Immunomodulatory action of jacalin from *Artocarpus integrifolia* and mannoprotein from *Saccharomyces uvarum* on the humoral immunity of laying hens

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**ABSTRACT**: Advances in the fields of glycobiology and immunology have provided many insights into the role of carbohydrate–protein interactions in the immune system. Jacalin of *Artocarpus integrifolia* (JCA) and structural mannoprotein of *Saccharomyces uvarum* (MPS) are molecules with immunomodulatory properties. JCA is an IgA human lectin binding molecule that causes the mitogenic stimulation of immune cells, production of cytokines, chemotaxis, and activation of leukocytes. Studies on the immunomodulatory properties of JCA and MPS in mammals and fish suggest that they have an action on antibody production. The aim of this study was to investigate the possible action of JCA and MPS on the production of specific antibodies in laying hens. For this, laying hens were inoculated with an intra abdominal injection of sheep red blood cells (SRBC) with either JCA (0.075 µg, 0.75 µg, and 7.5 µg) or MPS (20 µg and 100 µg). Levels of anti-SRBC antibodies of the IgY, IgM, and IgA classes were evaluated by ELISA. Results showed that JCA and MPS have immunomodulatory effects on levels of anti-SRBC IgM, IgA, and IgY. An immunostimulatory effect of JCA was observed in primary immune response on anti-SRBC IgY, while an inhibitory effect of JCA and MPS was observed in secondary immune response on the production of IgM and IgA anti-SRBC. These results suggested that MPS and JCA have immunomodulatory effects on antibody production and could be used in future studies on humoral immune response in poultry.

**Key words**: *Artocarpus integrifolia*, chickens, IgY, jacalin, mannoprotein, humoral immune response, *Saccharomyces uvarum*.
especially immunoglobulin Y (IgY) production (FERREIRA JÚNIOR et al., 2018; MUNHOZ et al., 2014). The main adjuvants used for the production of immunoglobulin IgY antibodies in laying hens are complete (CFA) and incomplete (IFA) Freund’s adjuvants. However, CFA is associated with tissue necrosis and ulceration, and IFA has a lower immunostimulatory effect (WANKE et al., 1996). Therefore, the identification of immunomodulatory molecules would be a useful tool to study humoral immune response and IgY production in chickens. However, few studies have explored the impacts of these molecules on immune responses in laying hens. It was shown that the ethanol extract of propolis has an adjuvant effect in laying hens and can be used for the development of novel adjuvants (FREITAS et al., 2011), while sulfated polysaccharides and carboxymethyl glucan have an adjuvant effect on the humoral and cellular immune response in chickens vaccinated against Newcastle disease and avian influenza (GUO et al., 2012; DARPOSSOLO et al., 2010).

Studies in mice have shown that a lectin extracted from the seed of Artocarpus integrifolia, called jacalin (JCA), as well as structural mannoprotein (MPS; extracted from Saccharomyces uvarum), have stimulatory effects on antibody production (ALBUQUERQUE et al., 1999; DARPOSSOLO et al., 2012). The JCA is a galactose-binding lectin that has specificity for human IgA-1 and has a stimulating action on macrophage-related antibody production (ALBUQUERQUE et al., 1999; FELIPE et al., 1995). Mannoprotein (MPS) is a ubiquitous component in the cell walls of yeasts and comprises approximately 15% proteins that covalently bind to a mannose-rich polysaccharide and a small amount of glucose. The main function of MPS is to modulate the permeability of the cell wall, and it is involved in water retention and protects against drying (KLIS et al., 2002). In mice, MPS is associated with increased IFN-gamma production by CD4+ T cells and antibody production (MENCACCI et al., 1994; DARPOSSOLO et al. 2012).

There is a great interest in the production of specific antibodies in laying hens for immunotherapy and immunodiagnostics to replace antibodies produced in mammalians (MONTINI et al., 2018; MARCQ et al., 2015; MARCQ et al., 2013; ETO et al., 2012). Laying hens represent an alternative to mammalians due to the possibility of obtaining large quantities of IgY from egg yolk at a low cost (MUNHOZ et al., 2014). Furthermore, IgY antibodies are functionally similar molecules to mammalian IgGs. They do not interact with the complement system or the Fc receptors in mammals; thereby, reducing their interference in immunoassay and passive immunotherapy protocols (MUNHOZ et al., 2014).

The aim of the research was to investigate the activity of JCA from A. integrifolia and MPS from S. uvarum on antibody production in laying hens.

