

Full Length Research Paper

Effects of three Chinese herbal crude polysaccharides on immunoglobulin A secreting cells and serum antibody titers in vaccinated chickens

Yan QIU^{1*}, Yuan-Liang HU², Fa-Ming DONG¹, De-Yun WANG² and Zhan-Qin ZHAO¹

College of Animal Technology, Henan Science and Technology University, Luoyang, 471003, P. R. China.
College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, 210095, P. R. China.

Accepted 18 December, 2019

This study was conducted to evaluate the effects of three Chinese herbal crude polysaccharides on immunoglobulin A secreting cells and serum antibody titers in vaccinated chickens. A total of 450 14-day-old chickens were randomly assigned to nine equal groups and all chickens were vaccinated. Concurrent with the first vaccination, chickens in groups 1 to 8 were intramuscularly injected with four crude polysaccharides among which the astragalus polysaccharide (APS) was selected as positive control at high and low doses, and group 9 (control group) with saline once a day for three successive days. The numbers of positive immunoglobulin A (IgA) secreting cells and the serum specific immunoglobulin G (IgG) antibody were determined by immunohistochemistry and micro method. The results showed that the individual administration of any of the three crude polysaccharides could significantly increase the number of IgA secreting cells, and the maximum numbers of increased IgA secreting cells in the cecum tonsil and duodenum in the three polysaccharides groups were 37.7 and 33.5 when compared with the controls, and those of the APS groups were 33.9 and 32.7. These three crude polysaccharides at appropriate doses also significantly enhance anti-Newcastle disease virus antibody titers, and the maximum antibody titer increase in the three polysaccharides groups was 1.6 log₂ when compared with the control group, and those of the APS groups was 1.7 log₂. These findings indicated that the appropriate doses of the three crude polysaccharides possess significant immune enhancing properties of mucosa and humoral immune responses, which have similar effect with astragalus polysaccharide, and may be useful as a new type of immune potentiator during vaccination.

Key words: Chinese herbal crude polysaccharides, vaccine, immunity, chickens.

INTRODUCTION

In recent years, many unknown and latent forms of infections have emerged in addition to the prevailing diseases. Among the various emerging diseases, viral

diseases in general and immunosuppressive viruses in particular are suspected as the etiological agents for a variety of clinical conditions in poultry. Newcastle disease (ND) virus is one of the most important avian infection agents, because of high mortality rates in acute infections caused by subclinical infections. Many researchers in Africa, Asia and Australia have identified ND as the major cause of mortality in chicken production. To protect chickens against ND virus, both live and inactivated vaccines have been used. The goal of vaccination is to generate a strong immune response providing long term protection against infection. However, it is difficult to provide full protection of chickens against virus infection. Thus, it is necessary to study and develop safer and more efficacious vaccine immune potentiators that are safe for

*Corresponding author. E-mail: qiuyan800429@yahoo.com.cn.
Tel/Fax: +86 379 64282341.

Abbreviations: APS, Astragalus polysaccharide; IRPS, isatis root polysaccharide; ARPS, achyranthes root polysaccharide; CYPS, Chinese yam polysaccharide; ND, Newcastle disease; IB, infectious bronchitis; HI, hemagglutination inhibition; IgG, immunoglobulin G; IgA, immunoglobulin A; CMF, calcium and magnesium-free; PBS, phosphate-buffered saline; DAB, diaminobenzidine.

chickens and have no risk of producing antigenic and pathogenic variants. The simultaneous application of a vaccine and an immune potentiator could improve the efficacy of vaccination.

Many Chinese herbal medicines and their ingredients have been reported to enhance immune responses (Hu, 1997; Xie, 2000; Ma et al., 2012), and thus have great potential in practical applications. Polysaccharides, one of the main classes of bioactive substances from Chinese herbal medicine, have been indicated to have wide pharmacological activities, especially broad immunomodulatory and antitumour effects. Thus, polysaccharides are regarded as biological response modifiers and attract attention, because of their natural origin, low toxicity in humans and animals, and long-standing use as folk medicines (Lu et al., 2003; Yon et al., 2006). It has been reported that astragalus, isatis root, achyranthes root and Chinese yam are common traditional Chinese medicinal plant widely used to enhance the body's natural defense mechanisms and the immune responses, and polysaccharides are the main effective components of immunological enhancement from these Chinese herbal medicines. Especially, the immune enhancement of astragalus polysaccharide has been reported by many researchers, and has been successfully used in livestock breeding industry (Cho et al., 2007; Cui et al., 2011; Sun and Xie, 2011; Zhao et al., 2008). In this study, we investigated the immunoenhancing effects of three kinds of crude polysaccharides with respect to mucosal and humoral immune responses following vaccination in chickens, astragalus polysaccharide as positive control. The aim of this study is to determine the potential of these three polysaccharides as a new type immune potentiator during vaccination.

