

Adjuvant effect of saponin on the immune responses to bovine serum albumin in hens

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Abstract

A glycoside the saponin was evaluated as a potential immunomodulator in various formulations. Hens were immunized three times orally with a bovine serum albumin (BSA) supplemented with 350 and 700 mg of the saponin. Birds in a positive control group were administered with solution of emulsified with an equal volume of Freund's incomplete adjuvant. BSA-specific IgA antibodies in saliva and IgY antibodies in yolk were examined by ELISA. It was found that the birds inoculated with BSA in addition to 350 mg of saponin had a higher amount of BSA-specific IgA antibodies in the saliva and BSA-specific IgY antibodies in yolk, than hens that received lower doses of the glycoside. However, the total IgY content in yolk was higher in BSA + 700 mg of saponin-treated hens as compared to chickens immunized with lower dose of this mixture. These results suggest that saponin is useful as an oral adjuvant or immunomodulator for hens.

Key words: hens, saponin, oral immunization, immune response.

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Introduction

A majority of laboratory animals are used as models of man in biomedical research. A significant proportion however, is used simply to produce biological reagents. The use of animals for antibody production may be associated with ethical problems and the immunization schemes and can be more or less aggressive depending on the site of administration, frequency of boosting, and type of immunopotentiating adjuvant. Large amounts of immunoglobulin Y (IgY) found in the egg yolk have led to the several immunological test systems as described early [1].

Mucosal immunity, in which secretory IgA antibodies play the main important role, is established *via* the mucosa-associated lymphoid tissues such as those in the respiratory and gastrointestinal tracts as indicated by [2]. The gut-associated lymphoid tissues (GALT), particularly the Peyer's patches, as inductive tissues, contain a large number of IgA precursor B cells, and the stimulation of them with orally administered antigens may lead to the dissemination of B and T cells to mucosal effector tissues such as the

lamina propria region of intestinal, respiratory, and genitourinary tracts and various secretory glands for subsequent antigen-specific IgA antibody responses as shown by [3].

This increasing interest is due to a tremendous historical legacy in folk medicine use of plants as medicines [4-10]. In the recent past, scientific studies on plants used in ethnomedicine have led to the discovery of many valuable drugs such as pilocarpine and vincristine among others. However, the effect of some extracts from medicinal herbs on immune system has not been studied in a systematic way.

The aim of the present study was to examine the immune response of laying hens to orally administered bovine serum albumin (BSA), with saponin as adjuvant.

Material and methods

The dry glycoside saponin was purchased from Merck (Germany) and was used as immunomodulator. Also a commercial preparation of BSA (Sigma, USA) was used as model antigen.

Hens

Nine 25-week-old outbred ISA hens were obtained from the breeding unit of the laboratory of animal resources of the Institute of State Research Institute Center for Innovative Medicine (Vilnius, Lithuania). The hens were kept singly in 1 m × 1 m floor pens equipped with nest boxes, in a standard animal room with a 17/7 h light/dark cycle (light long 25 lux). As bedding, chips of deciduous trees, after sterilization at 120°C and pressure 1.5 kg/cm² during 20 min, were used. The bedding was changed twice weekly. The temperature in the room was 16°C ± 2°C, with a relative humidity within the range of 60-70%. The chicken feed was based on granulated forage (“Kauno grūdai” AB Kaunas, Lithuania) which consisted of energetic (11.7 MJ/kg), crude protein (17%), crude oil (3%), and crude fibre (71%). The feed was balanced for vitamins and micronutrients, and amino acids. Water was provided *ad libitum*. We have performed the experiment after having received the permission from the Ethics Committee on the Use of Laboratory Animals, at the State Food and Veterinary Institute (No 0128, No 0162).

Study of antibody production

The study consisted of three treatment groups with three chickens in each group. The treatment groups were:

Group A: BSA + 350 mg saponin. The mixture containing 200 mg of BSA in 0.5 ml of phosphate-buffer saline (PBS), pH 7.2, was emulsified with an equal volume of olive oil (Extra Virgin, Carapelli Firenze, Italy). The BSA containing emulsion (1.0 ml) was mixed with 350 mg saponin.

