. cordata* group increased significantly, and the contents of HGB and HCT in the blood increased significantly. **[Conclusion]** CTX injection will reduce the growth performance, antioxidant and immune function of laying chicks. The addition of *M. cordata* extract could restore the growth performance, immune function and antioxidant function impaired by CTX.

Keywords Laying chicks; *Macleaya cordata* extract; Cyclophosphamide (CTX); Growth performance; Immune function

China is a big producer and consumer of poultry meat and eggs. According to statistics, the average stock of laying hens in China reached 1.07 billion in 2020, with an increase of 23 million hens compared with 2019 and a year-on-year growth of 2.20%, and the output of laying hens reached 18.956 5 million t[1]. In 2020, the number of yellow-feathered and white-feathered broilers slaughtered in China was about 9.3 billion, accounting for 55% of the total number of meat-type poultry slaughtered[2]. Before the prohibition of antibiotics, a large number of antibiotic growth promoters (AGPs) were used in poultry feed to promote their growth and prevent animal diseases. From July 1, 2020, feed enterprises in China must stop producing commercial feed containing growth-promoting drug feed additives (except traditional Chinese medicine). In this context, it is of great economic benefit and social significance to select safe and effective antibiotic substitute products in poultry production.

Macleaya cordata (Willd.) R. Br. is a herbaceous plant belonging to Papaveraceae, which grows all over the world, with great development potential. A large number of studies have shown that *M. cordata* extract has antibacterial, antioxidant and immunomodulatory effects, and is a good substitute for AGPs[3–4]. In 2012, the Ministry of Agriculture (later the Ministry of Agriculture and Rural Affairs) approved *M. cordata* as a Chinese veterinary drug feed additive[5]. The improvement of animal immune function is beneficial to the body's recognition and elimination of any foreign matter (virus, bacteria, *etc.*) invaded. Animals with low immune function are more likely to be infected with viruses that will cause diseases and death. Therefore, a large number of studies on livestock and poultry diets and additives mainly focus on the improvement of immunity[6]. At present, there have been related studies on the immune-enhancing effects of *M. cordata*, but all of them are based on healthy individuals, while the effects of *M. cordata* on immunosuppressed individ-

Received: 2022–04–29 Accepted: 2022–05–12
*Corresponding author. E-mail: 904606344@qq.com

uals are rarely explored and have not been reported systematically. In this study, with *M. cordata* extract as the test material, an immunosuppressive model was established to detect the growth performance and immune function indexes of laying chicks and to investigate the effects of *M. cordata* extract on growth performance and immune function of laying chicks.

1 Materials and Methods

1.1 Materials *M. cordata* extract used in the study was provided by Micolta Bioresource Inc. The active ingredient content was 3.75%, and the ratio of chelerythrine to sanguinarine was 1∶2. Cyclophosphamide (CTX) was purchased from Sigma Biotech Cooperation.

1.2 Experimental design and daily ration A total of 120 1-day-old laying chicks with the body weight of (38.76 ± 1.12) g were randomly divided into 3 groups with 40 replicates per treatment and 1 chick per replicate according to the principle of consistent body weight and half male and half female, namely control group, CTX group and *M. cordata* group. Each chick was raised in a single coop, and the trial lasted 21 d. The chicks in control group and CTX group were fed with basal diet, and those in *M. cordata* group were fed with the basal diet supplemented with 40 mg/kg *M. cordata* extract. On the 8th, 9th, 10th and 17th day of the trial, the chicks in CTX group and *M. cordata* group were intraperitoneally injected with 80 mg/kg BW CTX once every day. The chicks in control group were intraperitoneally injected with equal dose of normal saline. This trial was carried out in Huagai test base of Sichuan Tie Qi Li Shi Group from April to May, 2020, and a chicken house was selected for breeding. The basal diet used in the test was corn-soybean meal diet. The diet was formulated according to the standard design of laying hens in *Chicken Feeding Standard* (NY/T 33–2004) and the normal production process of Tie Qi Li Shi Group Mianyang B Factory. The diet composition and nutritional components are shown in Tab.1.