MATERIALS AND METHODS

Preparation of Chinese herbal crude polysaccharides

The four crude polysaccharides were extracted using water-decoction-ethanol-precipitation method as previously described (Xue, 1985). Total polysaccharide was measured by Vitriol-anthracene ketone, using glucose without H₂O as a standard control (Liu et al., 1994). The contents (%) of total astragalus polysaccharide (APS), isatis root polysaccharide (IRPS), achyranthes root polysaccharide (ARPS) and Chinese yam polysaccharide (CYPS) (comparable with those of glucose) were 65.1, 56.4, 54.3 and 72.8%, respectively. Based on our previous studies and on the polysaccharide content of the extracts prepared here, the four crude polysaccharides were diluted with deionized water into 2 concentrations as follows: 4 and 2 mg/ml for APS, 3 and 1.5 mg/ml for IRPS, 6 and 3 mg/ml for ARPS and CYPS, respectively. The diluted preparations were sterilized by pasteurization and tested for endotoxin by pyrogen tests (Veterinary Pharmacopoeia Commission of the People's Republic of China, 2000). Following confirmation that all polysaccharide crude extracts met the acceptable standard of Chinese Veterinary Pharmacopoeia (less than 0.5 EU/ml), the preparations were stored at 4°C until use.

Reagents

Mouse anti-chicken IgA antibody (dilution: 1:100), biotinylated rabbit anti-mouse immunoglobulin (IgG) antibody, horseradish peroxidase-labeled chain ovalbumin (all from Shenzhen Jingmei Biotechnology Co. Ltd., Shenzhen, China), normal goat serum (Henan Tumor Hospital Pathology Laboratories, Zhengzhou, China), developer diaminobenzidine (DAB, Sigma) purchased from Beijing Zhongshan Biotechnology Co. Ltd., Beijing, China; hydrogen peroxide, citric acid, sodium citrate are produced by Zhejiang Mitaka chemical reagent Co., Ltd., Lanxi, China; methanol, formaldehyde are produced by Hubei Xinda Li Biochemical Co., Ltd., Wuhan, China; sodium dihydrogen phosphate, disodium hydrogen phosphate are produced by Bei Jing Kang Pu Hui Wei Technology Co., Ltd., Beijing, China.

Vaccine

ND (Lasota strain)-IB (H₁₂₀ strain) live virus vaccine (No. 315) and ND-IB oil adjuvant vaccine (No. 551) were provided by the Institute of Veterinary Medicine, Animal Husbandry Bureau of Henan Province, Zhengzhou, China.

Birds and housing

One-day-old white Roman male chickens (layer type), purchased from Zhengzhou Ruixiang Co. Ltd., were housed in wire cages (60 × 60 × 100 cm) in climate controlled rooms at 36±1°C and kept under 24 h light at the beginning of the pretrial period, with ten chickens per wire cage. The temperature was gradually reduced to room temperature in spring and the light time was kept constant to 12 h per day. Chickens were fed with a commercial starter diet, provided by Feed Factory of Animal Husbandry Bureau of Henan province.

Experimental design

At 14 days of age, 450 chickens were vaccinated with ND-IB live virus vaccine by intranasal and intraocular administrations, and then were randomly divided into nine treatment groups of 50 chickens each, 5 cages per treatment. Their mean titer of maternal antibody against ND virus was 4.5 log₂ and the average body weight was 97.6 g. Each chicken in groups 1 to 8 was injected subcutaneously with 0.5 ml of one of the four crude polysaccharides at one of two concentrations, once a day for three successive days. In group 9, as the control, each chicken was injected with 0.5 ml saline at the same times as treatment groups. At 28 days of age, all chickens were vaccinated for the second time with ND-IB oil adjuvant vaccine by subcutaneous injection in the dorsal region of the cervix. On days 10, 20, 30, 40, 50, and 60 after the first vaccination, eight chickens were sampled randomly from each group to determine changes in the number of IgA secreting cells in the duodenum and cecum tonsil mucosa by immunohistochemistry (Yang et al., 2002), and to determine temporal changes of serum ND antibody titers by micro method (Thekisoe et al., 2004).

