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ANTI-*VATAIREA GUIANENSIS* LECTIN IGY ANTIBODIES PRODUCED IN IMMUNIZED CHICKEN EGGS: PRODUCTION, ISOLATION AND CHARACTERIZATION

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ABSTRACT

Lectins are a group of carbohydrate-binding proteins found in all organisms. Among these proteins, a species that stands out for having several biological activities is *Vatairea guianensis* lectin (VGL). Techniques involving antibodies can range from classic immunochemistry assays to advanced microscopy techniques. Thus, they can become valuable biotechnological tools since they are able to specifically recognize epitopes of the target molecules. Immunoglobulins are proteins whose main function is to bind with antigens foreign to the individual, in order to neutralize them. In the case of birds, the main class of immunoglobulins Y (IgYs) can be found not only in blood but also in egg yolks. This fact allows for quick and efficient purification of these molecules, facilitating their use as biotechnological tools. This work aimed to produce antibodies against VGL in chickens, implementing a methodology for the production biotechnological tools for the study of lectins. For that purpose, laying hens were immunized with VGL for 15 weeks at 10-day intervals. Collected eggs had the IgY purified and the produced antibody was able to recognize VGL as observed by western blotting. In this sense, the IgYs produced in this work constitute a powerful biotechnological tool available for future studies.

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INTRODUCTION

The Immunoglobulins (Ig), also known as antibodies, are proteins present in high concentration in the blood plasma of vertebrates (Schroeder & Cavacini, 2010). The main function of these molecules is to recognize antigens foreign to organisms, in order to neutralize them (Schroeder & Cavacini, 2010). Regardless of their class, Igs have a structure composed of two heavy polypeptide chains, whose molecular weight can vary from 50 to 70,000 Daltons, and two light polypeptide chains of molecular weight around 23,000 Daltons. The variation in immunoglobulins becomes even greater when investigating other groups of animals. In birds, reptiles, amphibians and some fishes, the immunoglobulin Y (IgY) is the most important class of antibodies (Munhoz *et al*., 2014).

Antibodies provide protection of the organism against microorganisms, allergens, toxins, among other molecules, contributing to its neutralization and subsequent elimination by the molecular and cellular machinery of the immune system (Forthal, 2014). In addition, these molecules are also considered essential biotechnological tools in several biological assays because they are able to specifically recognize epitopes of target molecules (Darwish, 2006). The main assays that are applied can include immunodiffusion, enzyme-linked immunosorbent assay (ELISA), Western blot, even advanced microscopy and immunohistochemistry techniques, being essential for studies to elucidate the biological activity of several proteins. Among the several classes of immunoglobulins, IgY stands out in terms of its sustainable production, good yields, and its molecular

specificity that is superior to that of IgG, which is produced from mammals and widely used in scientific works (Munhoz *et al*., 2014). Lectins are defined as proteins that present at least one non-catalytic domain capable of specific and reversible binding to mono- or oligosaccharides (Peumans & Van Damme, 1995). These proteins are widely distributed in nature being found in all organisms and viruses (Peumans & Van Damme, 1995). Inside the lectin group, those obtained from Legume plants are the most well studied. This is a group of structurally-related proteins that can present variation in the carbohydrate specificity and affinity (Lagarda-Diaz *et al*., 2017). Legume lectins are capable of eliciting various biological activities such as anticancer, anti-inflammatory, vasorelaxant, among others (Holanda *et al*., 2012). Despite having several reported effects and a high potential for applicability, the biological mechanisms related to these activities have not been fully determined and specific antibodies to lectins would be necessary for the design of tests to elucidate these mechanisms.

Vatairea guianensis lectin was purified from seeds and showed specificity for *N*-acetyl-D-galactosamine/D-galactose and derived sugars. Structurally, VGL is a glycoprotein with tetrameric oligomerization and molecular mass of 120 kDa. This lectin can elicit some biological activities such as vasorelaxation and binding to cancer antigens (Marques *et al*., 2017; Silva *et al*., 2012). In this context, the current manuscript aimed to produce, purify, and characterize antibodies anti-VGL from chicken eggs, for application in immunological assays.