Tab.1 Diet composition and nutritional components of laying chicks (air-dried basis)

Item	Content
Diet component//%	
Corn (grade 1)	56.68
Soybean meal (43%)	25.50
Rice bran meal	5.00
Extruded soybean	5.00
Corn gluten meal	3.00
Calcium hydrophosphate	1.90
Limestone (power)	1.15
Soybean oil	0.80
0.3% Laying chick premix[1)]	0.30
Salt	0.20
L- lysine hydrochloride (98.0%)	0.17
DL-methionine	0.15
50% Choline chloride	0.10
Mildew preventive	0.05
Total	100.00
Nutritional component[2)]	
Crude protein//%	19.97
Crude fat//%	4.51
Crude fiber//%	3.63
Calcium//%	0.96
Total phosphorus//%	0.74
Metabolic energy (poultry)//MJ/kg	12.15

Note: 1) Premix provides Fe 40 mg, Zn 100 mg, Mn 100 mg, Cu 15 mg, Se 0.35 mg, VA 10 000 IU, VD_3 3 920 IU, VE 70 IU, VB_1 2.8 mg, VB_2 8.4 mg, VB_6 3.5 mg, nicotinic acid 42 mg, D-pantothenic acid 23 mg, folic acid 3.5 mg, choline 800 mg per kg of complete feed; 2) Metabolizable energy (ME) is calculated based on the *Feed Composition and Nutritional Value Table in China* (30th Edition, 2019), while others are calculated values.

1.3 Feeding management This trial was carried out in a chicken house in Huagai test base of Sichuan Tie Qi Li Shi Group in April 2020. Before the test, biological control and safety measures were strictly performed; the chicken house was cleaned, and chicken coops and water bottles were disinfected. The room temperature, relative humidity and light were controlled according to standard feeding procedures. The chicks were allowed *ad libitum* to feed and water. During the experiment, the water intake, feed intake and mental state of the chicks were observed every day, and the chicken coop and feed tank were kept clean, dry and sanitary.

1.4 Measurement indexes and methods

1.4.1 Growth performance. At the beginning, on the 7th day and at the end of the test, the weight of chicks was measured after fasting for 12 h, and the average daily gain of each replicate was calculated. The feed intake of each treatment was recorded daily.

1.4.2 Detection of blood indexes. At the end of the test (the 21st day), 10 chicks were randomly selected from each group to collect two tubes of venous blood, which were 10 mL ordinary tube and 5 mL anticoagulant tube, respectively. The blood samples in 5 mL anticoagulant tube were stored at 4℃ for blood routine test. The blood samples in 10 mL ordinary tube were placed in water bath at 37℃ for 30 min and centrifuged at 3 500 r/min for 10 min, and the serum was collected and stored at −20℃ for testing. The blood samples in the anticoagulation tube were sent to Mianyang Central Hospital for routine blood test to detect the indexes including white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and platelet distributing width (PCT). Malondialdehyde (MDA), total superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC) and glutathione S-transferase (GSH-S) in the serum were determined by antioxidant kit (Nanjing Jiancheng Bioengineering Institute). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), glob-

ulin (GLOB) and total protein (TP) in the serum were determined by automatic biochemical analyzer (Shandong Biobase Biological Industry Co., Ltd., BK-500). The concentrations of immunoglobulin G (IgG), immunoglobulin M (IgM), interleukin-2 (IL-2), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) in the serum were determined by ELISA kit purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.3 Organ index. At the end of the test (the 21st day), 10 chicks were randomly selected from each group for weighing and slaughtering, and the liver, spleen and bursa of fabricius were removed. The liver, spleen and bursa of fabricius of each chick were weighed by an electronic balance, and the organ index was calculated with the weight of each chick as the denominator. Organ index=organ weight (g)/carcass weight (kg).