Group B: BSA +700 mg saponin. The BSA containing emulsion (1.0 ml) was mixed with 700 mg of the saponin.

Group C: (Positive control). The suspension containing 200 mg of BSA in 0.5 ml PBS was emulsified with an equal volume of Freund’s incomplete adjuvant (FIA) (Bio-Rad, USA) and the total volume of 1 ml was administered intramuscularly into four sites of the pectoral muscle of each hen. Inoculation was performed three times (at days 1, 14, and 28). Birds of groups A and B were administered by oral gavage with the antigen mixture in a dose of 1 ml/hen on days 1, 7 and 14. The birds were not anaesthetized during immunizations.

Saliva secretion was collected by absorbent filter papers (Whatman No.1, Sigma). Pre-weighed two wicks were placed under the tongue of the hen for approximately 20 s. The wicks were weighed to measure the amount of saliva. The saliva was extracted by adding 400 µl of PBS containing 0.1% Tween 20, pH 7.2, to the Eppendorff tube with the paper wicks and incubating the mixture with slow shaking at 20°C for 2 h. After this, the extract was used for analysis.

The purification of IgY from egg yolk was performed as described earlier [1].

BSA-specific IgA antibodies in saliva and BSA-specific IgY antibodies in yolk were analyzed by ELISA as described earlier [11].

The protein content (mg/ml) was measured photometrically at 280 nm and was calculated according to the Lambert-Beer law with an extinction coefficient of 1.34 for IgY by means of “BioPhotometer” (Eppendorf, Germany).

The antibody titers were expressed, as the reciprocal of the highest dilution of saliva or yolk and the optical density at 492 nm (Titertek Multiscan Plus MK II, Labsystems Finland)), which was 2-fold higher than that of the negative samples. The titers were converted to a base-2 logarithmic scale.

Statistical analysis

Statistical evaluation of the results was done by one-way analysis of variance ANOVA using PRISM Software (GraphPad Software, San Diego, CA, USA). The mean of the IgA, IgY antibody titers as well as the total IgY content were compared using unpaired Student’s *t*-test. All values were expressed as mean ± standard deviation and were considered to be statistically significant at *p* < 0.05.

Results

Figure 1 shows that the IgA titers in group A were significantly higher than those in groups B and C at 2, 5, 7 and 9 weeks after the last immunization. Notably, IgA titers were significantly increased within two to nine weeks after the last immunization only in group A. Furthermore, the titers of the IgA were below log₂ 3.0 before immunization (at day 0, Fig. 1).

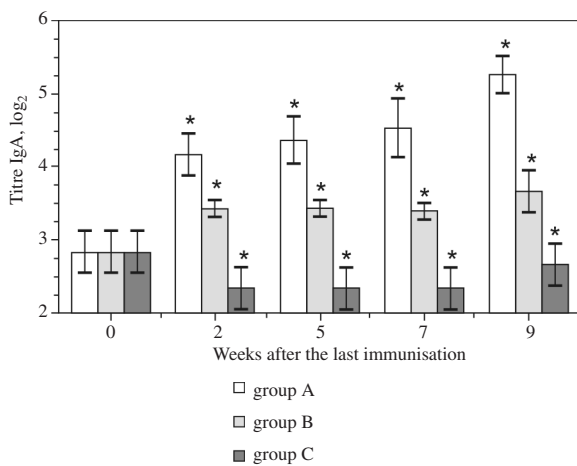


Fig. 1. Saliva IgA antibody response in hens after oral immunizations with BSA mixed with saponin (groups A and B) or immunized intra-muscularly (group C). Asterics indicate the values differing significantly from those of others groups on the same week

Generally, immunospecific anti-BSA IgG antibodies were detected in all groups. It was recorded that the IgG titers in group C (positive control) were significantly higher than those in other groups at all time-points of the observation after the last immunization (Fig. 2). Although, in groups A and B positive IgG responses were significantly increased within two to nine weeks after the last immunization. In fact, the titers of the IgG were below \log_2 5.0 before immunization (at day 0, Fig. 2).

