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Sulfated modification can enhance the adjuvant activity of astragalus polysaccharide for ND vaccine

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Abstract

Two hundred 14-day-old chickens were divided randomly into 10 groups. At the same time of vaccination with Newcastle disease vaccine, the chickens in six experimental groups were intramuscularly injected with three sulfated astragalus polysaccharides (sAPSs), $sAPS_{40}$, $sAPS_{50}$ and $sAPS_{60}$, at high or low doses, respectively, in two adjuvant control and two non-adjuvant control groups, non-sulfated APS and physiological saline, once a day for three successive days. On days 7, 14, 21 and 28 after vaccination, the blood samples were collected from brachial vein for determination of serum HI antibody. On days 14, 21, 28 and 35 after vaccination, the blood samples were collected by cardiopuncture for test of peripheral T lymphocyte proliferation. The results showed that three sAPSs could significantly enhance antibody titer and promote lymphocyte proliferation, which were better than that of non-sulfated APS with certain of dose– and time–effect relationships. At high dose, their effects on enhancing antibody titer were the better, at low dose, promoting lymphocyte proliferation. In general evaluation the efficacy of $sAPS_{60}$ was best and would be expected as a candidate of new-type immunopotentiator.

Keywords: Astragalus polysaccharide; Sulfated modification; Sulfated astragalus polysaccharide; Peripheral lymphocyte proliferation; Serum antibody titer

1. Introduction

Sulfated polysaccharide is a class of polysaccharides with sulfated group in its hydroxyl and possesses many different or stronger biological activities in comparison with non-sulfated polysaccharide, such as the anti-coagulation (Alban & Franz, 2000; Yang, Du, Huang, Wan, & Li, 2002), anti-virus (Amornrut et al., 1999; Ghosh et al., 2004; Talarico et al., 2004), anti-cancer (Nie, Shi, Ding, & Tao, 2006; Peng, Zhang, Zeng, & Kennedy, 2005), immune enhancement (Sugawara, Ishizaka, & Moller, 1982), anti-proliferation (Hoffman & Paper, 1993a) and so on. Along with the increasing pursuit for strong or new biological activities, the molecular modification and structure improvement of polysaccharide becomes an important research field. Many studies confirmed that the biological activities of the Chinese herbal medicinal polysaccharides could be improved or changed by the sulfated modification. Therefore, sulfated modification could be used to enhance the biological activities of Chinese herbal medicinal polysaccharides or obtain more sulfated polysaccharides.

Astragalus polysaccharide (APS) is one of main effective ingredients in *Astragali Radix* (Hoffman & Paper, 1993b). In our previous researches, we not only studied the immune enhancement (Kong, Hu, Rui, Wang, & Li, 2004) and adjuvant activity (Wang et al., 2005) of 10

Abbreviations: APS, astragalus polysaccharide; sAPS, sulfated astragalus polysaccharide; ND, Newcastle disease; HI, hemagglutination inhibition; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ConA, concanavalin A; DMSO, dimethyl sulfoxide.

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kinds of Chinese herbal medicinal ingredients, but also studied the antiviral activity of them (Hu et al., 2004), and found APS had significant immune enhancement, better adjuvant activity and the antiviral activity in some degrees. Later, total APS (APS_t) was extracted by one-step ethanol precipitation with 75% of working concentration and three fractional APSs (APS_{40}, APS_{50} and APS_{60}) were extracted by stepwise ethanol precipitation with 40%, 50% and 60% of working concentration. Four sulfated APSs, $sAPS_t$, $sAPS_{40}$, $sAPS_{50}$, $sAPS_{60}$ and sulfated epimedium polysaccharide (sEPS) were prepared by chlorosulfonic acid–pyridine method and their effects on infectious bursal disease virus (IBDV) infecting chicken embryo fibroblast (CEF) were compared. These results showed that sulfated modification could enhance the antiviral activity of polysaccharides (Huang et al., 2007; Lu, Wang, Hu, Huang, & Wang, 2008).

In present research, authors further compared the effects of three sAPSs with non-sulfated APS_t, as immune adjuvant, on serum antibody titers and peripheral lymphocyte proliferation in the chickens vaccinated with ND vaccine taking non-sulfated APS_t as control. The purpose of this research is to probe into the probability of sulfated modification to raise the immune enhancement of APS, select the best sulfated astragalus polysaccharide and offer theoretical evidence for development of new-type immunopotentiator.