1.5 Statistical analysis All data were sorted out via Excel 2016, and conducted one-way ANOVA using the GLM model in SAS 8.1 software. Each chick was used as the statistical unit of each index, and Duncan's multiple comparison was further performed in case of significant difference. $P<0.05$ indicated significant difference, and the results were displayed as mean ± standard error.

2 Results and Analysis

2.1 Effects of *M. cordata* extract on growth performance of immunosuppressed laying chicks As shown in Tab.2, the ADG of laying chicks in *M. cordata* group increased remarkably compared with control group during the period without CTX injection (0–7 d) ($P<0.05$). Compared with control group, the ADG and ADFI of chicks in CTX group decreased noticeably during the periods of 7–14, 14–21 and 7–21 d ($P<0.05$). The F/G in *M. cordata* group during the period of 7–14 d increased significantly compared with control group ($P<0.05$). Compared with control group, the ADG and ADFI of chicks in *M. cordata* group decreased significantly during the periods of 14–21 and 7–21 d ($P<0.05$). Compared with CTX group, the ADG of chicks in *M. cordata* group increased during the period of 7–14 d. Compared with CTX group, the ADG and ADFI of chicks in *M. cordata* group increased extremely ($P<0.01$) and the F/G decreased extremely during the periods of 14–21 and 7–21 d ($P<0.01$).

2.2 Effects of *M. cordata* extract on blood routine of immunosuppressed laying chicks As shown in Tab.3, compared with control group, the level of WBC in the blood decreased extremely in CTX group ($P<0.01$). Compared with CTX group, the levels of WBC, RBC, HGB, HCT and MCH in *M. cordata* group increased significantly ($P<0.05$). Compared with control group, the levels of HGB and HCT in the blood of *M. cordata* group increased remarkably ($P<0.05$).

2.3 Effects of *M. cordata* extracts on serum biochemistry of immunosuppressed laying chicks As shown in Tab.4, compared with control group, the activities of ALT and AST in the serum of chicks in CTX group increased significantly ($P<0.05$), while the contents of TP and GLOB in the serum decreased noticeably ($P<0.05$). Compared with CTX group, the activities of ALT and AST in the serum of chicks in *M. cordata* group decreased significantly

Tab.2 Effects of *M. cordata* extract on growth performance of immunosuppressed laying chicks

Item	Control group	CTX group	*M. cordata* group	*P*
0–7 d				
ADG//g	5.08±0.20 b	5.05±0.18 b	5.40±0.31 a	0.05
ADFI//g	12.24±1.13 b	12.12±1.14 b	12.98±1.31 a	0.04
F/G	2.41±0.03	2.40±0.05	2.41±0.04	0.38
7–14 d				
ADG//g	7.51±0.36 a	6.18±0.28 b	6.75±0.31 ab	0.03
ADFI//g	20.42±2.11 a	17.96±1.99 b	20.37±1.86 a	0.01
F/G	2.72±0.04 b	2.91±0.03 a	2.87±0.05 a	0.04
14–21 d				
ADG//g	8.20±0.23 b	7.81±0.18 c	8.84±0.19 a	0.03
ADFI//g	25.06±1.92 b	21.54±2.08 c	30.46±1.95 a	0.00
F/G	3.05±0.04 b	3.16±0.03 a	3.05±0.02 b	0.02
7–21 d				
ADG//g	7.86±0.27 b	7.00±0.26 c	8.28±0.25 a	0.01
ADFI//g	20.19±1.93 b	17.50±2.02 c	23.38±1.90 a	0.01
F/G	2.57±0.04 ab	2.70±0.03 a	2.62±0.04 b	0.01

Note: Different lowercase letters in the same row represent significant difference at 0.05 level; the results are expressed as mean ± standard error, n=10; the same below.