MATERIALS AND METHODS

Animals: Laying hens of the Dekalb White breed aged between 20 and 22 weeks have been purchased from farms located in cities near the city of Fortaleza, Brazil. The purchased chickens have been vaccinated against the main recurrent diseases in poultry. The chickens were housed in suspended accommodation cages in the poultry sector of the Zootechnics Department of the Federal University of Ceara. Cleaning has taken place daily and water and food will be offered ad libitum until the start of laying. The experiments started when the chickens were 24 weeks old, where the animals should weigh approximately 1.8 kg and reached the laying level of at least 5 eggs per week. Experimental protocols have been approved by the Ethics Committee of UFC following the guide of Care and Use of Laboratory Animals of the National Academy of Sciences (8th Edition, 2011).

Lectin purification: *Vatairea guianensis* lectin has been purified from seeds following the protocol published by (Silva *et al*., 2012). The purity of the sample has been accessed by SDS-Page (Laemmli, 1970).

Immunization of the chickens: For the application, a stock solution of VGL at 1 mg/mL in NaCl 150 mM was prepared and diluted to 200 and 400 µg/mL (in NaCl 150 mM) prior to application. In the first application only, the lectin was diluted in Freund adjuvant. 3 groups of 3 chickens have been applied for the experiment, one group receiving VGL at 200 µg/mL, a second group receiving 400 µg/mL and the third group receiving saline-only. The inoculation was performed using 1 mL syringes with needles with 21x8 mm. The inoculations have been carried out in the pectoral muscle at intervals of 10 days. The process was repeated 10 times (100 days of experiment). A volume of 1 mL was applied in each immunization.

Egg collection and IgY enrichment: The collection was carried out daily and started one week before the first immunization extending until two weeks after the last immunization (Amro *et al*., 2018). Therefore, the eggs were collected daily after the natural laying has been identified. Subsequently, they were stored in a refrigerator at 4 ºC for a maximum of 30 days. For each group, eggs collected over 7 days will be considered a sample. For the IgY purification process, 3 eggs or more from each sample had their yolks collected and were considered a pool. The other gems in the corresponding sample were stored in a pool as described above, for possible later use, if necessary. To separate and clarify the yolks, they were separated from the egg white, washed with distilled water and immediately wrapped in paper towels in order to remove as much albumin adhered to the surface. Subsequently, the membrane of each yolk has been pierced with a sterile needle and the content was transferred to a beaker. The 3 yolks (considered a pool) will be frozen until later use.

For the protein fraction obtention, a pool of yolks was thawed, diluted in ultrapure water in a 1:10 ratio and homogenized with the aid of a glass stick. Then, this mixture was acidified with a HCl solution until pH 5.0. Then, the acidified solution was centrifuged for 9,000 g for 10 minutes at 4 ºC. The pellet was discarded, and the supernatant was subjected to protein precipitation with ammonium sulfate in 0 - 30% saturation which was left to stand for 4 hours at 4 ºC. Then, the material will be centrifuged again, using the centrifugation parameters described above, the supernatant discarded and the precipitate, called F 0-30, dialysed exhaustively against ultrapure water followed by dialysis against 20 mM sodium phosphate buffer with potassium sulfate 0.5 M pH 7.5. Soluble proteins were quantified by the Bradford assay using BSA as standard protein (Bradford, 1976).

IgY purification: The antibody purification was achieved by affinity chromatography in a HiTrap® IGY Purification matrix (GE Healthcare®). For this, the F0-30 fraction has been applied in a previously equilibrated matrix (with sodium phosphate 20 mM with phosphate sulfate 0.5 M pH 7.5). Non-retained proteins were eluted with the equilibration solution while the retained protein was removed from the matrix with sodium phosphate buffer 20 mM pH 7.5. Fractions of 1 mL were collected and monitored by spectrophotometry at 280 nm wavelength. Fractions containing IgY were pooled, dialyzed against ultrapure water and freeze-dried for posterior use.