Immunohistochemical examination for IgA secreting cells

After sacrifice, a fragment of the duodenum from the same region and cecum tonsil from the same side of each chicken were excised, fixed in 10% neutral formalin solution and embedded in paraffin. Immunohistochemistry was performed on 0.6 mm thick paraffin sections. After deparaffinization and dehydration, endogenous peroxidase was inactivated by incubation with 0.3% hydrogen peroxide in methanol, and was washed in phosphate-buffered saline

(PBS, 0.01 M phosphate, 0.13 M NaCl, pH 7.4) for 10 min, then demasked by microwave oven treatment and citrate buffer. After washing in PBS, the sections were treated with 5% normal goat serum in PBS in a humid chamber for 30 min at room temperature to block non-specific binding. The sections were rinsed three times with PBS for 5 min and then stained separately with monoclonal mouse anti-chicken IgA antibody and the preparations were incubated at 4°C overnight. Tissues were rinsed in PBS for 5 min and then incubated for 30 min with biotinylated secondary antibody diluted (1:50) in PBS. After rinsing with PBS, sections were incubated with horseradish peroxidase-labeled chain ovalbumin for 30 min, washed with PBS and the reactions were made visible with DAB substrate. Sections were then counterstained with hematoxylin, rinsed with distilled water and cleared with xylene. All incubations were performed in a moist chamber. Control staining was carried out simultaneously, in which the primary antibody was replaced with PBS.

Hemagglutination inhibition examination for serum specific IgG antibody

Blood samples (1.0 ml per chicken) were drawn into Eppendorf tubes from the main brachial vein of the chicken and allowed to clot at 37°C for 2 h prior to collecting serum. Serum was separated by centrifugation and stored at -20°C for use. Briefly, after inactivation of serum at 56°C for 30 min, two-fold serial dilution of serum in a 96-well, V-shaped bottom microtiter plate containing 50 µl PBS was performed, and 50 µl of ND virus antigen (4 hemagglutinin (HA) units) was added to all the wells except for the last row, as controls. Serum dilutions ranged from 1:2 to 1:2048. The antigen serum mixture was incubated for 10 min at 37°C. Then 50 µl of a 1% rooster erythrocyte suspension was added to each well and re-incubated for 30 min. Positive serum, negative serum, erythrocytes, and antigens were included as controls. The highest dilution of serum causing complete inhibition of erythrocyte agglutination was considered the endpoint. The geometric mean titer was expressed as reciprocal log₂ values of the highest dilution that displayed anti-ND virus hemagglutination inhibition.

Statistical analysis

The sections were observed using an LEICA microscope (Model DM2000, Germany, purchased from Leica Microsystems Trading Ltd. Shanghai, China) and analyzed by Qwin image analysis system of LEICA image workstations (CD 2000, Germany, purchased from Leica Microsystems Trading Ltd., Shanghai, China). Twenty different fields of view were chosen per section, with 8 sections per group and analysis of positive IgA secreting cells which appear as brown (Figure 1) was calculated by number. The number of IgA secreting cells in regional units was used for the statistical analysis of the data.

Data are expressed as mean ± standard deviation for analysis; single factor analysis of variance (ANOVA) test was performed using Statistical Package for Social Sciences (SPSS) to determine the difference among herbal polysaccharides and control groups. $P < 0.05$ was considered significant for all analyses.

RESULTS

Increased numbers of IgA secreting cells in duodenum of treated chickens

On day 20 after the first vaccination, the numbers of IgA secreting cells in the duodenum in the APS_L group were significantly elevated when compared with controls ($P <$

0.05). On day 30, the numbers of IgA secreting cells in APS_H, APS_L, IRPS_L, ARPS_H and CYPH groups were significantly elevated when compared with the controls ($P < 0.05$), and the largest mean number of IgA secreting cells was 139.1 in IRPS_L group, and 133.2 in APS_H, which in control group was 101.4. On days 40 and 50, the numbers of IgA secreting cells in APS_L, IRPS_L, ARPS_H and CYPH groups were significantly elevated when compared with the controls ($P < 0.05$), and the largest mean number of IgA secreting cells in CYPH and IRPS_L groups was 128.9 and 120.9, respectively, and was 131.4 and 118.6 in APS_L, which in control group was 97.5 and 88.1. On day 60, the numbers of IgA secreting cells in IRPS_L and CYPH groups were significantly elevated when compared with controls ($P < 0.05$), and the largest mean number of IgA secreting cells in IRPS_L group was 99.3, and 97.6 in APS_L, which in control group was 75.7 (Table 1).

