

Effects of *Macleaya cordata* Extract on the Growth Performance and Immune Function of Squabs: Postprint

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Abstract

This experiment aimed to investigate the effects of *Macleaya cordata* extract on growth performance, organ indices, serum biochemical indices, and immune function in squabs. A total of 280 healthy 10-day-old squabs with similar body weight were selected and randomly allocated into 7 groups using a single-factor completely randomized design, with 10 replicates per group and 4 squabs per replicate. The control group (Group A) was fed a basal diet, while the treatment groups (Groups B, C, D, E, F, and G) were fed the basal diet supplemented with 30, 40, and 50 mg/kg *Macleaya cordata* powder, 40 mg/kg *Macleaya cordata* extract PE50, 40 mg/kg *Macleaya cordata* extract BE60, and 150 mg/kg chlortetracycline, respectively. The experiment consisted of a 5-day preliminary feeding period followed by a 15-day experimental period. The results showed: 1) No significant differences in average daily weight gain or feed conversion ratio were observed among all groups ($P>0.05$). 2) No significant differences in thymus index or bursa of Fabricius index were found among all groups ($P>0.05$). The spleen index in Group E was extremely significantly higher than that in the control group ($P<0.01$), while Group D was significantly higher than the control group ($P<0.05$). The pancreas index in Group D was significantly higher than that in the control, B, and F groups ($P<0.05$). 3) The total protein content in Group E was significantly higher than that in Group G ($P<0.05$). The low-density lipoprotein content in Group B was significantly higher than that in the control group ($P<0.05$). 4) The immunoglobulin G content in Group D was extremely significantly higher than that in the control and B groups ($P<0.01$) and significantly higher than the other groups ($P<0.05$); the interleukin-6 content in Group E was significantly lower than that in the control group ($P<0.05$). These results indicate that *Macleaya cordata* extract can enhance immune organ indices and increase serum total protein, low-density lipoprotein, and immunoglobulin G contents in squabs, thereby improving systemic immunity.

Full Text

Effects of *Macleaya cordata* Extracts on Growth Performance and Immune Function of Squabs

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Abstract

This study investigated the effects of *Macleaya cordata* extract on growth performance, organ indices, serum biochemical parameters, and immune function in squabs. A total of 280 healthy ten-day-old squabs with similar body weight were randomly allocated into seven groups using a single-factor completely randomized design. Each group comprised ten replicates with four squabs per replicate. The control group (Group A) received a basal diet, while experimental groups (Groups B, C, D, E, F, and G) received the basal diet supplemented with 30, 40, or 50 mg/kg *Macleaya* powder; 40 mg/kg *Macleaya cordata* extract PE50; 40 mg/kg *Macleaya cordata* extract BE60; or 150 mg/kg chlortetracycline, respectively. A 5-day pre-trial period was followed by a 15-day formal experimental period. The results showed: (1) No significant differences in average daily weight gain or feed-to-gain ratio were observed among groups ($P > 0.05$). (2) No significant differences in thymus or bursa indices were found among groups ($P > 0.05$). The spleen index in Group E was extremely significantly higher than that in the control group ($P < 0.01$), while Group D showed a significantly higher spleen index compared to the control ($P < 0.05$). The pancreatic index in Group D was significantly higher than that in the control, Group B, and Group F ($P < 0.05$). (3) Total protein content in Group E was significantly higher than in Group G ($P < 0.05$). Low-density lipoprotein content in Group B was significantly higher than in the control group ($P < 0.05$). (4) Immunoglobulin G content in Group D was extremely significantly higher than in the control and Group B ($P < 0.01$) and significantly higher than all other groups ($P < 0.05$). Interleukin-6 content in Group E was significantly lower than in the control group ($P < 0.05$). These findings indicate that *Macleaya cordata* extract can enhance immune organ indices and increase serum levels of total protein, low-density lipoprotein, and immunoglobulin G, thereby improving the immune function of squabs.

