Effects of *Abrus cantoniensis* Hance Extract on Blood Lipid of Laying Hen Feed with High Energy and Low Protein Diet

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Abstract [**Objectives**] To explore the effects of *Abrus cantoniensis* Hance (ACH) extract on blood lipid indicators of laying hen fed with high energy and low protein diet. [**Methods**] Sixty 90-day-old laying hens were randomly divided into five groups: the blank control group (basic diet), the model group (high-energy and low-protein diet, HELPD), the low-dose group (HELPD + 0.5 g ACH extract per hen, LACH), and the medium-dose group (HELPD + 1 g ACH extract per hen, MACH), high dose group (HELPD + 2 g ACH extract per hen, HACH). The ACH extract was administrated by drinking water for 48 d. [**Results**] Different doses of ACH could improve the pathological changes induced by high energy and low protein. ACH extract had no significant effect on blood routine indicators of laying hens ($P > 0.05$). The contents of total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) in the model group were significantly higher than those in the control group ($P < 0.05$), while the content of high density lipoprotein cholesterol (HDL-C) was significantly lower than that in the control group ($P < 0.05$). There was no significant difference in blood lipid between LACH group and model group ($P > 0.05$). In MACH and HACH groups, the contents of TC, TG and LDL-C were significantly lower than those in the model group ($P < 0.05$), and the content of HDL-C was significantly higher than that in the model group ($P < 0.05$). [**Conclusions**] The ACH extract can regulate the HELPD-induced dyslipidemia in laying hens.

Key words *Abrus cantoniensis* Hance (ACH), High-energy and low-protein diet (HELPD), Laying hen, Lipid

1 Introduction

Fatty liver hemorrhagic syndrome (FLHS) is a major nutritional and metabolic disease of laying hens, and it is characterized by lipid metabolism disorder. FLHS is not easy to be detected and treated early because of its inconspicuous clinical manifestations and sporadic nature. At present, the clinical prevention and control of FLHS is mainly based on prevention, lacking special treatment drugs and perfect prevention and control mechanism[1-3]. Abri Herba is the dried whole plant of *Abrus cantoniensis* Hance (ACH). The main chemical components of ACH include flavonoids, polysaccharides, triterpenoids and so on. ACH has the advantages of wide distribution and low price[4-6]. Besides, ACH has the effects of clearing away heat and toxic materials, promoting diuresis, removing jaundice, soothing the liver and relieving pain. In recent years, the role of ACH in reducing lipid content in plasma and liver, promoting lipid metabolism and improving liver tissue structure has been fully confirmed. However, the effect of ACH extract on FLHS is rarely reported. In view of this, we explored the effects of ACH extract on lipid metabolism in laying hens fed with high-fat and low-protein diet, and to provide a reference for the further development of traditional Chinese medicine preparation for the prevention and treatment of fatty liver syndrome in laying hens.

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2 Materials and methods

2.1 Preparation of ACH extract The whole dried plant (collected from Yulin, Guangxi) was cut, soaked in 10 times of water for 1 h, and decocted for 2 h. The filtrate was separated, and 5 times of water was decocted for 1 h. The two filtrates were combined and concentrated to 3 g/mL of crude drug concentration, separately packaged and stored at 4 ℃ for later use.

2.2 Experimental design The FLSH model was induced according to the methods reported in the literature[7-8]. The basal diet of laying hens was ordinary complete formula diet, and the high-fat and low-protein diet increased the content of corn by 6%, decreased the content of wheat bran by 0.8%, decreased the content of soybean meal by 9.42%, and added 4.22% soybean oil. The composition and nutrient level of the experimental diets are shown in Table 1.

Sixty 90-day-old Jingfen laying hens were randomly divided into control group, model group and three ACH treatment groups (low, medium and high), with 12 hens in each group. The laying hens in the blank control group were fed with ordinary basic complete diet every day; the laying hens in the model group were fed with HELPD every day; the laying hens in the low dose group (LACH) were fed with high fat diet + 0.5 g per hen ACH extract every day; the laying hens in the medium dose group (MACH) were fed with high fat diet + 1 g per hen ACH extract every day; high dose group (HACH) was fed with high fat diet + 2 g per hen ACH extract every day. The ACH extract was administered with water once a day. The laying hens were reared on the clean cage separate cages, fed and drank freely, the relative humidity and temperature in the house were (23 ± 2) ℃, the illumination time was 15 h/d, and the experimental period was 48 d.