Increased numbers of IgA secreting cells in cecum tonsils of treated chickens

On day 20 after the first vaccination, the numbers of IgA secreting cells in the cecum tonsil in the treatment groups were elevated when compared with the controls, and there was no significant difference ($P > 0.05$). On day 30, the numbers of IgA secreting cells in APS_L, IRPS_H, IRPS_L, ARPS_H and CYPH groups were significantly elevated when compared with the controls ($P < 0.05$), and the largest mean number of IgA secreting cells in IRPS_L group was 138.3, and was 141.4 in APS_L, which in the control group was 108.7. On days 40 and 50, the numbers of IgA secreting cells in APS_L, IRPS_L, ARPS_H and CYPH groups were significantly elevated when compared with the controls ($P < 0.05$), and the largest mean number of IgA secreting cells in IRPS_L group was 128.9 and 110.2, and was 122.5 and 108.8 in APS_L group, which in the control group was 95.4 and 85.5. On day 60, the numbers of IgA secreting cells in APS_L, IRPS_L and CYPH groups were significantly elevated when compared with the controls ($P < 0.05$), and the largest mean number of IgA secreting cells in IRPS_L group was 105.3, and for APS_L group was 102.2, which in control group was 77.1 (Table 2).

Dynamic changes in serum ND virus-specific IgG antibody titers

On day 10 after the first vaccination, ND virus-specific IgG antibody titers among the 9 groups showed no significant difference ($P > 0.05$). For APS, on days 20, 30, 40, and 50, ND virus-specific IgG antibody titers in the APS_L group were higher when compared with the controls significantly ($P < 0.05$). And on day 60, the titers in APS_H and APS_L groups were higher when compared with the controls significantly ($P < 0.05$); the antibody titer in APS_L group was 8.9 log₂, which in control group was 7.2 log₂; the antibody titer increased in the APS_L group was the

Table 1. Dynamic changes in the number of IgA secreting cells in the duodenum of vaccinated chickens.

Group	D ₁₀	D ₂₀	D ₃₀	D ₄₀	D ₅₀	D ₆₀
IRPS _H	72.3±11.2 ^a	99.1±14.3 ^b	119.6±16.1 ^b	119.2±17.7 ^b	97.8±13.6 ^b	90.2±12.9 ^b
IRPS _L	77.8±14.7 ^a	105.5±15.2 ^b	139.1±19.5 ^a	127.1±18.2 ^a	120.9±15.5 ^a	99.3±12.6 ^a
ARPS _H	75.9±10.9 ^a	108.9±14.5 ^b	135.5±18.3 ^a	125.5±17.5 ^a	114.7±14.5 ^a	95.9±14.4 ^{ab}
ARPS _L	73.6±11.6 ^a	97.1±13.9 ^b	118.8±15.4 ^b	114.6±17.9 ^b	95.7±13.2 ^b	87.6±13.8 ^b
CYPS _H	71.4±13.1 ^a	110.6±15.9 ^{ab}	136.4±19.8 ^a	128.9±17.3 ^a	115.2±14.9 ^a	98.4±14.1 ^a
CYPS _L	75.1±12.8 ^a	98.7±13.5 ^b	114.2±15.6 ^b	110.8±16.8 ^b	101.3±15.8 ^b	84.7±13.2 ^b
APSH	79.2±14.2 ^a	96.9±13.3 ^b	133.2±21.9 ^a	122.3±18.4 ^b	105.1±15.1 ^b	85.9±15.2 ^b
APSL	74.7±12.4 ^a	117.3±17.4 ^a	129.7±13.4 ^a	131.4±18.9 ^a	118.6±14.8 ^a	97.6±14.6 ^{ab}
Control group	74.9±13.7 ^a	95.3±14.8 ^b	101.4±17.7 ^b	97.5±15.1 ^b	88.1±14.3 ^b	75.7±12.9 ^b

Column data marked without the same superscripts differ significantly (P<0.05). D, day; APS, astragalus polysaccharide which is the positive control; IRPS, isatis root polysaccharide; ARPS, achyranthes root polysaccharide; CYPS, Chinese yam polysaccharide.

Table 2. Dynamic changes in the number of IgA secreting cells from cecal tonsils of vaccinated chickens.

Group	D ₁₀	D ₂₀	D ₃₀	D ₄₀	D ₅₀	D ₆₀
IRPS _H	69.7±12.9 ^a	93.6±14.6 ^a	128.7±17.9 ^a	106.3±16.9 ^b	99.1±15.4 ^b	92.6±13.2 ^b
IRPS _L	68.4±11.8 ^a	102.8±15.2 ^a	138.3±19.3 ^a	128.9±16.2 ^a	110.2±17.3 ^a	105.3±16.1 ^a
ARPS _H	70.3±12.7 ^a	99.2±14.3 ^a	127.8±14.8 ^a	119.8±15.7 ^a	107.9±16.6 ^a	97.5±15.2 ^{ab}
ARPS _L	60.8±11.1 ^a	95.3±13.7 ^a	117.9±15.6 ^b	113.4±17.1 ^{ab}	96.3±15.8 ^{ab}	83.8±13.3 ^b
CYPS _H	65.5±11.5 ^a	98.4±14.1 ^a	129.6±16.3 ^a	120.7±16.4 ^a	106.5±15.1 ^a	99.9±12.4 ^a
CYPS _L	67.9±12.2 ^a	91.6±13.9 ^a	112.3±16.8 ^b	101.5±16.1 ^b	92.4±14.2 ^b	85.9±13.5 ^b
APSH	62.2±10.8 ^a	100.7±14.9 ^a	120.1±16.5 ^{ab}	103.2±15.9 ^b	97.6±14.5 ^b	88.5±13.1 ^b
APSL	64.9±11.3 ^a	94.5±13.8 ^a	141.4±18.7 ^a	122.5±15.5 ^a	108.8±16.7 ^a	102.2±14.3 ^a
Control group	65.3±11.9 ^a	89.9±13.6 ^a	108.7±17.7 ^b	95.4±15.3 ^b	85.5±14.7 ^b	77.1±12.8 ^b