Tab.3 Effects of *M. cordata* extract on blood routine of immunosuppressed laying chicks

Item	Control group	CTX group	*M. cordata* group	*P*
WBC//10^9/L	197.22±6.86 a	156.50±9.86 b	212.40±5.25 a	0.00
RBC//10^9/L	1.61±0.21 ab	1.35±0.16 b	2.09±0.07 a	0.02
HGB//g/L	82.60±10.26 b	68.00±7.98 b	109.80±3.84 a	0.01
HCT//%	21.48±2.75 b	17.88±2.01 b	27.96±1.06 a	0.01
MCV//fL	134.36±0.88	132.60±0.64	134.14±1.80	0.55
MCH//pg	51.56±0.56 ab	50.14±0.56 b	52.52±0.40 a	0.02
MCHC//g/L	385.00±4.94	379.20±5.23	392.6±2.32	0.14
RDW-SD//fL	10.44±0.85	10.66±0.79	9.04±0.34	0.25
PLT//10^9/L	9.60±2.06	12.40±2.40	17.20±3.77	0.20

($P<0.05$), while the contents of TP and GLOB in the serum increased remarkably ($P<0.05$).

2.4 Effects of *M. cordata* extracts on serum antioxidant activity of immunosuppressed laying chicks As shown in Tab.5, compared with control group, the serum MDA content in CTX group increased significantly ($P<0.05$). Compared with CTX group, the serum MDA content in *M. cordata* group decreased remarkably ($P<0.05$).

2.5 Effects of *M. cordata* extracts on serum immune factors of immunosuppressed laying chicks As shown in Tab.6, compared with control group, the contents of IgG, IgM, IL-1β and IL-10 in CTX group decreased significantly ($P<0.05$). The contents of IgG, IgM, IL-1β, IL-6 and IL-10 in *M. cordata* group were significantly higher than those in CTX group ($P<0.05$).

2.6 Effects of *M. cordata* extracts on organ index of immunosuppressed laying chicks As shown in Tab.7, compared with CTX group, the organ index of chicks in *M. cordata* group decreased significantly ($P<0.05$) and the bursa of fabricius index increased remarkably ($P<0.05$). The bursa of fabricius index in *M. cordata* group was higher than that in control group ($P<0.05$).

Tab.4 Effects of *M. cordata* extracts on serum biochemistry of immunosuppressed laying chicks

Item	Control group	CTX group	*M. cordata* group	*P*
ALT//U/L	6.39±0.40 b	7.80±0.51 a	5.78±0.32 b	0.01
AST//U/L	288.59±11.54 b	321.27±12.46 a	268.62±4.12 b	0.01
TP//g/L	31.61±1.16 a	26.76±1.26 b	31.16±0.69 a	0.01
ALB//g/L	14.16±0.76	12.52±0.69	13.91±0.27	0.17
GLOB//mmol/L	17.45±0.69 a	14.24±0.65 b	17.25±0.43 a	0.00

Tab.5 Effects of *M. cordata* extracts on serum antioxidant activity of immunosuppressed laying chicks

Item	Control group	CTX group	*M. cordata* group	*P*
SOD//U/mg	514.34±21.45	431.26±32.16	459.17±34.05	0.18
MDA//nmol/mg	4.15±0.35 b	7.13±1.26 a	4.50±0.43 b	0.04
GSH-Px//μg/mg	596.04±59.69	400.88±62.53	490.55±73.50	0.15
GSH-S//U/mg	58.44±2.96	61.68±9.55	54.84±5.09	0.76
T-AOC//U/mg	0.70±0.05	0.66±0.04	0.71±0.04	0.67

Tab.6 Effects of *M. cordata* extracts on serum immune factors of immunosuppressed laying chicks