2. Materials and methods

2.1. Extraction and purification of APSs

APS_t, $sAPS_{40}$, $sAPS_{50}$ and $sAPS_{60}$ were prepared in our laboratory. Briefly, *Astragali Radix* was decocted three times with water and condensed into decoction corresponding 1 g materia medica/ml. APS_t was extracted by one-step ethanol precipitation that is ethanol was added into above-mentioned decoction to make ethanol concentration reach to 75% (v/v). Three fractional APSs, APS_{40}, APS_{50} and APS_{60}, were extracted from the APS_t solution by stepwise precipitation with ethanol at 40%, 50% and 60% of working concentration in turn.

They were purified, respectively, by Sevag's method (Qin, Huang, & Xu, 2002) and active carbon adsorption, then chromatography with macroporous adsorption resins D101 and ADS-7 column, DEAE cellulose A-25 column and Sephadex G-75 column (Zhao, 1994) in turn to remove protein, pigment and other impurity.

2.2. Sulfated modification of APSs

Three sulfated APSs (sAPSs), $sAPS_{40}$, $sAPS_{50}$ and $sAPS_{60}$, were prepared by chlorosulfonic acid–pyridine method and the modified conditions were based on our preparative experiment. In brief: The mixture of chlorosulfonic acid and pyridine complex (1:6) was prepared in ice bath. Then, 400 mg of one of APSs was added and stirred for 1 h at temperature 95 °C. The compound was dissolved in 100 ml ice-cold water, cooled to room temperature, neutralized with saturated NaOH solution and precipitated with 95% ethanol. The sediment was re-dissolved with water and dialyzed in dialysis sack against tap water for 48 h and distilled water for 12 h, then lyophilized to obtain sulfated APS (sAPS). Three sAPSs, $sAPS_{40}$, $sAPS_{50}$ and $sAPS_{60}$, were prepared. Their degrees of substitution (DS) determined by Antonopoulos' method (Dodgson & Price, 1962) were 1.15, 1.265 and 1.545, respectively, and FT-TR spectra recorded by Nicolet fourier transformation infra-red (FT-IR) 360 spectrophotometer (Nicolet) and KBr pellets method displayed the characteristic of sulfate ester (Ghosh et al., 2004).

Three sAPSs and one non-sulfated APS_t were diluted with deionized water (Key laboratory of Nanjing Agricultural University) into high (8 mg/ml) and low (4 mg/ml) two concentrations according to our previous experiment (Kong et al., 2004; Wang et al., 2005). The diluted preparations were sterilized by pasteurization and detected for endotoxin by pyrogen tests (Veterinary Pharmacopoeia commission of the People's Republic of China, 2000). When the endotoxin amount was up to the standard of Chinese Veterinary Pharmacopoeia (less than 0.5 EU/ml), they were stored at 4 °C for the test (Kong et al., 2004; Wang et al., 2005).

2.3. Reagents

Alsever's solution was obtained by dissolution of glucose 2.05 g, sodium citric acid 0.80 g and sodium chloride 0.42 g in 100 ml of deionized water, regulating pH to 7.2 with 0.1% citric acid and filtering through a 0.22 μm syringe filter, stored at 4 °C. Rooster erythrocytes suspension was prepared by separating 5 ml of blood in 20 ml of Alsever's solution, washing 3 times and diluting into 1% with physiological saline. It was stored at 4 °C (valid for 1 week).

RPMI-1640 (Gibco) supplemented with benzylpenicillin 100 IU/ml, streptomycin 100 IU/ml and 10% fetal bovine serum, was used for washing and re-suspending the cells, diluting the mitogen and culturing the cells. Concanavalin A (ConA, Sigma), as the T-cell mitogen, was dissolved into 2.5 μg/ml with RPMI-1640. The 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Amresco Co.) was dissolved into 5 mg/ml with calcium and magnesium-free (CMF) phosphate-buffered saline (PBS, pH 7.4). These reagents were filtered through a 0.22 μm syringe filter. ConA solution was stored at −20 °C, MTT solution at 4 °C in dark bottles. Lymphocytes Separation Medium (Ficoll–Hypaque, ρ: 1.077 ± 0.002, No. 20001220) was the product of Hengxing Biostix Shanghai Inc. Dimethyl sulfoxide (DMSO) was produced by Zhengxing Institute of Chemical Engineering in Suzhou.