Column data marked without the same superscripts differ significantly (P<0.05). D, day; APS, astragalus polysaccharide which is the positive control; IRPS, isatis root polysaccharide; ARPS, achyranthes root polysaccharide; CYPS, Chinese yam polysaccharide.

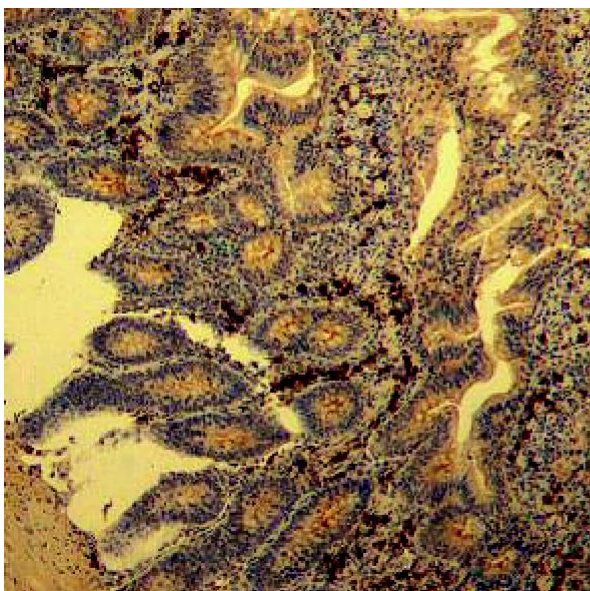


Figure 1. The section by Immunohistochemical staining which were observed using optical microscope. Distribution of SIgA positive cells which appears brown in the chicken duodenum and the section was observed at 400x magnification.

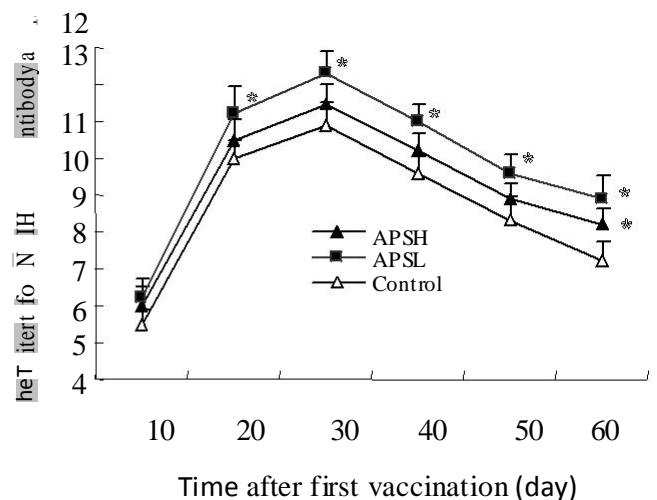


Figure 2. Dynamic changes of serum specific IgG antibody titers (Log₂) in APS (positive control) and control groups. Data represent the mean ± SD. *P<0.05 compared with controls. ND, New castle disease; APS, astragalus polysaccharide; APS_H, high dose astragalus polysaccharide; APS_L, low dose astragalus polysaccharide.

maximum (1.7 log₂) when compared with the controls (Figure 2). For IRPS, on days 20, 40, and 50, the titers in

IRPS_L group were higher when compared with the controls significantly ($P < 0.05$). On days 30 and 60, the titers in IRPS_H and IRPS_L groups were higher when compared with controls significantly ($P < 0.05$). And on days 60, the antibody titer in IRPS_L group was 8.8 log₂, which in control group was 7.2 log₂; the antibody titer increased in IRPS_L group was the maximum (1.6 log₂) when compared with the controls (Figure 3). For ARPS, on days 20, 30, 40, 50, and 60, the titers in ARPS_H group were higher when compared with the controls ($P < 0.05$). And on days 60, the antibody titer in ARPS_H group was 8.5 log₂, which in control group was 7.2 log₂; the antibody titer increased in the ARPS_H group was the maximum (1.3 log₂) when compared with the controls (Figure 4). For CYPS, on days 20, 30, 40, 50, and 60, the titers in the CYPS_H group were higher when compared with controls significantly ($P < 0.05$). And on days 60, the antibody titer in CYPS_H group was 8.4 log₂, which in control group was 7.2 log₂; the antibody titer increased in CYPS_H group was the maximum (1.2 log₂) when compared with controls (Figure 5). It showed that IRPS is the most similar to APS in raising ND virus-specific IgG antibody titers.