Keywords: Macleaya cordata extract; growth performance; organ index; biochemical index; immune function

The primary active components in Macleaya cordata are alkaloids such as sanguinarine and chelerythrine, which exhibit diverse pharmacological effects including antibacterial, insecticidal, antitumor, and liver function improvement properties [1-5]. Macleaya cordata is widely distributed in China with abundant resources, easy cultivation, and simple management, providing ample supply and raw material security for its development and utilization. Current research on Macleaya alkaloids primarily focuses on clinical applications, veterinary medicine, and biopesticides. In animal production, these extracts have been shown to significantly improve growth performance in early-weaned piglets, reduce feed-to-gain ratios, and decrease weaning stress responses. However, few reports exist on their effects on animal immunity and biochemical parameters, and no studies have been reported on their effects in squabs. This experiment aimed to investigate the effects of different Macleaya cordata extract products on growth performance, immune organ indices, serum biochemical parameters, and immune markers in squabs, to determine the optimal supplementation level for squab development, and to further understand the different effects and mechanisms of action of Macleaya cordata extracts, providing a reference basis for their better application in animal production.

1.1 Experimental Materials

Macleaya powder (Meiyouzhuang) contained Macleaya cordata extract as its active ingredient, with sanguinarine and chelerythrine comprising 2.25% of the product (sanguinarine content 1.50%) and starch as the remaining component, purchased from Hunan Meikedu Biological Resources Co., Ltd. PE50, a Macleaya cordata extract, contained 30% protopine and -allocryptopine, 20% -allocryptopine, and 50% HSO and OH. BE60, another Macleaya cordata extract, contained 40% sanguinarine, 20% chelerythrine, and 40% HSO. Both PE50 and BE60 were provided by the Hunan Provincial Engineering Research Center for Traditional Chinese Medicine Extraction. Chlortetracycline (“Xitefei Veterinary Chlortetracycline” produced by Zhumadian Huazhong Zhengda Co., Ltd.) contained 15% active ingredient.

The breeding pigeon feed was “639 Breeding Pigeon Compound Feed” produced by Guangzhou Yuehong Feed Co., Ltd. Whey protein powder was high-protein whey powder imported from F&A Dairy Products, Inc. by Shanghai Yijun Trading Co., Ltd. Plant oil was “Jinle Grade A Soybean Oil” from Jiusan Grain and Oil Industry Group Co., Ltd. Yeast powder was “Hekangwang Beer Yeast Powder” produced by Pufike Biotechnology. Microelements were “Microelement Amino Acid Chelates” from BeBon Company. Protein digestive enzyme was “Jinhai Brand Compound Enzyme” from Huanghua Bohai Veterinary Drug Co., Ltd. Multi-vitamins were “Subu 14” from Nanning Huijianong Feed Co., Ltd., containing water-soluble vitamins A, D, E, C, B₁, B₂, B₆, B₁₂, etc., with an absorption rate of 45%-60%.

1.2 Experimental Design

A single-factor completely randomized design was employed. A total of 280 ten-day-old squabs were randomly divided into seven groups, each consisting of ten replicates with four squabs per replicate. The control group (Group A) was fed the basal diet (Table 1) and health sand (Table 2), while experimental groups (Groups B, C, D, E, F, and G) received the basal diet supplemented with 30, 40, or 50 mg/kg *Macleaya* powder; 40 mg/kg PE50; 40 mg/kg BE60; or 150 mg/kg chlortetracycline, respectively. A 5-day pre-trial period was conducted during which all groups were fed the basal diet. The formal experiment began when squabs reached 15 days of age and lasted for 15 days.

1.3.1 Experimental Diets

The composition and nutrient levels of the basal diet are presented in Table 1.

1.3.2 Management Practices

The experiment was conducted at Liangli Ecological Breeding Farm in Puji Town, Liuyang City. The facility utilized natural ventilation and electric heating temperature control, with squabs raised in online cages. Artificial feeding was performed using an improved 100 mL large syringe three times daily, with health sand provided once approximately one hour after the second meal. The entire experimental facility was disinfected with diluted potassium permanganate solution every two days, with feces cleaned and pests eliminated simultaneously.