2.4. Vaccine

ND vaccine (LaSota strain, No. 051202) was offered by Nanjing pharmaceutical and apparatus factory of China Animal Husbandry Industry Company.

2.5. Animals

One-day-old White Roman chickens (male), purchased from Tangquan Poultry Farm, were housed in wire cages (60 × 100 cm) in air-conditioned rooms at 37 °C and lighted for 24 h at the beginning of pretrial period. The temperature was gradually declined to the room temperature and the light time to 12 h per day, which were kept constant in the following days. Chickens were fed with the commercial starter diet provided by the feed factory of Jiangsu Academy of Agricultural Science.

2.6. Experimental design

Two hundred chickens were randomly divided into 10 groups. On 14 days old, their average ND-HI titer of maternal antibody was 3.5 $\log_2$ and the average body weight was 125 g. The chickens in Groups 1–9 were vaccinated with 0.5 ml of ND-IV vaccine by intranasal and intraocular routes, in Group 10, 0.5 ml of physiological saline, as blank controls. Simultaneously, the chickens in Groups 1–6 were intramuscularly injected, respectively, with 0.5 ml of $sAPS_{40}$, $sAPS_{50}$ and $sAPS_{60}$ at high or low concentrations, in Groups 7 and 8, 0.5 ml of APS_t at high or low doses as adjuvant controls, in Group 9, 0.5 ml of physiological saline as non-adjuvant (NA) controls, once a day for three successive days. On days 7 (D_7), 14 (D_{14}), 21 (D_{21}) and 28 (D_{28}) after the vaccination, 10 chickens randomly from each group were sampled for determination of serum hemagglutination inhibition (HI) antibody titer by micro-method (Thekisoe, Mbati, & Bisschop, 2004; Xu, 1998). On D_{14}, D_{21}, D_{28} and D_{35}, four chickens randomly from each group were sampled for test of peripheral T lymphocyte proliferation by MTT assay.

2.7. Sample collection and assay

2.7.1. Serum HI antibody assay

Blood samples (1.0 ml per chick) were drawn into Eppendorf tubes from the brachial vein of chicken and allowed to clot at 37 °C for 2 h. The serum was separated by centrifugation and stored at −20 °C for HI antibody assay. Briefly, after the serum was inactivated at 56 °C for 30 min, twofold serial dilution were made in a 96-well V-shaped bottom microtiter plate containing 50 μl of CMF–PBS in each well, then 50 μl of NDV antigen (4 HA units) was added into all the wells except for the last row as the controls. Serum dilutions ranged from 1:2 to 1:2048. The plate was incubated at 37 °C for 10 min, then 50 μl of 1% rooster erythrocytes suspension was added to each well and continued to incubate for 30 min. A positive serum, a negative serum, erythrocytes and antigens were also included as controls. The highest dilution of serum caused complete inhibition was considered the endpoint. The geometric mean titer was expressed as reciprocal $\log_2$ values of the highest dilution that displayed HI (Thekisoe et al., 2004; Xu, 1998).

2.7.2. Peripheral lymphocyte proliferation assay

Blood samples (5 ml per chicken) were collected by cardiopuncture and transferred immediately into aseptic capped tubes with sodium heparin, then diluted with an equal volume of Hanks' solution and carefully layered on the surface of lymphocyte separation medium. After 20 min of centrifugation at 800*g*, a white cloud-like lymphocytes' band was collected and washed twice with RPMI 1640 media without fetal bovine serum. The resulting pellet was re-suspended to 2.5×10^6/ml with RPMI 1640 media and incubated in 96-well culture plates, 80 μl/well, each sample seeded four wells. Then another 20 μl/well of ConA was added using for the purification of lymphocytes. The plates were incubated at 39.5 °C for 48 h in a humid atmosphere of 5% CO_2. After 44 h of the incubation period, 20 μl of MTT (5 μg/ml) was added into each well, and the plates were re-incubated for 4 h. Then the plates were centrifuged at 1000*g* for 10 min at room temperature. The supernatant was removed carefully and 100 μl of DMSO were added into each well to dissolve the formazan crystals. The plates were shaken for 5 min to dissolve the crystals completely. The absorbance at a wave length of 570 nm (A_{570}) of lymphocyte cells in each well was measured by microliter enzyme-linked immunosorbent assay reader (Model DG-3022, East China Vacuum Tube Manufacturer). The mean A_{570} values were used as the indicator of peripheral T lymphocyte proliferation (Bao, 1998; Barta, Barta, & Pierson, 1992; Thiagarajan, Ram, & Bansal, 1992; Wang & Li, 2002; Wang et al., 2005).