MATERIALS AND METHODS

Animals

In total, 42 White Leghorn hens were used at 40 weeks of life from the Farm School of the State University of Maringá, PR. The animals were kept in cages at room temperature (RT) with food and water ad libitum. The procedure followed the recommendations of the ethics committee for animal experiments at the Universidade Estadual de Londrina, Brazil.

Immunomodulators JCA and MPS

The JCA sample was extracted from the aqueous extract of the seeds of A. integrifolia. The extract was kindly provided by M.C. Roque-Barreira. The MPS of S. uvarum was obtained as described in DARPOSSOLO et al. (2012).

Treatment of laying hens

Initially, 42 hens were distributed randomly into seven groups (6 hens/group). The animals in groups I (GI) and II (GII) were inoculated with phosphate buffered saline (PBS; 0.15 M, pH 7.2) and 5% SRBC in PBS, respectively. The animals in groups JCAI, JACII, and JACIII received 0.075 µg, 0.75 µg, and 7.5 µg of JCA plus 5% SRBC in PBS, and the animals in groups MPSI and MPSII were inoculated with 20 µg and 100 µg of MPS plus 5% SRBC in PBS, respectively. All inoculations were performed intra abdominally on day 1 and were repeated on day 28. On days 7 and 35 of the experiment, serum samples were obtained and stored at –20 °C until use.

Immunoassays for the determination of levels of anti-SRBC IgY, IgM, and IgA

Initially, microplates (Costar® 3590) were sensitized with 100 µl of protein extract of SRBC at 2.5 µg/mL, as described by LADICS, (2007), and kept overnight at 4 °C. Plates were washed with PBS–0.05% and blocked with PBS and 5% skim powdered milk for 1 hour at RT. Then plates were washed again and the serum samples (1:100) added. After 1 hour at room temperature, plates were washed
and peroxidase-conjugated with anti-IgY, anti-IgM, or anti-IgA (Bethyl Laboratories, Inc., Montgomery, TX, USA) at dilutions of 1:40,000, 1:5,000, and 1:8,000, respectively; and incubated for 1 hour at RT. Plates were washed and substrate/chromogen $\text{H}_2\text{O}_2$ (F. Maia, Cotia, SP, Brazil)/3,3',5,5'-tetramethylbenzidine (TMBZ; Sigma-Aldrich Co., St. Louis, MO, USA)/sodium acetate buffer (0.1 M, pH 5.0) was added and the reaction stopped by the addition of 1N $\text{H}_2\text{SO}_4$. Optical density was determined using a Multiskan reader (Thermo Plate, TP-Reader, Rayto Life, and Analytical Sciences Co. Ltd, Shenzhen, People’s Republic of China) at 450 nm (KILLPACK & KARASOV, 2012). All analyses were performed in duplicate and each plate contained two antigen-free and serum-free wells, two antigen-free wells with serum, and two wells with antigen and without serum as controls.

**Statistical analysis**

Results were analyzed using one-way analysis of variance (ANOVA) by a post hoc Tukey test or Kruskal–Wallis test followed by Dunn’s multiple comparison test. A value of $P<0.05$ was considered statistically significant. Prism version 5.01 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses.

**RESULTS AND DISCUSSION**

Jackfruit (*Artocarpus heterophyllus*, family Moraceae) is a monoecious evergreen tree that grows in several tropical countries. Previously, the purification of human IgA was very tedious, and affinity chromatography on immobilized JAC has become a convenient procedure for its isolation. In recent years, several studies have demonstrated that JAC is an important as a lectin with diverse applications, ranging from the isolation of human IgA1 to applications in acquired immune deficiency syndrome (AIDS) research (KABIR, 1998; MADRUGA et al., 2014).

The main effect of JCA on the mammalian immune system is on lymphocytes. JCA is a potent stimulator of T lymphocyte CD4+ and has an adjuvant effect on antibody responses against soluble antigens and protozoa Trypanosoma cruzi in mice (Albuquerque et al., 1999). The mechanism of action of JCA on the humoral immune response apparently involves stimulation of a Th2 response due to its interaction with the CD45 molecule of B and T lymphocytes, followed by phosphorylation of mitogen-activated protein kinase p38 and the secretion of interleukin (IL)-4 (TAMMA et al., 2006a; TAMMA et al., 2006b; BABA et al., 2007).