DISCUSSION

The mucosal immune system is equipped with unique innate and acquired defense mechanisms, which provide a first line of protection against ingested infectious agents (Mick and Karin, 2006). Secretory IgA is the major antibody isotype present in mucosal secretions and has many functions, both direct and indirect, that prevent infective agents such as bacteria and viruses from breaching the mucosal barrier (Egmond et al., 2001; Russell and Sibley, 1999). Therefore, IgA secreting cells are important for the protection of mucosal surfaces. Changes in the numbers of IgA secreting cells are one of the standards used to estimate mucosal immunity. In this study, the presence of positive IgA secreting cells was detected from duodenum and cecum tonsils of chickens by immunohistochemistry. We found that the numbers of positive IgA secreting cells per unit area from tissues of treatment groups were much higher than those from control groups at most time points, especially in the APS_L, IRPS_L, ARPS_H and CYPS_H treatment groups. This suggests that APS, IRPS, ARPS and CYPS might promote the differentiation and proliferation of IgA secreting cells in the intestinal mucosa of chickens. This demonstrated that Chinese herbal polysaccharides could effectively stimulate mucosal immune responses to resist external microbial invasion. Interestingly, we found that the effects of APS and IRPS at low doses were better than ARPS and CYPS at high doses. This phenomenon concurs in part with a study by Zhang et al. (2007), where two compound adjuvants (cMIA I and cMIA II) promoted IgA secreting cells and intestinal intraepithelial lymphocytes in chickens vaccinated with attenuated Newcastle-disease vaccine.

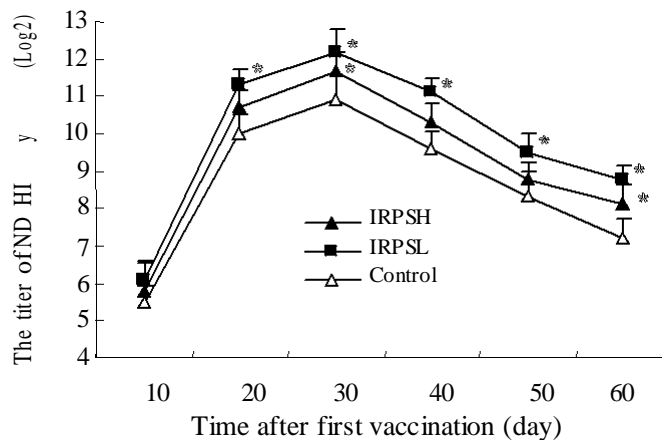


Figure 3. Dynamic changes of serum specific IgG antibody titers (Log₂) in IRPS and control groups. Data represent the mean \pm SD. * $P < 0.05$ compared with controls. ND, New castle disease; IRPS, isatis root polysaccharide; IRPS_H, high dose isatis root polysaccharide; IRPS_L, low dose isatis root polysaccharide.

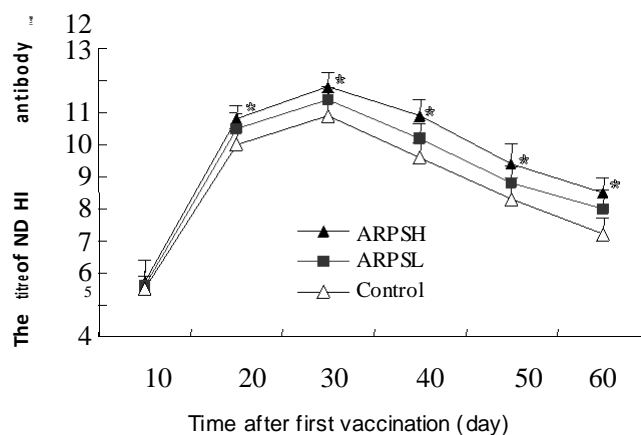


Figure 4. Dynamic changes of serum specific IgG antibody titers (Log₂) in ARPS and control groups. Data represent the mean \pm SD. * $P < 0.05$ compared with controls. ND, New castle disease; ARPS, achyranthes root polysaccharide; ARPS_H, high dose achyranthes root polysaccharide; ARPS_L, low dose achyranthes root polysaccharide.