2.8. Statistical analysis

Data are expressed as means ± SD. Duncan's multiple range test was used to determine the difference among sAPSs adjuvant and control groups. Differences between means were considered significant at $p < 0.05$.

3. Results

3.1. The dynamic changes of serum antibody titer

The dynamic changes of antibody titer were listed in Table 1. On day 14 after vaccination, the antibody titers in $sAPS_{40}$ at high dose group and $sAPS_{50}$ at high and low dose groups were significantly higher than those in non-adjuvant group and APS_t at high and low dose groups ($p < 0.05$). On day 21, except for $sAPS_{40}$ at low dose group, the antibody titers in all groups were significantly higher than that in non-adjuvant group ($p < 0.05$), and the antibody titer in $sAPS_{60}$ at high dose group was significantly

Table 1
The dynamic changes of serum ND-HI antibody titer (log_2)

Groups	D_7	D_{14}	D_{21}	D_{28}
$sAPS_{40H}$	5.4 ± 0.547^{ab}	7.0 ± 0.707^{a}	7.5 ± 0.547^{bc}	3.5 ± 0.836^{d}
$sAPS_{40L}$	5.5 ± 0.836^{a}	5.8 ± 0.836^{b}	6.7 ± 0.516^{dc}	5.7 ± 0.516^{d}
$sAPS_{50H}$	4.3 ± 0.516^{cd}	7.3 ± 0.516^{a}	8.2 ± 0.752^{bc}	6.0 ± 0.00^{b}
$sAPS_{50L}$	4.8 ± 0.752^{abcd}	7.0 ± 0.632^{a}	7.5 ± 0.547^{bc}	6.8 ± 0.447^{a}
$sAPS_{60H}$	4.6 ± 0.547^{bcd}	4.6 ± 0.547^{c}	9.2 ± 0.983^{a}	6.2 ± 0.447^{ab}
$sAPS_{60L}$	4.2 ± 0.836^{d}	5.8 ± 0.983^{b}	7.8 ± 0.752^{bc}	6.8 ± 0.752^{a}
APS_{tH}	5.2 ± 0.752^{abc}	4.5 ± 0.547^{c}	8.7 ± 0.547^{ab}	3.4 ± 0.547^{d}
APS_{tL}	5.3 ± 0.516^{ab}	5.7 ± 0.816^{b}	7.8 ± 0.516^{bc}	3.8 ± 0.447^{cd}
NA control	5.5 ± 0.547^{a}	6.0 ± 0.547^{b}	6.2 ± 0.447^{de}	5.5 ± 0.577^{b}
Blank control	1.5 ± 0.547^{e}	1.5 ± 0.547^{d}	3.5 ± 0.577^{f}	4.4 ± 0.547^{c}

Data within a column with different superscripts differ significantly ($p < 0.05$). H, high dose; L, low dose; NA, non-adjuvant.

higher than that of APS_t at low dose group. On day 28, the antibody titers in $sAPS_{50}$ and $sAPS_{60}$ at low dose were significantly higher than those of non-adjuvant group and APS_t at high and low dose groups ($p < 0.05$).

3.2. The dynamic changes of peripheral lymphocyte proliferation

The dynamic changes of A_{570} values in all groups were listed in Table 2. On day 14 after the vaccination, the A_{570} values in $sAPS_{40}$ and $sAPS_{60}$ at high dose groups and $sAPS_{40}$, $sAPS_{50}$, $sAPS_{60}$ and APS_t at low dose groups were significantly larger than that in non-adjuvant group ($p < 0.05$), and the A_{570} values in $sAPS_{40}$ and $sAPS_{60}$ at low dose groups were significantly larger than those in APS_t at high and low dose groups ($p < 0.05$). On day 21, the A_{570} values in three sAPSs at low groups were significantly larger than those in non-adjuvant and APS_t at high and low dose groups ($p < 0.05$). On day 28, the A_{570} values in three sAPSs at high and low dose groups were significantly larger than that in non-adjuvant ($p < 0.05$), and the A_{570} value in $sAPS_{60}$ at low dose group was significantly larger than those in APS_t at high and low dose groups ($p < 0.05$). On day 35, the A_{570} values in $sAPS_{50}$ and APS_t at high dose groups were significantly larger than that in non-adjuvant ($p < 0.05$), and the A_{570} value in $sAPS_{50}$ at high dose group was significantly larger than that in APS_t at low dose group ($p < 0.05$).