Western blotting: In order to assess the specificity of the polyclonal anti-VGL antibody, a western blotting assay has been performed (Mahmood & Yang, 2012). For this, the lectin from *Vatairea guianensis* was separated using a 12% SDS-PAGE gel. In order to monitor both the electrophoretic run and the transfer of proteins, the molecular marker TrueColor High Range Protein Marker S2600 (Sinapse ®) has been used. The electrophoretic run was carried out under standard conditions, according to the method previously described. Then, the proteins adsorbed on the gel were transferred to the nitrocellulose membrane (GE Healthcare®) using the SE300 miniVE Blot System (Hoefer ®) with a voltage of 25 V and current at 300 mA for 3 h. The 25 mM Tris-HCl, 183 mM

glycine and 20% methanol solution will be used as the transfer solution. Both the electrophoresis gel, as well as the membrane and the other parts of the transfer system, were previously conditioned with this same solution. After the transfer, the membrane was incubated for 16 h with TTBS buffer (100 mM Tris-HCl, pH 7.5, 150 mM NaCl and 1% Tween 20) plus 0.5% skimmed milk in order to block sites of nonspecific interactions. Then, the membrane was incubated for one hour with the polyclonal anti-VGL antibody at a ratio of 1:100 in a solution containing TTBS and 0.5% skimmed milk. After the incubation, three washes of the membrane (of 15 minutes each) were performed with TTBS to then proceed with the second incubation with the secondary antibody. The assay has applied chicken anti-IGY (IGG) antibody conjugated to alkaline phosphatase (A9171, Merck®) at a ratio of 1:1000 with an hour's incubation in TTBS with 0.5% skimmed milk. After incubation, three washes of the membrane were performed with TTBS followed by development with SIGMAFAST ™ BCIP® / NBT (B5655, Merck®). The membrane labeling for *Vatairea guianensis* lectin indicates the specificity of the anti-VGL antibody.

RESULTS

Maintenance and immunization of the chickens: The chickens have been kept in cages, as previously described. All animals remained healthy throughout the immunization process, showing no abnormalities in their development and egg posture. At the sites of injection of the antigen there were no signs of pain when pressing, discomfort or edema. This could be the result of the short time of manipulation and inoculation (5 min maximum) and the skills of the handler that have reduced the stress levels of the chickens. During the 15 weeks of the experiment, eggs have been collected from each experimental group. The egg production was stable with about 19 eggs per group per week. For each week, 3 eggs were selected at random for IgY purification.

IgY purification: The produced anti-VGL IgY was purified in two steps from egg yolks, the first step consisted of an ammonium sulphate precipitation followed by affinity chromatography, in which the antibody was retained in the matrix and eluted with phosphate buffer (Fig 1). Soluble protein content was about 8 mg/mL. SDS-Page presented 2 bands corresponding to 68 kDa and 28 kDa indicating the purity of IgY preparation (Fig 2).

Western blotting: Results from western blotting have demonstrated the specificity of the polyclonal anti-VGL IgY antibody (Fig 3). During the SDS-Page run, 2 aliquots of different preparations of VGL have been detected by applying the reaction involving the alkaline phosphatase signaling the binding between the IgY antibody produced in the present work in 1:100 proportion to the secondary antibody. By showing the antigen-antibody affinity, the current results indicate the possibility of application of this antibody in analysis with immunoassays in processes such as vasorelaxation and cancer (Korekane *et al*., 2012; Wu & Haab, 2010).

DISCUSSION

The present study produced and partially purified antibodies of the IgY class against the lectin extracted from seeds of *Vatairea guianensis* (VGL). Therefore, the protein in question was used as an antigen to challenge laying hens.

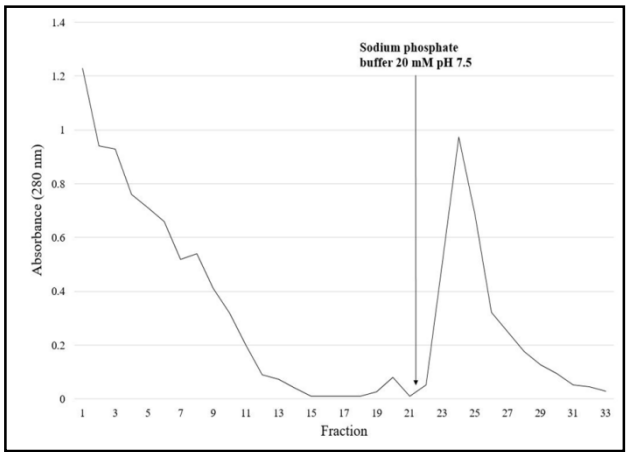

Figure 1. Affinity chromatography of the protein fraction on a HiTrap® IGY Purification matrix previously equilibrated with sodium phosphate 20 mM with phosphate sulfate 0.5 M pH 7.5. The unbound fraction was eluted with the equilibration solution and the retained protein was eluted with sodium phosphate buffer 20 mM pH 7.5. Fractions of 1 mL were collected and monitored by absorbance at 280 nm