1.4 Sample Collection and Preparation

At 31 days of age, squabs were fasted in the morning and six squabs were randomly selected from each group. Live body weight was recorded before slaughter via carotid artery exsanguination. Blood was collected in 10 mL centrifuge tubes (7 mL per tube), capped, placed on a rack, and allowed to stand for 2 hours. Serum was separated by centrifugation when a light yellow liquid precipitated and stored at -20 °C for later analysis. After slaughter, squabs were scalded with 50-80 °C water, de-feathered, and carcass weight was recorded. The spleen, pancreas, bursa of Fabricius, and thymus were excised, surface blood was wiped off, and organ weights were recorded. Approximately 50 g of breast muscle was collected, labeled, and stored at -20 °C for future analysis.

1.5.1 Growth Performance Measurement

At the beginning and end of the feeding trial, squabs were weighed to record initial and final body weights. Average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) were calculated for each replicate, and mortality rates were recorded.

1.5.2 Immune Organ Index and Immune Function Measurement

At the end of the experiment (31 days of age), squabs were fasted and weighed in the morning, then blood was collected and feathers were removed before dissection. The thymus, spleen, and bursa of Fabricius were collected, surface liquid was wiped off with absorbent paper, and immune organ weights were recorded. Six squabs were randomly selected from each group, weighed, and euthanized by carotid and venous exsanguination. The spleen, thymus, and bursa of Fabricius were dissected and weighed.

Immune organ indices were calculated using the following formulas:

Thymus index (mg/g) = thymus weight (mg) / squab body weight (g)

Spleen index (mg/g) = spleen weight (mg) / squab body weight (g)

Serum immunoglobulin G (IgG), immunoglobulin A (IgA), interleukin-6 (IL-6), and interleukin-1 (IL-1) contents were determined using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer' s instructions.

1.6 Statistical Analysis

Experimental data were expressed as mean \pm standard deviation. One-way ANOVA was performed using SPSS 17.0 statistical software, and Duncan' s multiple comparison test was used for post-hoc analysis.

2.1 Effects of *Macleaya cordata* Extracts on Growth Performance of Squabs

As shown in Table 3 , no significant differences were observed in average daily weight gain or feed-to-gain ratio among all groups ($P > 0.05$), indicating that *Macleaya cordata* extract had no significant effect on the growth performance of squabs.

2.2 Effects of *Macleaya cordata* Extracts on Organ Indices of Squabs

Table 4 shows that no significant differences were found in thymus or bursa indices among groups ($P > 0.05$). Group E exhibited the highest spleen index, followed by Group D, with Group E being extremely significantly higher than the control ($P < 0.01$) and Group D significantly higher than the control ($P < 0.05$). No other significant differences were detected among groups ($P > 0.05$). The pancreatic index in Group D was significantly higher than that in the control, Group B, and Group F ($P < 0.05$), with no other significant differences observed ($P > 0.05$). These results demonstrate that Groups D and E markedly increased the spleen index, while Group D enhanced the pancreatic index in squabs.

2.3 Effects of *Macleaya cordata* Extracts on Serum Biochemical Indexes of Squabs

According to Table 5 , no significant differences were observed in albumin or high-density lipoprotein levels among groups ($P > 0.05$). Total protein content in Group E was significantly higher than in Group G ($P < 0.05$), with no other significant differences detected ($P > 0.05$). Low-density lipoprotein content in Group B was significantly higher than in the control group ($P < 0.05$), while no other significant differences were found ($P > 0.05$).

2.4 Effects of *Macleaya cordata* Extracts on Serum Immune Indexes of Squabs

As presented in Table 6 , immunoglobulin G content in Group D was extremely significantly higher than in the control and Group B ($P < 0.01$) and significantly higher than all other groups ($P < 0.05$). No significant differences were observed in immunoglobulin A or interleukin-1 levels among groups ($P > 0.05$). Interleukin-6 content in Group E was significantly lower than in the control group ($P < 0.05$), with no other significant differences detected ($P > 0.05$).

