



Effects of Polysaccharide Extracted from Traditional Chinese Medical Herbs on Lymphocyte Transformation Rate and AI-HI Antibody Titer in Chicks

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Abstract

[Object]: Detect whether different concentrations of Chinese herbs compound polysaccharides (CPS), astragalus polysaccharides (APS) and angelica polysaccharides (ASP), epimedium herb polysaccharides (EPS) have effects on the immunity function of healthy Roman chicken. [Method]: 260 one-day-old chickens were divided into thirteen groups randomly, 20 birds each group. The physiological saline, Chinese herbs compound polysaccharides, APS, ASP or EPS had been hypodermically injected for seven days continuously, and the blood was drawn on the 7th, 14th, 21st, 28th, 35th, 42nd, 49th and 56th day to evaluate the activity of the translation rate of blood lymphocyte and AI-HI antibody titers in chickens. [Result]: The results of the experiment showed that the translation rate of blood lymphocyte and AI-HI antibody titers increased markedly after the use of Chinese herbs compound polysaccharides, APS, ASP and EPS to the chickens. Chinese herbs compound polysaccharides had more effective function improving the translation rate of blood lymphocyte and AI-HI antibody titers than others. [Conclusion]: The Chinese herbs compound polysaccharides, APS, ASP and EPS could promote the immunity function of the chickens. The Chinese herbs compound polysaccharides were the strangest one among them.

Keywords: Chinese herbs compound polysaccharides, Translation rate of blood lymphocyte, Antibody titer

1. Introduction

Chicken is sensitive to many infectious diseases, such as Newcastle Disease, Avian Influenza (AI) and Infectious Bursa Diseases, which caused high mortality and economic loss world widely. But there are no effective drugs to cure these diseases and the most effective way is to use Vaccines to prevent the occurrence of these. But them another problem comes which is that, vaccines is not always as effective as people expected.

Sometimes, because of weak immunity, poor quality, short protective terms or virus mutation immunity failure which often appears all can cause immunity failure(Chen X, 2002, p18-20). One way to solve it is to find a safe and effective immunopotentiator. Many papers in recent years showed that polysaccharides can enhance immunity and have no obvious side effect. So they are potential immunopotentiator. But the majority scholars only aim at single polysaccharide like angelica sinensis polysaccharide (Yang T H, 2005, p782-783), astragalus polysaccharide (Li S P, 2005, p51-54), lycium barbarum polysaccharide (Tao D Y, 2007, p6816-6818) and so on, to conduct the research, or several polysaccharide composed by a certain percentage of polysaccharide (Wang D Y, 2005, p3704-3708) conducted a study. The traditional Chinese medicine compound prescription polysaccharide (Yan G Q, 1998, p31-32) had not reported as the immunity intensifier's research. In this study, we focused on effect of compound polysaccharides (CPS), astragalus polysaccharides (APS), angelica polysaccharides (ASP), epimedium herb polysaccharides (EPS) on

immunity in health Roman chicken. Because the four selected polysaccharides are neglected by others, although other polysaccharides have been investigated by many studies. In the study, we check the effect of the four polysaccharides. In the next step our goal is to observe whether their application to the new discovered medicines can enhance their effects.

2. Materials and methods

2.1 Traditional Chinese medicine

The traditional Chinese medicine compound prescription by radix astragali, poria, radix rehmanniae preparata, fructus psoraleae, radix polygoni multiflori, herba epimedii, radix angelicae sinensis, radix codonopsis, rhizoma chuanxiong, fructus crataegi and radix ophiopogonis were bought from Shihezi Pharmaceuticals Company in Xinjiang, smashed. The extraction compound polysaccharides (CPS), astragalus polysaccharides (APS), angelica polysaccharides (ASP), epimedium herb polysaccharides (EPS) were processed in Shihezi University. The polysaccharides content is 42.82%, 27.35%, 27.73%, 32.30% respectively. The injection dose in the experiment refers to the actual pure polysaccharide, entering into the chick's body after calculating. Dissolve the APS with distilled water before injecting. Then the solution is autoclaved at 115°C for 30min.