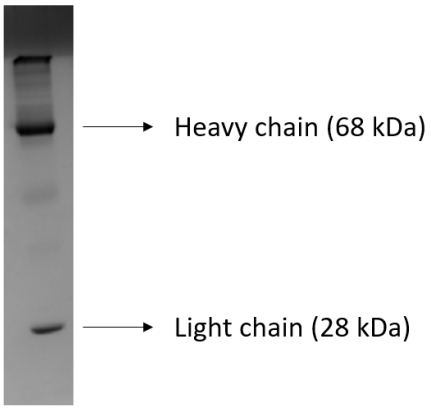

Figure 2. SDS-Page of IgY depicting the heavy and light chains.

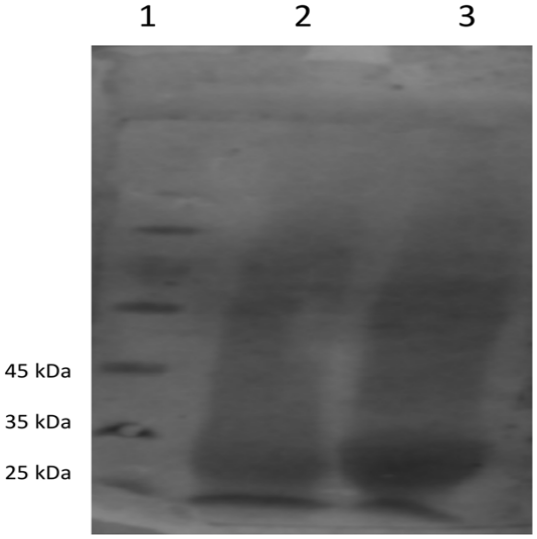

Figure 3. Western blotting with the Anti-VGL antibody produced in the current work. 1 - TrueColor High Range Protein Marker S2600; 2 and 3 - *Vatairea guianensis* lectin bound with the IgY

The hens used were from the Dekalb White line and the anti-VGL polyclonal IgY antibodies extracted from the egg yolk. Li *et al*. (1998) (Li *et al*., 1998) carried out a comparative study between white chickens of the Single Comb White Leghorn line and red chickens of the Rhode Island Red line for the production of specific IgY antibodies against bovine serum albumin (BSA). The authors demonstrated that the protein concentration and the amount of anti-BSA antibodies, for each mL of pure yolk, was similar in both strains. Chickens can be immunized through different routes depending on the immunization protocol (Eto *et al*., 2012). However, for the production of polyclonal IgY, in most birds, they are immunized intramuscularly with protein antigens and Freund's adjuvants (Araújo *et al*., 2010). In that study, the chickens were immunized via the intramuscular route which, according to Wooley and Landon (1995) (Woolley & Landon, 1995), results in high levels of antibodies 28 days after immunization. In addition, the resulting antibody may have a 10-fold higher specificity when compared to chickens immunized with the same antigen subcutaneously. Furthermore, chickens immunized by the intramuscular route continue to produce specific antibodies for more than 200 days (Horton *et al*., 1985). Likewise, chickens can tolerate the use of common immunological adjuvants, as the oily adjuvant used in this study.