3.1 Effects of *Macleaya cordata* Extracts on Growth Performance of Squabs

Li et al. [6] reported that feeding 5.0 mg/kg *Macleaya cordata* extract to five-way crossbred weaned piglets significantly increased daily weight gain and feed intake while reducing the feed-to-gain ratio. Cai et al. [7] added different doses of *Macleaya cordata* extract to the basal diet of three-way crossbred weaned piglets, with 30 mg/kg chlortetracycline in the antibiotic control group, and found that *Macleaya cordata* extract improved daily weight gain and feed intake while reducing feed-to-gain ratio, outperforming the antibiotic group, with an optimal dose of 0.675 mg/kg. Man et al. [8] investigated the effects of different doses of *Macleaya cordata* extract in diets for 35-day-old weaned piglets and reported that the 5.0 mg/kg group showed superior daily weight gain, feed intake, and feed-to-gain ratio compared to both control and antibiotic groups. Rao et al. [9] demonstrated that *Macleaya cordata* extract promoted growth performance in piglets, increasing daily weight gain by 17.26% and daily feed intake by 12.32% while decreasing feed-to-gain ratio by 4.5%. In contrast, the current study showed no significant differences among groups, likely because squabs cannot self-feed and were artificially gavage-fed, resulting in consistent feed intake across all groups.

3.2 Effects of *Macleaya cordata* Extracts on Immune Organ Indices of Squabs

The fundamental function of immune organs is to secrete immune substances (cytokines, immune molecules, etc.), synthesize immune cells, and execute cellular and humoral immune functions. The thymus, spleen, and bursa of Fabricius

are primary sites for immune cell formation, differentiation, and antibody production, serving as important immune organs whose organ indices can reflect immune function strength [10-11]. Generally, increased immune organ weight results from cellular growth, development, and proliferation. This study found that *Macleaya cordata* extract had no significant effect on thymus or bursa indices but significantly increased spleen index in Groups D and E. Since the spleen contains abundant lymphocytes and macrophages with a high proportion of B lymphocytes closely related to humoral immunity, these results preliminarily suggest that *Macleaya cordata* extract enhances humoral immune function in squabs.

He et al. [12] reported that sanguinarine from *Macleaya cordata* relatively increased portal plasma flow rate and enhanced net absorption of essential and total amino acids in piglets. Li et al. [13] reported that *Macleaya* alkaloids promoted proliferation of porcine intestinal epithelial cells within a certain concentration range, speculating that improved animal growth performance might result from enhanced intestinal cell proliferation and differentiation, thereby strengthening intestinal digestion, absorption, immune barrier, and stress response functions. The pancreas is a vital digestive organ, and the current study's finding that Group D showed increased pancreatic index compared to the control may be attributed to improved nutrient digestion and absorption, as increased pancreatic weight enhances pancreatic juice secretion and promotes food digestion and absorption. This could also explain previous reports of *Macleaya cordata* extract increasing animal feed intake.

3.3 Effects of *Macleaya cordata* Extracts on Immune Function of Squabs

Immunoglobulins are animal proteins with biological functions including antigen binding, complement activation, and immune regulation, produced by immune system lymphocytes and convertible to antibodies upon antigen induction. Immunoglobulins are primarily classified as IgG, IgA, and IgM. IgG can bind to antigen-bearing target cells, triggering killer cell cytotoxicity and playing a crucial role in defense against invading microorganisms in open organs and local immunity. In this study, serum IgG levels in Groups C, D, E, and F were elevated compared to the control. Additionally, Group E showed significantly higher total protein content than Group G, and Group B exhibited significantly higher low-density lipoprotein content than the control. We hypothesize that *Macleaya cordata* extract enhances blood total protein and IgG levels, inhibiting and neutralizing pathogens, fixing complement, and thereby killing bacteria and viruses, which confers its antibacterial, antiviral, and insecticidal functions.

Supplementation of *Macleaya cordata* extract in squab diets positively affects immune organ development and immune cell factor levels, thereby enhancing overall immunity, with the most pronounced effects observed at 50 mg/kg *Macleaya* powder supplementation.

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