2.2 Reagent

The live vaccine of the Avian Influenza - Newcastle Disease reorganizes (rL-H5) was provided by the Shihezi veterinary station. Avian Influenza H5 the hypotype HI antigen was provided by the Xinjiang army corps raising livestock veterinary services main terminal. Cell nutrient fluid: takes the TC199 culture medium to dissolve according to the instruction booklet in the 1000ml doubled distilled water, filters after the fungus loads separately, restored at 4°C. Joins 10% calf blood serum near the time (56°C the 30min deactivation, filtration eliminates fungus) and penicillin 100U/ml, chain mildew element 100g/ml, and adjusts pH with 60.0g/L NaHCO₃ to 7.2~7.4; The plant haemagglutinin (PHA) 10mg/ml filters has eliminated the fungus.

2.3 Equipment

DNP-9082 electric heating constant temperature incubator (Shanghai fine great test installation Limited company, Shanghai); PHS-3C precise PH counts (Shanghai instrument 3rd factories, Shanghai); DGW-99 table model high speed miniature centrifuge (Ningbo new iris biotechnology Limited liability company, Ningbo); HHS21-6 electric heating constant temperature water-bath (Beijing Chang An Scientific instrument factory, Beijing); OLYMPUS-CX21FS1 biology microscope (Guangzhou bright beautiful science and technology Limited company, Guangzhou); Ultra-clean work table.

2.4 Experimental design

Experimental chickens are from the chick's farm in Shihezi city, and their health is tested clinically. To divide 260 one-day-old chickens into 13 groups at random and each group has 20 chickens. Use the medication on them when they're one day old. The program is as follows: group 1 is the normal control, and receive 0.2ml/d normal saline, group 2~13 receive 0.2ml/d i.p. 12.5mg/ml, 25 mg/ml, 50 mg/ml compound polysaccharides(CPS), astragalus polysaccharides(APS), angelica polysaccharides(ASP), epimedium herb polysaccharides(EPS) respectively. The inject continuously for 7 days.

2.5 Breeding and management

The breeding method, condition, environment, feeder quality and husbandry of four groups' chicks are unified. The chickling on 7th the age with the live vaccine (rL-H5) the drop nose, and the spot eye immunity, on 21st the age carries on the second immunity.

2.6 Lymphocyte transformation rate determination (Lin Q H, 1999, p188-189)

In the 7, 14, 21, 28, 35, 42, 49 and 56 days after treatment, we take 10 chickens from each group to prepare the blood stochastically separately, and antifreeze with the sodium citrate. Takes 0.1ml, add 1.8ml TC199 nutrient fluid, and PHA 0.1 ml, to 0.1ml anti-hemogglutination samples, and then mix uniform postpositioned 37°C nurtures 72h warm. Shake them one time a day. Take them out after the raise had ended blot the majority of supernates, join 8.3g/L NH₄Cl 4ml to mix uniform, and set them at 37°C water bath 10min. After taking out, 2000r/min centrifugal 5min, abandons clear, the settling adds the 5ml fixture, sets at room temperature 10min. The offcenter abandons clear, takes 2~3 drop of settling in ice-cold and has on the moisture content slide glass, causes it to unfold evenly, after rapid drying up, the dye liquor dyes 15min with Giemsa Ranye. Then laundering, dry, oil mirror inspection. Observes 200 lymphocytes, the counting transformation cell number. According to the equation below equation:

$$\% \text{ lymphocyte transformation rate} = \frac{\text{transformed lymphocyte}}{(\text{transformed lymphocyte} + \text{untransformed lymphocyte})} \times 100$$

2.7 AI-HI antibody examination

In experiments 7, 14, 21, 28, 35, 42, 49, 56 days, takes 10 chickens from each group to pick the blood stochastically, the

separation blood serum, examines the AI-HI antibody titer with the β - micromethod. The result expressed with the log2 geometry average value.