As seen from Fig. 3, the total IgY content in Groups A, B and C varied in the range 7.1-11.2, 12.9-51.0 and 9.5-46.5 mg/ml of egg yolk, respectively. Interestingly, that IgG concentrations in yolk were observed to peak within 5 to 9 weeks after the last immunization. In contrast, the total IgY content in yolk peaked at 5 week in groups B and C, and then it was reduced at 7 week in Group C.

Discussion

In this study, we focused on the use a saponin as a potential mucosal adjuvant. For these experiments, we used a model of oral immunization previously demonstrated to be effective by others and our laboratory [12-14].

In our studies, we have demonstrated that oral immunization with BSA in combination with glycoside saponin could also prime the immune system for both secretory and systemic booster antibody responses to later repeated peroral immunizations. Furthermore, addition of a saponin to the immunization mixture had an effect on the enhancement of the hens immune response over a long time. It is important, that the high anti-BSA antibody titers in saliva indicate, that the induction of antibody responses had taken place in the mucosa of the upper respiratory tract.

The IgY concentration follows clear age-dependent kinetics that was confirmed by authors [15, 16].

In general, current phenomenon may be reflect an biological activity of chicken B lymphocytes, in view of a rhythmic production of different amounts of IgY.

To obtain a stable, water-in-oil emulsion, various kinds of emulsions with different oil samples were evaluated. With this type of emulsion, the antigen in the internal aqueous phase is released slowly into the biological fluids. Basically, the emulsification system used for the immunization includes an oil phase, an aqueous phase, and an emulsifying agent, which should favor the formation of water-in-oil emulsion [17].

In the present study, probably the water-in-oil emulsion contained high local concentration of olive oil trapped in a droplet together with antigen that is normally deposited in a mucosal tissue. These findings suggest that repeated immunization with the BSA in combination with the saponin as mucosal adjuvant may induce the mechanism of the immune memory and develop the immune response in animals for a long time as described earlier [8]. Our results show that water-in-oil emulsions based on mixture

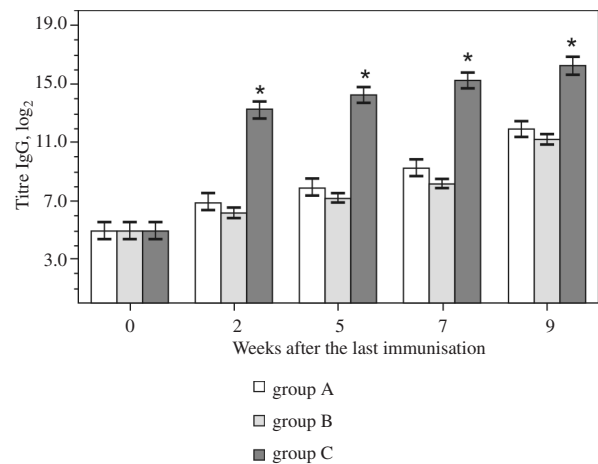


Fig. 2. Yolk IgG antibody response in hens after oral immunizations with BSA mixed with saponin (groups A and B) in comparison to the positive control (group C). Asterisks indicate the values differing significantly from those of others groups on the same week

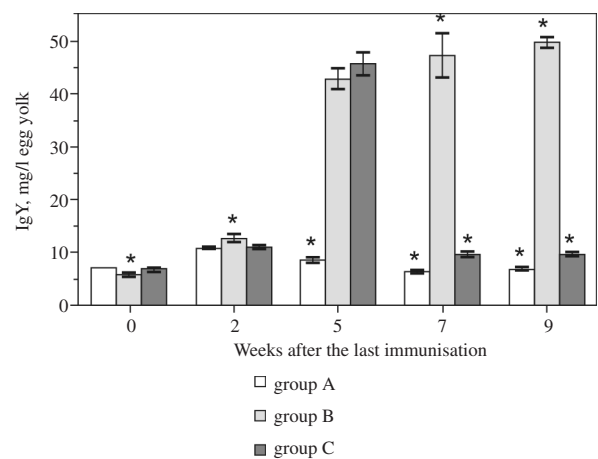


Fig. 3. Time-dependent changes in the total IgY content. Asterisks indicate groups the values of differ significantly from those of others groups on the same week

containing biologically active components as saponin may be effective when used in various formulations as immunostimulating complexes.

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