Item	Control group	CTX group	*M. cordata* group	*P*
IgA//mg/mL	3.03±0.53	3.59±0.66	4.08±0.33	0.40
IgG//mg/mL	53.52±8.97 a	26.52±1.57 c	33.92±2.22 b	0.01
IgM//mg/mL	8.60±0.61 a	5.35±0.28 b	7.76±0.52 a	0.00
IL-1β//pg/mL	69.52±9.10 a	44.45±3.04 b	58.26±2.82 a	0.03
IL-2//pg/mL	46.14±1.40	48.80±3.27	51.14±1.22	0.30
IL-6//pg/mL	65.41±12.18 ab	50.04±3.12 b	85.86±6.71 a	0.03
IL-10//pg/mL	228.55±13.92 a	111.84±10.16 b	182.54±24.88 a	0.00

Tab.7 Effects of *M. cordata* extracts on organ index of immunosuppressed laying chicks

Item	Control group	CTX group	*M. cordata* group	*P*
Liver index	30.43±0.47 ab	32.36±0.27 a	27.18±0.31 b	0.05
Spleen index	1.78±0.04	1.77±0.10	1.98±0.22	0.13
Bursa of fabricius index	4.70±0.61 b	4.16±0.28 b	5.68±0.52 a	0.01

3 Discussion

Immune stress is a condition in which the body loses its immune homeostasis due to the action of external forces. Activation and inhibition are two opposite states of immune stress. The immune stress caused by overinjection of vaccines in actual production is immune overactivation, while mycotoxin poisoning can cause immunosuppression. CTX injection is commonly used to establish animal immunosuppression model in scientific research. A large number of studies have found that the successful establishment of animal immunosuppression models will reduce the growth performance of animals and inhibit immune-related indexes[9-12]. In this test, CTX injection reduced the growth performance of laying chicks, restrained the proliferation of immune cells, and inhibited the ability of immune cells to secrete immune factors. The results were consistent with the results of He[10], Jones [13], Chen *et al.*[14], Tripathi *et al.*[15], indicating that the immunosuppressive model of CTX on laying chicks was established successfully.

Our research results showed that the supplementation of *M. cordata* extract in the diet of immunosuppressed laying chicks could significantly improve the ADG and ADFI of laying chicks, but it also increased the F/D. Liu *et al.*[12] put forward that immune stress could down-regulate plasma epinephrine activity, serum angiotonia II and amylase activities, and had negative effects on energy utilization of broilers. This is consistent with the result of the increase of F/G caused by CTX injection in this test. The supplementation of *M. cordata* extract reduced the F/G and promoted the ADFI and ADG of chicks, suggesting that *M. cordata* extract could improve the growth performance of immunosuppressed laying chicks and alleviate the negative effects of CTX on the growth of chicks. Khadem *et al.*[16] found that the growth performance of broilers

gradually increased with the increasing dose of *M. cordata* extract in the diet, which was consistent with the conclusion of this study. Different types of WBC participate in the body's defense response in different ways. Studies have shown that immunosuppression will reduce the level of WBC in the body, showing decreased body resistance[17]. Girard *et al*.[18] injected CTX into mice to establish an immunosuppressive model, and WBC in mice reduced significantly. Interleukin is produced by WBC, and the decrease of WBC will also lead to the decrease of interleukin content, which is consistent with the results of this study. In this test, the supplementation of *M. cordata* extract significantly improved the decrease of WBC and interleukin levels in the serum caused by immunosuppression, while the levels of RBC, HGB, HCT and MCH increased accordingly. RBC not only participates in the transport of nutrients in the body, but also has immune function [19]. Vrba *et al*.[20] found that sanguinarine regulates the expression of heme oxygenase 1 (HO-1) by activating Nrf2 signaling pathway, but chelerythrine can not affect this pathway. Therefore, the study found that compared with control group, the supplementation of *M. cordata* extract could increase the level of RBC and HGB in the blood, which probably because sanguinarine in *M. cordata* extract involved in this pathway.