Merz et al. (1981) reported that humoral immune responses played important roles in the host's defense against ND virus infection. The specific antibodies could neutralize or inactivate the free virus by binding to virus surface glycoproteins, thus inhibiting the attachment of virus to cells, and blocking viral spread. The dynamic changes of specific serum IgG antibody titers reflect the state of humoral immunity in the animals. Our results showed that the antibody titers in most treatment groups at many time points were significantly higher than in the control group. Titers in the APS_L, IRPS_L, ARPS_H and CYPS_H groups at five time points were significantly higher than in controls, indicating that low dose IRPS and high

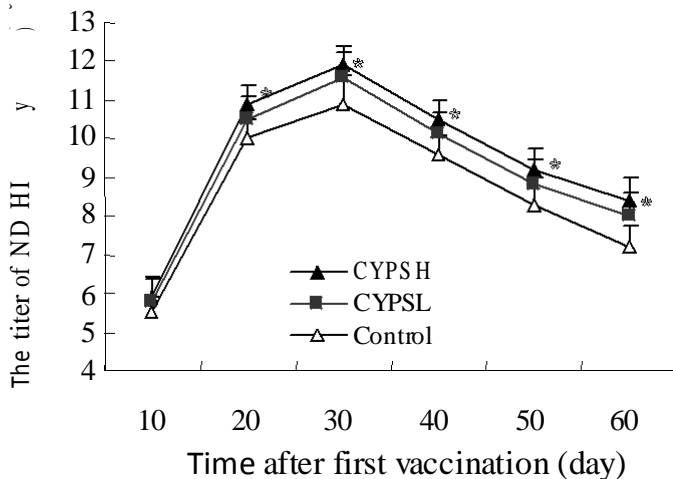


Figure 5. The dynamic changes of serum specific IgG antibody titers (Log₂) in CYPS and control groups. Data represent the mean ± SD. *P<0.05 compared with controls. ND, New castle disease; CYP, Chinese yam polysaccharide; CYP_{SH}, high dose Chinese yam polysaccharide; CYP_{SL}, low dose Chinese yam polysaccharide.

dose ARPS and similar to APS, CYPS had the best effect on enhancing humoral immunity. Antibody titers in low dose APS and IRPS and high dose ARPS and CYPS chickens up to 60 days old were still higher than 8.4 Log₂, while the titer in control group was 7.2 Log₂. This indicated that APS, IRPS, ARPS and CYPS at suitable doses could maintain higher antibody titers, because of a slower decline of antibody titer. Gu et al. (2005) reported that Chinese herbal medicine compound polysaccharides could promote the development of immune organs in chickens. The main immune organ for specific antibody production is the thymus and bursa of Fabricius (Alam et al. 1997). Thus, these three Chinese herbal crude polysaccharides could strengthen humoral immunity in vaccinated chickens through promoting development of immune organs.

These results showed that immune-enhancing effects of the three different crude polysaccharides are very similar to APS. In our previous preliminary test, a variety of traditional Chinese medicine were selected and extracted to observe the immunomodulatory effects on mice, and then these crude polysaccharides which had the better effect were selected to observe its immune-enhancing effect in chickens, when compared with APS. This may be the reasons why the experimental results are very similar, but it indicated that the suitable doses of these crude polysaccharides were not the same, and the effect of IRPS which was most similar to APS at low doses were slightly better than ARPS and CYP at high doses. Similar research also verified the remarkable potential benefits of crude polysaccharides derived from Chinese medicines (Chen et al., 1997; Sun et al., 2005).

Conclusion

This study confirmed that low dose IRPS and high dose ARPS and CYP could significantly promote the differentiation and proliferation of IgA secreting cells in the intestinal mucosa and increase serum ND virus-specific IgG antibody titers, and thus, enhance mucosal and humoral immune responses. It takes longer time to inhibit the multiplication of virus when compared with antiviral drug, but Chinese herbal crude polysaccharides has the advantages of natural, safe, less toxic or side effect at suitable dose, because the antiviral efficacy of these Chinese herbal crude polysaccharides is achieved through enhancing the body's immune system. Therefore, Chinese herb polysaccharides should be used for the prevention of viral diseases, rather than treatment. These three crude polysaccharides may form the basis for a new immune potentiating drug in the domestic animal and poultry industry. The dosage used is an important factor and must be considered in the development of a Chinese herbal medicinal immune potentiating drug. Further study on the mechanism of protective vaccination effects of the three Chinese herbal polysaccharides is underway.