2.8 Statistical analysis

Statistical analyses were carried out by One-way-ANOVA, Tukey and Dunnett's *posthoc* tests by using SPSS 12.0.1 Statistical Package (SPSS Inc., Chicago, IL). P values less than 0.05 ($p < 0.05$) were regarded as statistically significant. All the values are presented as mean $\pm$ SEM (the standard error of the mean).

3. Results

The result of table 1: each polysaccharide elevated lymphocyte transformation at the checkpoint 7th, 14th, 21st, 28th, and arrived at the peak at 28th. Compared with control, by 56th day, only 25mg/ml compound polysaccharides (CPS) showed significant difference ($P < 0.05$).

The result of table 2: each polysaccharide elevated AI-HI titers, AI-HI titers arrived at the peak at 28th and 35th. Compared with control group, even at 56th, compound polysaccharides (CPS), 25mg/ml astragalus polysaccharides (APS) have the significant effect ($P < 0.01$).

4. Discussion

In the study, compound polysaccharides (CPS), astragalus polysaccharides (APS), angelica polysaccharides (ASP), epimedium herb polysaccharides (EPS) enhanced the proliferation of lymphocyte and AI-HI. This result is in accordance with others. The result showed that the four polysaccharides can enhance herb's immunity. The findings of Xu Y (Xu Y, 2000, p434-437), Wang D Y (Wang D Y, 2006, p194-196), Gu X L (Gu X L, 2005, p813-820) and Li N (Li N, 2004, p61-64) coincide with our experimental result, which shows that the four polysaccharides can enhance herb's immunity.

At the three checkpoints 7th, 14th, 21st, the detection index of each experimental group was elevated which compared with the normal control group. At 28th day, it reached the peak, in the experiment, effect of 25mg/ml groups was better than 12.5mg/ml and 50mg/ml groups in all of the four polysaccharides groups. It indicated that there was not a positive correlation between concentration and improvement of immunity and its mechanism is expected to be further studied.

Cell immunity is mediated by T cells; lymphocyte transformation rate was an important index for evaluating cell immunity. The results of this study indicated that compound polysaccharides(CPS), astragalus polysaccharides(APS), angelica polysaccharides(ASP), epimedium herb polysaccharides(EPS) elevated lymphocyte transformation rate. By 49th day, lymphocyte transformation rate recovered to normal level in ASP, EPS and 12.5mg/ml APS, 50mg/ml APS groups.

In the study, vaccines were used together with compound polysaccharides(CPS), astragalus polysaccharides(APS), angelica polysaccharides(ASP) or epimedium herb polysaccharides(EPS) could elevated antibody titer(CPS) of Avian Influenza, and this result was in accordance with others(Kong X F, 2004, p468-472). Polysaccharides especially 25mg/ml APS, 12.5mg/ml and 25mg/ml compound polysaccharide (CPS) advance production, accelerate Rise Velocity of antibody, and also prolong effective period of antibody. All of these results showed that these polysaccharides can use as immunopotentiator.