4. Discussion

The dynamic change of antibody titer reflects the humoral immunity state in the animal. The humoral immunity is one of the major ways to resist infectious diseases. The ND antibody titer is proportional to ND protection rate. When the antibody titer is higher than 5 log_2, the chicken can be protected from ND, lower than 4 log_2, will suffer from recessive infection (Wang, Liu, Bai, & Fang, 1999). Our determination results demonstrated that antibody titers of most polysaccharides adjuvant groups at many time points were significantly higher than those of non-adjuvant group, which suggested that the sulfated and non-sulfated APS could enhance the humoral immunity. To make a comparison between sulfated APS and non-sulfated APS, it could be seen that the antibody titers of $sAPS_{40H}$, $sAPS_{50H}$ and $sAPS_{50L}$ groups on day 14, $sAPS_{60H}$ group on day 21 and $sAPS_{50L}$ and $sAPS_{60L}$ groups on day 28 after vaccination were significantly higher than those of APS_t at high and low dose groups. These indicated that the efficacy of sulfated polysaccharide was better than that of non-sulfated polysaccharide.

According to the number of antibody titer being significantly higher than that of non-adjuvant group at four time points, four polysaccharides at two dose were arranged into $sAPS_{50L}$ (3), $sAPS_{40H}$, $sAPS_{50H}$ and $sAPS_{60L}$ (2), APS_{tH}, APS_{tL} and $sAPS_{60H}$ (1). The peak value of antibody titer in $sAPS_{60H}$ group was higher than that in APS_t

Table 2
Dynamic changes of lymphocyte proliferation in every group (A_{570} value)

Groups	D_{14}	D_{21}	D_{28}	D_{35}
$sAPS_{40H}$	0.316 ± 0.076^{cd}	0.214 ± 0.081^{cd}	0.331 ± 0.084^{c}	0.183 ± 0.084^{bc}
$sAPS_{40L}$	0.626 ± 0.096^{a}	0.448 ± 0.028^{ab}	0.444 ± 0.063^{bc}	0.141 ± 0.049^{c}
$sAPS_{50H}$	0.167 ± 0.057^{ef}	0.178 ± 0.065^{cd}	0.501 ± 0.011^{ab}	0.262 ± 0.017^{a}
$sAPS_{50L}$	0.263 ± 0.067^{cd}	0.548 ± 0.030^{a}	0.394 ± 0.013^{c}	0.153 ± 0.045^{bc}
$sAPS_{60H}$	0.218 ± 0.052^{de}	0.132 ± 0.019^{d}	0.488 ± 0.098^{b}	0.195 ± 0.081^{bc}
$sAPS_{60L}$	0.434 ± 0.018^{b}	0.342 ± 0.015^{b}	0.592 ± 0.018^{a}	0.184 ± 0.048^{bc}
APS_{tH}	0.161 ± 0.038^{ef}	0.194 ± 0.035^{cd}	0.377 ± 0.078^{bc}	0.208 ± 0.019^{ab}
APS_{tL}	0.266 ± 0.012^{cd}	0.27 ± 0.093^{bc}	0.292 ± 0.042^{c}	0.145 ± 0.028^{c}
NA control	0.111 ± 0.017^{f}	0.21 ± 0.033^{cd}	0.185 ± 0.055^{d}	0.163 ± 0.071^{c}
Blank control	0.149 ± 0.043^{f}	0.123 ± 0.035^{d}	0.151 ± 0.010^{d}	0.152 ± 0.045^{c}

Data within a column with different superscripts differ significantly ($p < 0.05$). H, high dose; L, low dose; NA, non-adjuvant.

group. These results also confirmed that the efficacy of sulfated polysaccharide was better than that of non-sulfated polysaccharide, that is, the adjuvanticity of the APS could be improved by the sulfated method.