Table 1 Composition and nutrient level of the experimental diet

	Item	Basic diet	HELPD
Composition//%	Corn	64.00	70.00
	Wheat bran	2.00	1.20
	Soybean meal	24.00	14.58
	Soybean oil	0.00	4.22
	Calcium	8.00	8.00
	Premix	2.00	2.00
	Total	100.00	100.00
Nutrient level	Metabolic energy//MJ/kg	11.25	13.02
	Crude protein//%	15.86	12.00
	Available phosphorus//%	0.51	0.46
	Arginine//%	1.03	0.74
	Methionine//%	0.37	0.32
	Valine//%	0.77	0.58
	Methionine + Cysteine//%	0.67	0.56

NOTE The premix provides 2.5 mg of copper, 20 mg of iron, 15 mg of manganese, 17.5 mg of zinc, 4 mg of iodine, 6 mg of sodium selenite, 2.5 mg of cobalt gasification, 50 mg of methionine, 2.00 mg of chromium pyridine, 15.00 mg of multivitamins, 10.00 mg of phytase, 7.50 mg of kallinopine, 2 mg of antioxidants, 50.00 mg of choline, 200 mg of salt, 500 mg of calcium phosphate, and 76 mg of zeolite powder.

2.3 Instruments and reagents BC-5140 automatic animal blood routine analyzer (Shenzhen Mindray Bio-medical Electronics Co., Ltd.); DP8018-VET animal version automatic biochemical analyzer (Guangzhou Dongtang Electronic Technology Co., Ltd.). Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) detection kits were purchased from Shanghai Jianglai Biotechnology Co., Ltd.

2.4 Sample collection On the 48th day of the experiment, all chickens were fasted for 12 h, and blood was collected from the hearts of all chickens, and part of the blood was anticoagulated for the detection of blood routine indicators; part of the blood was coagulated, centrifuged at 1 500 r/min for 10 min, and the serum was separated and stored at −20 ℃ for the detection of blood lipid indicators. The livers were collected, trimmed and fixed in 10% formalin solution, and the fixative was changed every other day. The fixed tissues were used for paraffin sections.

2.5 Determination indicators and methods

2.5.1 Blood routine and blood lipid indicator. The blood routine indicators were measured by BC-5140 automatic blood routine analyzer[9]. White blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW) were measured. The contents of total cholesterol (TC), triglyceride (TG), high density lipoprotein (HLD-C) and low density lipoprotein (LDL-C) were determined according to the instructions of the kit.

2.5.2 Histopathological observation of liver. The fixed liver tissues were routinely embedded in paraffin, stained with hematoxylin and eosin, and sealed with a digital image acquisition system to observe the pathological changes of the liver.

2.6 Data statistics and analysis The data were analyzed by SPSS 24.0 statistical software (t test), and the results were expressed as "mean ± standard deviation", and $P < 0.05$ indicated significant difference.

3 Results and analysis

3.1 Clinical symptoms and pathological changes During the experiment, the clinical manifestations of laying hens were observed, and it was found that after 20 d of the experiment, some laying hens in the model group showed different degrees of loss of appetite, preference for lying, lethargy, lameness, slight fading of comb, and even death. After necropsy, it was found that the liver of laying hens became light in color or even broken. At the end of the experiment, all the chickens in the model group were observed to have yellow, swollen, fragile and greasy liver; abdomen and mesentery accumulated a large amount of fat; there were also fat deposits and bleeding spots on the surface of intestine, liver, heart and gizzard. Chickens in blank control group were normal by necropsy. The color of liver in different doses of ACH groups was lighter than that in negative control group, but darker than that in model group. The severity of gross pathological changes in LACH group was greater than that in MACH and HACH groups, but the pathological changes between MACH and HACH were not significant.

3.2 Effects of ACH extract on blood routine indicators of laying hens (Table 2) It can be seen from Table 2 that there was no significant difference in blood routine indicators between the model group and the control group ($P > 0.05$). Compared with the model group, the MCV of the LACH group hens was significantly decreased ($P < 0.05$), and the other indicators were not significantly different ($P > 0.05$). Compared with the control group and the model group, the number of RBC in the MACH group was significantly increased ($P < 0.05$). Compared with the model group, the number of WBC in the HACH group was significantly increased ($P < 0.05$), and there was no significant difference in other indicators ($P > 0.05$).