References

- Chen, X, & Gao, S. (2002). Immunodepression and immune failure in poultry. *Journal of Animal Science and Veterinary Medicine*, 21(2), 18-20.
- Gu, X L, Li, H Q, Wang, J D, & Jiang, J J. (2005). Effects of compound polysaccharide extracted from traditional Chinese medical herbs on the immunity function in chickens. *Scientia Agricultura Sinica*, 38(4), 813-820.
- Kong, X F, Hu, Y L, Li, X R, Wang, D Y, Zhang, B K, Liu, J G, & Wang, X T. (2004). Effects of nine Chinese herbal medicinal ingredients on serum HI antibody titer after vaccination of NDV IV strain vaccine in chicks. *Acta Veterinaria et Zootechnica Sinica*, 35(4), 468-472.
- Li, N, She, R P, Han, L J, & Wang, K Z. (2004). Effect of astragalus root extractions on chicken growth and immunological function. *Chinese Journal of Veterinary Science and Technology*, 34(5), 61-64.
- Li, S P, & Zhao, X J. (2005). Effects of synbiotics of astragalus polysaccharide and profitable microbe on growth performance and immune function of chick. *Chinese Agricultural Science Bulletin*, 21(6), 51-54.
- Lin, Q H. (1999). *Immunology experiment*. Wuhan: Wuhan University press, pp.188-191
- Tao, D Y. (2007). Immunomodulation effect of lycium barbarum polysaccharide. *Journal of Anhui Agri.Sci*, 35(22), 6816-6818.
- Wang, D Y, Hu, Y L, & Sun, J L. (2005). Comparative study on adjuvanticity of compound Chinese herbal medicinal ingredients. *Vaccine*, 23(28), 3704-3708.

Wang, D Y, Hu, Y L, Sun, J L, Zhang, B K, & Liu, J G. (2006). Effect of Chinese herbal medicinal ingredient prescription on peripheral T lymphocyte transformation and serum antibody titer of chickens inoculated with ND vaccine. *Chinese Journal of Veterinary Science*, 26(2), 194-196.

Xu, Y, Mu, X S, Wang, G, Sun, P Y, Zhang, B F, & Chen, Y J. (2000). The effects of epimedium polysaccharides on the immune function of immune-depressed mice. *Journal of Shenyang Pharmaceutical University*, 17(6), 434-437.

Yan, G Q, Gu, X L, Wang, W, & Li, J. (1998). Effect of Chinese herbs immunopotentiator on immune function of chicks. *Chinese Journal of Veterinary Science and Technology*, 28(2), 31-32.

Yang, T H, Jia, M, & Mei, Q B. (2005). Effects of angelica sinensis polysaccharide on cell-mediated immunity. *Chinese Journal of Cellular and Molecular Immunology*, 21(6), 782-783.

Table 1. The effect of different density polysaccharides on the translation rate of blood lymphocyte

Groups	PS.CO mg/ml	7th day exp %	14th day exp %	21st day exp %	28th day exp %	35th day exp %	42nd day exp %	49th day exp %	56th day exp %
saline	0	40.6±1.435	40.8±1.497	40.8±0.583	41.0±1.612	41.2±1.158	40.8±1.158	40.6±0.748	40.8±1.068
APS	12.5	43.2±0.860	44.6±0.927*	44.8±0.860**	45.2±0.860**	44.8±0.860*	44.0±0.707*	42.8±0.860	40.0±1.000
APS	25	45.2±1.114**	45.8±1.594**	49.2±1.241**	55.6±1.077**	48.8±1.158**	46.0±0.707**	43.4±0.927*	41.2±1.241
APS	50	42.4±0.927	44.4±0.927*	44.6±0.927**	45.0±0.707**	44.4±0.927*	43.6±1.030*	42.0±0.707	40.4±0.927
ASP	12.5	41.4±1.077	44.4±0.927*	44.4±0.927*	44.0±0.707*	45.6±1.030**	43.4±0.927*	41.6±1.208	40.6±1.077
ASP	25	44.4±0.927*	45.2±1.655**	48.6±1.030**	47.2±1.497**	45.6±1.030*	44.2±1.356*	42.2±0.583	40.8±1.281
ASP	50	41.6±1.208	44.2±0.860*	43.8±0.860*	44.0±0.707*	43.8±0.860	43.0±0.707*	41.2±0.860	40.2±0.860
EPS	12.5	40.8±1.158	44.0±0.707	44.2±0.860*	44.0±0.707*	43.8±1.158	43.4±0.927*	40.8±0.860	40.4±1.228
EPS	25	40.4±0.707*	45.0±1.225**	46.0±1.000*	49.6±1.435**	45.0±1.000**	44.0±0.707*	41.4±1.228	40.6±1.030
EPS	50	41.0±0.707	44.2±0.860*	44.4±0.927*	44.2±0.860*	44.0±0.707*	43.8±0.860*	41.2±0.860	40.8±1.068
CPS	12.5	44.4±0.927*	44.6±0.927*	46.0±0.707**	52.2±0.860**	46.0±0.707**	44.8±0.870**	43.6±1.600*	41.6±0.927
CPS	25	45.6±1.720**	46.2±1.158**	50.2±1.281**	62.2±1.030**	54.6±0.927**	49.0±0.707**	44.0±0.707*	44.2±0.860*
CPS	50	44.2±1.158*	44.8±1.241*	47.8±0.860**	51.0±0.707**	45.2±1.068**	43.8±0.860*	43.4±0.927*	41.4±1.077