In addition to research conducted with mammals, studies on fish have also demonstrated that JCA exhibits stimulatory action. AHMED et al. (2018) evaluated the capacity of JCA to stimulate antibody production in zebrafish. To verify this hypothesis, the authors performed a radial immunoassay (RIA) using heat-killed A. hydrophila and anti-serum obtained from zebrafish. In their experiments, the anti-serum was collected from infected fish, which demonstrated increased precipitation 21 days post-treatment with JCA; these data suggested a time-dependent effect.

In the current study, three different concentrations were used to evaluate whether JCA action was associated with humoral immunity. Results are shown in figures 1 and 2. Figure 1 presents the levels of anti-SRBC IgM, IgA, and IgY in serum 7 days after the challenge. The results demonstrated that in primary immune response, there was an immunostimulatory effect of JCA on the production of IgY antibodies (Fig 1C), while in secondary immune response there was an immune inhibitory effect of 0.75 and 7.5 µg of JCA on IgA antibodies production (Fig 2B). These results suggest that JCA is able to influence antibody production in laying hens.

We also evaluated structural mannoprotein molecules (MPS), which are the complex and multifunctional outer layer of the cell wall of yeasts (Cid et al., 1995). In chickens, there are few studies on mannose receptors on immune cells; but the presence of these receptors in macrophages has been described (ROSSI & HIMMELHOCH, 1985). It has been reported that the receptor responsible for transporting IgY to the egg yolk is a family member of mannose receptors (HE & BJORKMAN, 2011). However, the role of these receptors in the immune response of birds remains unknown.

Results obtained when evaluating the impacts of specific antibodies produced in animals inoculated with the MPS/SRBC of *S. uvarum* are shown in Figures 1 and 2. Results demonstrated a reduction in the levels of anti-SRBC IgM and IgA in all animals treated with MPS in relation to control group II after the second inoculation. There was a significant reduction in the levels of anti-SRBC IgM in the group inoculated with 100 µg of MPS (Figure 2A) and inhibitory production of anti-SRBC IgA in animals treated with 20 µg of MPS. This MPS-related effect was not observed in the production of anti-SRBC IgY 7 days after the challenge (Figure 1). When analyzing the levels of IgY, we did not observe a significant increase in antibody levels in MPS treated groups in relation to the control group inoculated with SRBC. These results suggested that MPS from...
S. uvarum is able to influence the exchange of Ig classes in laying hens, inhibiting the production of IgM and IgA. Although, few studies on the molecular mechanisms involved in the production of specific antibodies have been conducted with chickens, there is evidence that class switching in chickens occurs preferentially in germinal centers and is dependent on the activation signals received by dendritic cells, as in mammals (IMAMURA et al., 2009).

In mammals, these molecules are recognized by mannose receptors located in host macrophages and neutrophils. These receptors participate in the process of phagocytosis and the release of inflammatory mediators (TOROSANTUCCI et al., 1997; STAH! & EZEKOWITZ, 1998). In mice, when MPS was inoculated with the antigen, the results demonstrated significant increases in the levels of antigen-specific antibodies (DARPOSSOLO et al., 2012). In chickens, although, there are no studies on the effect of MPS inoculation on the humoral immune response, supplementation of their diet with mannose oligosaccharides resulted in increased production of antigen-specific antibodies (HASSAN & RAGAB, 2007).

In the current study, we observed suppressive action of JCA from A. integrifolia and MPS from S. uvarum on the humoral immune response in laying hens. Investigation of the mechanisms associated with the suppressive effects of JCA or effect on production of stimulator IgY of MPS on the humoral immune response may reveal features of the immune response in avians that may be important for the development of new adjuvants for use in poultry.

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Figure 2 - Secondary immune response of anti-sheep red blood cells (SRBC) IgM (A), IgA (B), and IgY (C) to SRBC. Means ± standard deviation (SD) of OD of anti-SRBC antibodies.

*Significantly different from group I (PBS) and group MPSII (SRBC + Mannoprotein 100 µg) by Kruskal Wallis and post hoc Dunn’s test, P<0.05.

**Significantly different from GI (PBS), JCAII (SRBC + Jacalin 0.75 µg); JCAIII (SRBC + Jacalin 7.5 µg), and MPSI (SRBC + Mannoprotein 20 µg) by Kruskal Wallis and post hoc Dunn’s test, P<0.05.

***Significantly different from group I (PBS) by one way ANOVA and post hoc Tukey test, P<0.05.
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BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

Protocol 21/11 of the ethics committee for animal experiments at the Universidade Estadual de Londrina.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

AUTHORS’ CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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