When injected into the body, CTX needs to be activated by liver metabolism. The activation process of CTX consumes the liver microchromase P450, increases the content of reactive oxygen species, rapidly depletes glutathione (GSH), and causes lipid peroxidation, resulting in liver cell injury[21]. In this test, compared with control group, the MDA content in CTX group increased significantly, and the liver index increased by 6.34%. Minor liver injury can lead to elevated levels of ALT and AST in the blood[22]. At the same time, when liver injury occurs, the protein content in the blood will decrease, and the immunoglobulin will also decrease significantly[23]. In this study, injection of CTX led to the increase of ALT and AST levels in the blood of laying chicks, which was consistent with the results of previous studies[20]. The serum ALT, AST and MDA levels decreased and the liver index also decreased significantly after the supplementation of *M. cordata* extract. Studies have found that *M. cordata* components have the ability to scavenge free radicals and have good antioxidant activity, which can prevent oxidative damage and carbonylation damage of proteins and inhibit lipid and DNA oxidative damage[24-25]. This suggests that *M. cordata* extract has antioxidant properties and repairs liver injury, thus reducing ALT and AST levels in the blood.

4 Conclusions

The results showed that CTX inhibited the immune capacity and decreased the growth performance of chicks. *M. cordata* regulated the immune system of laying chicks, which had the functions of improving the immune capacity of immunosuppressed chickens induced by cyclophosphamide, maintaining the normal function of immune cells, improving antioxidant capacity, protecting liver and other tissues, and maintaining the health and growth of the body.

References

[1] LI N, LIU Q, ZHANG LJ, *et al.* Overview of the development of livestock and poultry seed industry in Liaoning province during the 13th five-year plan period[J].Modern Journal of Animal Husbandry and Veterinary Medicine, 2021(6): 88–91. (in Chinese).

[2] WU M, ZHANG LF.Based on a new starting point, embark on a new journey and jointly contribute to the high-quality development of the poultry industry [J]. BeiFang MuYe, 2021 (9): 7–10. (in Chinese).

[3] CHEN J, KANG B, ZHAO Y, *et al.* Effects of natural dietary supplementation with macleaya cordata extract containing sanguinarine on growth performance and gut health of early weaned piglets[J]. J Anim Physiol An N, 2018, 102(6): 1666–1674.

[4] BAVARSADI M, MAHDAVI AH, ANSARI-MAHYARI S, *et al.* Effects of different levels of sanguinarine on antioxidant indices, immunological responses, ileal microbial counts and jejunal morphology of laying hens fed diets with different levels of crude protein [J]. J Anim Physiol An N, 2017, 101(5): 936–948.

[5] ZHANG R, WANG XW, ZHU JY, *et al.* Dietary sanguinarine affected immune response, digestive enzyme activity and intestinal microbiota of Koi carp (*Cryprinus carpiod*)[J]. Aqua culture, 2019, 502: 72–79.

[6] LIU YL, ZHONG L, CHEN T, *et al*. Dietary sanguinarine supplementation on the growth performance, immunity and intestinal health of grass carp (*Ctenopharyngodon idellus*) fed cottonseed and rapeseed meal diets[J]. Aquaculture, 2020(528): 735521.

[7] RAWLING MD, MERRIFIELD DL, DAVIES SJ. Preliminary assessment of dietary supplementation of Sangrovit on red tilapia (*Oreochromis niloticus*) growth performance and health[J]. Aquaculture, 2009, 294(1–2): 118–122.

[8] GUAN G, DING S, YIN Y, *et al.* Macleaya cordata extract alleviated oxidative stress and altered innate immune response in mice challenged with enterotoxigenic *Escherichia coli*[J]. Sci China Life Sci, 2019, 62(8): 1019–1027.

[9] IBRAHIM HM, MOHAMMED-GEBA K, TAWFIC A A, *et al.* Camel milk exosomes modulate cyclophosphamide-induced oxidative stress and immuno-toxicity in rats[J]. J Funct Foods, 2019, 10(11): 7523–7532.