The A_{570} value is correlated to the number of live cells. The bigger A_{570} value is, the stronger lymphocyte proliferation is. It is the indicator of lymphocytes proliferation reflecting the cellular immunity state in the animal. The experimental results showed that most A_{570} values of eight polysaccharide adjuvant groups at certain time points were significantly larger than that of non-adjuvant group, which revealed that the sulfated and non-sulfated APS could promote the lymphocyte proliferation and improve the cellular immunity. Comparison of sulfated APS with non-sulfated APS groups showed that the A_{570} values of $sAPS_{40L}$ and $sAPS_{60L}$ groups on day 14, $sAPS_{40L}$, $sAPS_{50L}$ and $sAPS_{60L}$ groups on day 21 and $sAPS_{60L}$ group on day 28 after vaccination were significantly larger than those of APS_t at high and low dose groups. These indicated that the efficacy of sulfated polysaccharide was better than that of non-sulfated polysaccharide in enhancing cellular immunity.

The A_{570} peak values of eight polysaccharide adjuvant groups were arranged from high to low as following: $sAPS_{40L}$, $sAPS_{60L}$, $sAPS_{50L}$, $sAPS_{50H}$, $sAPS_{60H}$, APS_{tH}, $sAPS_{40H}$ and APS_{tL}. In all of four time points, the numbers of A_{570} values significantly larger than that of non-adjuvant group in $sAPS_{40L}$, $sAPS_{50L}$ and $sAPS_{60L}$ groups were more than that in APS_t group. These results further showed that the efficacy of sulfated polysaccharide was better than that of non-sulfated polysaccharide, which confirmed again that sulfated modification could enhance adjuvanticity of APS.

We also found that the adjuvant activity of each polysaccharide presented certain dose–effect and time–effect relationships. In aspect of raising the antibody titers, $sAPS_{40}$, $sAPS_{50}$ and $sAPS_{60}$ at high dose was better than low dose. In aspect of promoting the T lymphocyte proliferation, $sAPS_{40}$, $sAPS_{50}$ and $sAPS_{60}$ at low dose was better than high dose. The efficacy of $sAPS_{40}$ at high and low dose in earlier stage after administration, $sAPS_{50}$ at low dose in intermediate stage and $sAPS_{50}$ at high dose and $sAPS_{60}$ at high and low dose in later stage was better. By comparison among three sAPSs, it could be seen that $sAPS_{60}$ possessed the strongest adjuvanticity.

The activity of sulfated polysaccharide strongly depended on the degree of substitution (DS) (Alban, Schauerte, & Franz, 2002). When DS was within scope of 1.5–2.0, the biological activities of sulfated polysaccharide was the best (Wang & Guang, 2000). This also is confirmed by our experiment. To compare three sAPSs synthetically, $sAPS_{60}$ presented the best efficacy because it had the highest DS (1.545) which was just within 1.5–2.0. As for the mechanisms of sulfated polysaccharides in immunoenhancement, it may be contribute to binding to the receptor of lymphocytes and macrophages, activating lymphocytes and macrophages by a series of identification and transfer of bio-information and then exerting their immunomodulating activity (Miao et al., 2002). It was reported that sulfated polysaccharides from Seaweed (SPS) could inhibit the apoptosis of lymphocytes induced by oxidative stress, which made G_1-phase cells enter S-phase through lowering the expression of oncogene-NF-kB and P_{53} (Wei et al., 2002). Sulfated polysaccharides could enhance the phagocytic function, promote peritoneal macrophages to produce interleukin-1 (Wei et al., 2001), induce the lymphocyte proliferation and interleukin-2 secretion (Wang, Zhou, Xing, & Guan, 1994), thus enhance the specific and non-specific immune function of organism. Whether sulfated APS has these actions awaits our further investigation.

This research studied the effects of three sAPSs on serum antibody titers and peripheral T lymphocyte proliferation in chicken vaccinated with ND vaccine from humoral immunity and cellular immunity aspect. The results confirmed that sulfated modification could enhance the adjuvant activity of APS, $sAPS_{60}$ had the best efficacy and would be expected as a candidate of new-type immunopotentiator. Further study on its vaccination protective effect is underway.

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