Note: Compared with the control group, in same Column, * P<0.05, ** P<0.01.

Table 2. The effect of different density polysaccharides on AI-HI antibody titers

Groups	PS.CO mg/ml	7th day exp	14th day exp	21st day exp	28th day exp	35th day exp	42nd day exp	49th day exp	56th day exp
saline	0	3.8±0.200	5.0±0.707	6.4±0.245	7.2±0.200	7.0±0.707	6.2±0.200	5.4±0.245	4.2±0.200
APS	12.5	4.4±0.245	6.0±0.707*	7.6±0.245**	8.4±0.245**	8.2±0.200**	7.2±0.200*	6.2±0.200*	5.0±0.316*
APS	25	4.8±0.374*	6.2±0.447**	7.8±0.200**	8.6±0.245**	8.4±0.245**	7.4±0.245**	6.4±0.245**	5.2±0.200**
APS	50	4.2±0.200	6.0±0.707*	7.2±0.200*	8.2±0.200**	8.0±0.000**	7.0±0.316*	6.2±0.200*	4.8±0.200
ASP	12.5	4.0±0.316	5.8±0.447*	7.2±0.374*	8.2±0.200**	8.0±0.316**	7.0±0.316*	6.0±0.316	4.8±0.200
ASP	25	4.4±0.245	6.0±0.707*	7.6±0.245**	8.2±0.200**	8.0±0.316**	7.0±0.316*	6.2±0.200*	5.0±0.316*
ASP	50	4.2±0.200	5.6±0.548	7.4±0.245**	8.0±0.316*	7.8±0.200*	6.8±0.374	5.8±0.200	4.6±0.245
EPS	12.5	4.2±0.200	5.8±0.447*	7.4±0.245**	8.0±0.316*	7.8±0.200*	6.8±0.200	5.8±0.200	4.6±0.245
EPS	25	4.4±0.245	6.0±0.707*	7.6±0.245**	8.2±0.200**	8.0±0.316**	7.0±0.316*	6.2±0.200*	5.0±0.00*
EPS	50	3.8±0.374	5.8±0.447*	7.2±0.200*	8.0±0.316*	7.6±0.245	6.8±0.200	5.6±0.245	4.4±0.245
CPS	12.5	4.6±0.400	6.2±0.447**	7.8±0.200**	8.6±0.245**	8.4±0.245**	7.4±0.245**	6.4±0.245**	5.2±0.200**
CPS	25	5.2±0.374**	6.4±0.548**	8.0±0.316**	8.8±0.200**	8.6±0.245**	7.8±0.374**	6.6±0.245**	5.4±0.245**
CPS	50	4.8±0.374*	6.0±0.707*	7.6±0.245**	8.4±0.245**	8.2±0.200**	7.2±0.200*	6.4±0.245**	5.2±0.200**

Note: Compared with the control group, in same Column, * P<0.05, ** P<0.01.