Table 2 Effects of ACH extract on blood routine indicators of laying hens

Group	WBC// $\times 10^9$ L	RBC// $\times 10^{12}$ L	HGB//g/L	HCT//%	MCV//fL	MCH//pg	MCHC//g/L	RDW//%
Control	387.47 ± 2.97^{ab}	2.22 ± 0.12^{b}	76.20 ± 2.02	27.43 ± 4.41	114.69 ± 0.89^{ab}	30.55 ± 0.87	287.03 ± 2.92	38.92 ± 1.24
Model	385.57 ± 1.57^{b}	2.24 ± 0.14^{b}	74.43 ± 2.89	25.32 ± 1.49	116.37 ± 1.20^{a}	30.15 ± 0.57	287.70 ± 2.56	38.74 ± 1.03
LACH	382.73 ± 5.26^{b}	2.35 ± 0.18^{ab}	76.73 ± 2.58	26.67 ± 2.36	113.69 ± 2.10^{b}	30.20 ± 0.54	285.98 ± 7.19	39.11 ± 0.71
MACH	386.70 ± 6.90^{ab}	2.50 ± 0.08^{a}	75.90 ± 2.55	26.40 ± 2.07	114.96 ± 1.50^{ab}	29.97 ± 0.65	286.97 ± 1.74	38.40 ± 1.16
HACH	391.88 ± 4.98^{a}	2.29 ± 0.17^{b}	76.17 ± 2.26	25.38 ± 1.06	115.29 ± 1.76^{ab}	30.13 ± 0.77	285.98 ± 1.78	38.21 ± 1.05

NOTE Data in the same row with no letter or alternate letters on the shoulder indicate no significant difference ($P > 0.05$), different lowercase letters indicate significant difference ($P < 0.05$), and different uppercase letters indicate extremely significant difference ($P < 0.01$). The same below.

3.3 Effects of ACH extract on blood lipid of laying hens As indicated in Table 3, compared with the control group, the contents of TC, TG and LDL-C in the model group were significantly increased, and the content of HDL-C was significantly decreased ($P<0.05$); there was no significant difference in blood lipid between LACH group and model group ($P>0.05$). Compared with the model group, the levels of TC, TG and LDL-C in the MACH group were significantly decreased, while the level of HDL-C was significantly increased ($P<0.05$). The contents of TC, TG and LDL-C in the HACH group were significantly lower than those in the model group, while the content of HDL-C was significantly higher than that in the model group ($P<0.05$).

3.4 Effect of ACH extract on the liver of laying hens From Fig. 1, it can be seen that in the control group, the liver cell structure of the chicken was complete, the boundary was clear, and the cytoplasm was uniform. In the model group, the structure of chicken liver tissue was disordered, there were many vacuoles in the liver cells, the gap between the liver cords became larger, and the structure was blurred. In LACH group, the structure of liver tissue was disordered, and there was a small amount of vacuolar degeneration in hepatocytes; in MACH and HACH groups, the structure of liver tissue was clear, the structure of hepatocytes was complete, and there was no significant vacuolar degeneration.

Table 3 Effects of ACH extract on blood lipid of laying hens

Group	TC//mmol/L	TG//mmol/L	HDL-C//mmol/L	LDL-C//mmol/L
Contrl	33.60 ± 3.86^{c}	2.35 ± 0.49^{d}	$1\,210\pm192^{a}$	5.76 ± 0.51^{c}
Model	59.80 ± 6.05^{a}	5.02 ± 0.55^{a}	742 ± 68^{c}	8.89 ± 0.88^{a}
LACH	59.10 ± 6.10^{a}	5.05 ± 0.42^{a}	840 ± 120^{bc}	8.70 ± 0.72^{a}
MACH	47.00 ± 6.97^{c}	3.63 ± 0.64^{c}	870 ± 91^{a}	7.62 ± 0.56^{c}
HACH	37.80 ± 7.81^{b}	2.96 ± 0.56^{b}	$1\,150\pm146^{b}$	6.88 ± 0.72^{b}