[10] HE XY, YANG J, GUO YM. Effects of different dietary oil sources on immune function in cyclophosphamide immunos uppressed chickens[J]. Anim Feed Sci Tech, 2007, 139 (3/4): 186–200.

[11] ZHOU Y, CHEN X, YI R, *et al*. Immunomodulatory effect of tremella polysaccharides against cyclophosphamide-induced immunosuppression in mice[J]. Molecules, 2018, 23(2): 239.

[12] LIU L, QIN DK, WANG XF, *et al*. Effect of immune stress on growth performance and energy metabolism in broiler chickens [J]. Food Agr Immunol, 2015, 26(1/2): 194–203.

[13] JONES RC, NAYLOR CJ, AL-AFALEQ A, *et al.* Effect of cyclopho sphamide immunosuppression on the immunity of turkeys to viral rhinotracheitis [J]. Res Vet Sci, 1992, 53(1): 38–41.

[14] CHEN D, CHEN G, DING Y, *et al.* Polysac-

charides from the flowers of tea (*Camellia sinensis* L.) modulate gut health and ameliorate cyclophosphamide-induced immunosuppression[J]. J Funct Foods, 2019(61): 103470.

[15] TRIPATHI DN, JENA GB. Intervention of astaxanthin against cyclophosphamide-induced oxidative stress and DNA damage: a study in mice[J]. Chem-biol Interact, 2009, 180(3): 398–406.

[16] KHADEM A, SOLER L, EVERAERT N, *et al.* Growth promotion in broilers by both oxytetracycline and *Macleaya cordata* extract is based on their anti-inflammatory properties [J]. Br J Nutr, 2014, 112(7): 1110–1118.

[17] MERYMAN HT. Transfusion-induced alloimmunization and immunosuppression and the effects of WBC depletion[J]. Transfus Med Rev, 1989, 3(3): 180–193.

[18] GIRARD D, REGAN PA, MILISEN WB, *et al.* Influence of immunosuppression on the pharmacokinetics and pharmacodynamics of azithromycin in infected mouse tissues[J]. J Antimicrob Che moth, 1996, 37(1): 21–35.

[19] SONVEAUX P, LOBYSHEVA I I, FERON O, *et al.* Transport and peripheral bioactivities of nitrogen oxides carried by red blood cell hemoglobin: role in oxygen delivery[J]. Physiology, 2007, 22(2): 97–112.

[20] VRBA J, OROLINOVA E, ULRICHOVA J. Induction of heme oxygenase-1 by macleaya cordata extract and its constituent sanguinarine in RAW264. 7 cells[J]. Fitoterapia, 2012, 83(2): 329–335.

[21] LEAR L, NATION R L, STUPANS I. Effects of cyclophosphamide and adriamycin on rat hepatic microsomal glucuronidation and lipid peroxidation [J]. Biochem Pharmacol, 1992, 44(4): 747–753.

[22] RASYID SA, LIO TMP. Analysis of serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase levels in tuberculosis patients who are undergoing oat treatment in Kendari City General Hospital, Kota Kendari, Indonesia [J]. Curr Infect Dis Rep, 2020, 12(11): 75–77.

[23] HONMA K, KAMIKUBO M, MOCHIZUKI K, *et al.* Insulin-induced inhibition of gluconeogenesis genes, including glutamic pyruvic transaminase 2, is associated with reduced histone acetylation in a human liver cell line [J]. Metabolism, 2017(71): 118–124.

[24] LI CM, YANG XY, ZHONG YR, *et al.* Chemical composition, antioxidant and antimicrobial activity of the essential oil from the leaves of macleaya cordata (Willd) R. Br[J]. Nat Prod Res, 2016, 30(4): 438–442.

[25] GUAN G, DING S, YIN Y, *et al.* Macleaya cordata extract alleviated oxidative stress and altered innate immune response in mice challenged with enterotoxigenic *Escherichia coli* [J]. Sci China Life Sci, 2019, 62 (8): 1019–1027.