ACKNOWLEDGEMENTS

This research was supported by the Foundation of National Science and Technology Pillar Program (2008BADB4B06) and the Foundation for Doctors from Henan University of Science and Technology (09001240). The authors are grateful to all staffs in the Veterinary Microbiology Laboratory of Henan Agricultural University for their experimental assistance.

REFERENCES

Alam KMT, Lslam MA, Rahman MM (1997). Antibody response and challenged against New castle disease. *Bangladesh Vet. J.* 31:23-27.

Chen YQ, Chen JX, Zhang TS (1997). The antitumor effect and the influence on immunity function of the four polysaccharides. *Chin. J. Cancer.* 3:198-200.

Cho WC, Leung KN (2007). *In vitro* and *in vivo* immunomodulating and immunorestorative effects of *Astragalus membranaceus*. *J. Ethnopharmacol.* 113(1):132-141.

Cui W, Wu GX, Zhang ZL (2011). Effects of *Achyranthis bidentatae Radix* and *Achyranthes bidentata* Polysaccharides on Enhancing Immune Function. *Chin. J. Exp. Trad. Med. Formul.* 16:141-143.

Egmond MV, Damen CA, Spriell ABV (2001). IgA and IgA Fc receptor. *Trends Immunol.* 22:205-211.

Gu XL, Li HQ, Wang JD (2005). Effects of Compound Polysaccharide Extracted from Traditional Chinese Medical Herbs on the Immunity Function in Chickens. *Scientia Agric. Sinica* 4:813-820.

Hu YL (1997). Progress in the study of immunopharmacology of Chinese herbal medicine. *Chin. J. Immunol.* 3:96-98.

Liu XP, MA Z, Wang XY, Wang Y (1994). Studies on Huangqi polysaccharide oral liquid. *J. Chin. Med. Mater.* 6:40-43.

Lu XT, Dai JH, Liao M, Liu N (2003). Advances of studies on immunoregulative activities of polysaccharides. *Prog. Vet. Med.* 24:10-12.

Ma HD, Deng YR, Tian Z, Lian ZX (2012). Traditional Chinese Medicine

- and Immune Regulation. *Clin. Rev. Allergy Immunol.* 24:1-4.
- Merz DC, Scheid A, Choppin P (1981). Immunological studies of the functions of paramyxovirus glycoprotein. *Virology* 28:208-221.
- Mick B, Karin H (2006). The postnatal development of the mucosal immune system and mucosal tolerance in domestic animals. *EDP Sci.* 37:443-453.
- Russell MW, Sibley DA (1999). IgA as an anti-inflammatory regulator of immunity. *Oral Dis.* 5:55-57.
- Sun HL, Miao DY, Gong YM, Zhang PJ (2005). Effect of extractive amylose from tragacanth, rock alga and cole on the immunity with ND live vaccine in chickens. *Chin. J. Prev. Vet. Med.* 5:205-208.
- Sun, Xie B (2011). Progress in pharmacological study of Chinese yam. *Trad. Chin. Drug Res. Clini. Pharmacol.* 3:353-355.
- Thekisoe MMO, Mbatia PA, Bisschop SPR (2004). Different approaches to the vaccination of free ranging village chickens against Newcastle disease in Qwa-Qwa. *South Afr. Vet. Microbiol.* 101:23-30.
- Veterinary Pharmacopoeia Commission of the People's Republic of China (2000). *Veterinary pharmacopoeia of the People's Republic of China.* Chemical Industrial Press, Beijing, China. pp. 72-73.
- Xue YL (1985). *Handbook of laboratory experiments in plant physiology.* Shanghai Science and Technology Press, Shanghai, China, pp. 136-138.
- Xie XQ (2000). Exploitation and application of new-type preparation of Chinese herbal medicine. *People Sanitary Press, Beijing, China.* pp. 377-383.
- Yang Q, Lian GJ, Huang GQ (2002). Effect of cysteamine on the modulation of sIgA cells and intraepithelium lymphocytes in chicken intestine. *J. Nanjing Agric. Univ.* 25:89-92.
- Yon ZT, Lina ZH, Peter C (2006). Physicochemical properties and antitumor activities of water-soluble native and sulfated hyperbranched mushroom polysaccharides. *Carbohydr. Res.* 341:2261-2269.
- Zhang XF, Zhang XW, Yang Q (2007). Effect of compound mucosal immune adjuvant on mucosal and systemic immune responses in chicken orally vaccinated with attenuated Newcastle disease vaccine. *Vaccine* 25:3254-3262.
- Zhao YL, Wang JB, Shan LM, Jin C, Ma L, Xiao XH (2008). Effect of Radix isatidis polysaccharides on immunological function and expression of immune related cytokines in mice. *Chin. J. Integr. Med.* 14(3):207-211.