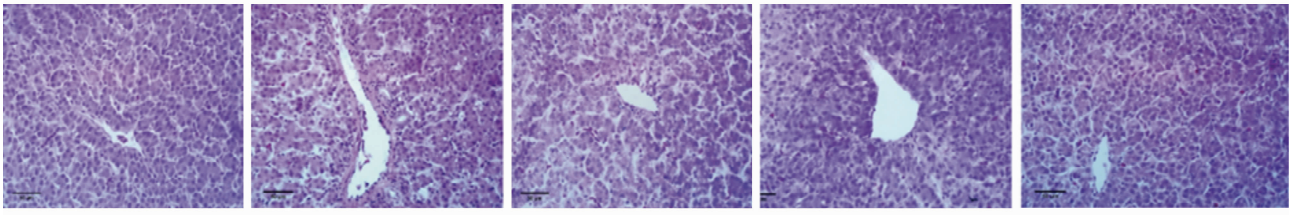

Fig. 1 HE staining of liver

4 Discussion

4.1 Effects of HELPD on blood lipid of laying hens Nutrient imbalance, excess energy, low protein content and insufficient trace elements in feed can lead to lipid metabolism disorders in laying hens, resulting in elevated blood lipid levels, liver cell degeneration, and further lead to the formation of fatty liver. In this study, hens were fed with HELPD to induce dyslipidemia. Clinically, FLHS is mainly characterized by lipid metabolism disorders, while HELPD leads to an increase in plasma fatty acids. Carbohydrates that are not used by animals are converted into fat accumulation (mainly TG) in the body, and the fat accumulated in the body is difficult to be excreted due to insufficient protein content and lack of apolipoproteins, which leads to lipodystrophy. Cage rearing can reduce the amount of exercise and energy consumption of laying hens, which is more likely to promote the occurrence of fatty liver syndrome in laying hens[7-8,10-11]. Findings of Peng *et al.*[12] and Rozenboim *et al.*[13] have shown that HELPD can lead to FLSH. In this experiment, the model group showed clinical manifestations such as loss of appetite, lethargy, preference for lying, lameness, *etc.* Combined with blood biochemical indicators and histopathological results, it can be said that the model was successful.

4.2 Effects of ACH extract on blood routine of laying hens Blood routine examination is an important basis for evaluating animal health. At present, there is no report about the effects of feeding HELPD on the blood routine of laying hens. In this experiment, there was no significant difference in the blood routine indicators between the model group and the control group, indicating that the high-fat diet had little effect on the blood routine indicators of laying hens. Most of the studies on the lipid-lowering and hepatoprotective effects of ACH extract have focused on the study of serum biochemical indicators such as blood lipids and cholesterol[14]. The study of Chen Xiaobai *et al.*[15] showed that ACH extract could reduce the mean corpuscular volume (MCV) and blood flow velocity of hyperlipidemia model rats. It is speculated that this effect will also occur in the formation of FLSH in laying hens. In the three treatments of ACH extract, the WBC content in the HACH group was significantly higher than that in the model group, the RBC content in the MACH group was significantly higher than that in the model group, and the MCVM in the LACH group was significantly lower than that in the model group. There was no significant difference in other blood routine indicators between the three doses of ACH treatment and the model group, indicating that the ACH extract had no significant effect on the blood routine of FLSH laying hens.

4.3 Effects of ACH extract on blood lipid of laying hens ACH contains flavonoids, alkaloids, triterpenoids, anthraquinones, polysaccharides and other chemical components. Abraline and hypaphorine were the main alkaloids, and schaftoside and Vicenin II were the main flavonoid C-glycosides. Pharmacological activity studies have found that ACH has hypolipidemic, antioxidant and hepatoprotective effects, and its hypolipidemic and hepatoprotective effects are related to its total flavonoids active components[16]. Total flavonoids can remove the accumulated oxygen free radicals in the body in time and have antioxidant capacity[17-19]. Therefore, it is speculated that the lipid-lowering and hepatopro-

tective effects of ACH may be related to the antioxidant mechanism[6,20-21]. Yuan Xujiang *et al.*[22] found that the active components of ACH can enter the blood in a free state to exert their efficacy in the experimental study of molecular docking screening of the active components of ACH for lipid-lowering and liver-protecting. The results of blood biochemical indexes in this test showed that compared with the control group, the contents of TC, TG and LDL-C in the model group were significantly increased, and the content of HDL-C was significantly decreased, which was consistent with the literature[8,12,23]. Except for HDL-C, there were significant differences in other blood lipid indicators among the high, middle and low dose groups. The medium and high doses of ACH extract had a greater effect on the four indicators of FLSH blood lipids of laying hens, and the indicators were closer to those of the control group, which was consistent with the results of literature[24], indicating that the regulation effect of ACH on blood lipids of rats was similar to that of laying hens. Lei Qingyao[24] observed the effects of ACH extract on the expression levels of ROCK and CD14 in the liver tissue of rats with nonalcoholic fatty liver disease (NAFLD), and found that ACH extract could reduce the content of ROCK and CD14, and protect the liver tissue to a certain extent. Therefore, it is speculated that ACH may protect the liver tissue of laying hens through liver tissue-related kinases. This experiment did not study the mechanism of ACH in reducing blood lipids, and the effective substances and pathways of ACH should be further clarified in the later study combined with the characteristics of lipid metabolism in laying hens.

5 Conclusions

There was no significant effect of ACH extract on blood routine indicators of laying hens fed with HELPD. The medium and high doses of ACH extract could increase the content of HDL-C, decrease the content of TC, TG and LDL-C, and eliminate the vacuolar degeneration of hepatocytes caused by HELPD, and the effect of the medium dose of ACH extract was better than that of the high dose, suggesting that the ACH extract (2 g per hen) had a good therapeutic effect on FLSH in laying hens. To some extent, this study provides a reference for the clinical application of ACH and the mechanism of action of FLSH in laying hens.

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