Normally, the laying capacity of chickens is not affected by the injection of the antigen (de Paula *et al*., 2011; Matheis & Schade, 2011). On the other hand, some authors report a frequent side effect in chickens, which is the transient drop in egg production during the immunization period (Hatta *et al*., 1997). The distribution of the inoculum in different places reduces local inflammation and can avoid a reduction in egg production (Schwarzkopf & Thiele, 1996) possibly by dividing the inflammatory process in several points, being less aggressive. Local inflammatory reactions are a concern, although they are not always seen in chickens. The results demonstrate that these reactions were not noticeable in the experiment conducted. This fact is possibly due to the low concentration of the antigen and the application of the antigen at various points. In addition, no significant drop in egg laying was noted. The transovarial passage of IgY takes approximately 5 days (Mohammed *et al*., 1998). The half-life of IgY circulating in adult birds is approximately 36 to 65 hours. This is much shorter than the sheep IgG half-life, which is approximately 15 days. According to several authors, serum specific antibodies are transported to the yolk with a delay of 5-6 days or 3-4 days. The delay may be involved with eggs subjected to their final processing, during which the IgYs are transferred to the yolk together with other selected proteins. The amount transferred appears to be independent of the size of the egg (Patterson *et al*., 1962; Woolley & Landon, 1995).
The amount of IgY produced by a chicken can be 20 to 40 g per year (Ferreira Júnior *et al*., 2012). The concentration of it in egg yolks is constant and it is possible to obtain about 100 mg in a single egg. The productivity of IgYs antibodies in chickens can be 5 to 10 times greater than the productivity of IgGs in rabbits (200mg IgG / 40mL blood). Within this quantity of IgYs produced, only about 3 to 3.2% of the antibodies are specific to the antigens administered (Akita & Li-Chan, 1998). Throughout the experiment it is believed that a relatively high amount of IgYs was produced. IgY antibodies appear in the chicken serum approximately 4 days after inoculation of the antigen, reaching a maximum title in 6 to 8 days, and declining after that.

The antibody titer can be strongly increased by booster immunizations. According to the results of Petterson *et al*. (1962) (Patterson *et al*., 1962), some chickens respond with mammalian-like antibody kinetics, while others respond to the same immunization protocol with an increase in antibody titer following the first immunization, but fail to bring about any significant change after the second immunization. There are many methods for purifying immunoglobulins from eggs. The main ones include: Precipitation methods using ammonium sulfate or sodium sulfate (Akita & Li-Chan, 1998), polyethylene glycol (PEG) (Polson *et al*., 1980), caprylic acid (McLaren *et al*., 1994) and carrageenan (Chang *et al*., 2000), chromatographic methods: affinity chromatography (Lesley & Groskreutz, 1997), ion exchange chromatography (Schade *et al*., 2014), hydrophobic interaction chromatography (Schade *et al*., 2014), among others. All the techniques mentioned above, as well as the protocols derived from these methodologies, have proven to be efficient for the purification of Immunoglobulin Y. In the 19th century it was discovered that the immune serum could be used to treat infectious diseases (Munhoz *et al*., 2014). In the early 20th century, serotherapy was used to treat a wide variety of bacterial infections, including those caused by *Corynebacterium diphtheria*e, in addition to applications for treatment and diagnosis, there are a number of possibilities for IgY applications. In the specific case of IgYs produced in our study, one can apply them in immunohistochemical studies and several others.

Conclusion

The immunization of laying hens with *Vatairea guianensis* lectin (VGL) induced the production of polyclonal antibodies against this protein. The antibody was successfully produced and purified. In this sense, the IgYs produced in this work constitute a powerful biotechnological tool to be made available for future studies and open doors for their application in lectinology as well as in other histochemical studies.

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REFERENCES

Akita, E. M., & Li-Chan, E. C. 1998. Isolation of bovine immunoglobulin G subclasses from milk, colostrum, and whey using immobilized egg yolk antibodies. Journal of Dairy Science, 811, 54–63.

Amro, W. A., Al-Qaisi, W., & Al-Razem, F. 2018. Production and purification of IgY antibodies from chicken egg yolk. Journal, Genetic Engineering & Biotechnology, 161, 99–103.

Araújo, A. S., Lobato, Z. I. P., Chávez-Olórtegui, C., & Velarde, D. T. 2010. Brazilian IgY-Bothrops antivenom: Studies on the development of a process in chicken egg yolk. Toxicon: Official Journal of the International Society on Toxinology, 554, 739–744.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the

principle of protein-dye binding. Analytical Biochemistry, 72, 248–254.

Chang, H. M., Lu, T. C., Chen, C. C., Tu, Y. Y., & Hwang, J. Y. 2000. Isolation of immunoglobulin from egg yolk by anionic polysaccharides. Journal of Agricultural and Food Chemistry, 484, 995–999.

Darwish, I. A. 2006. Immunoassay Methods and their Applications in Pharmaceutical Analysis: Basic Methodology and Recent Advances. International Journal of Biomedical Science: IJBS, 23, 217–235.

de Paula, V. S., da Silva, A. dos S., de Vasconcelos, G. A. L. B. M., Iff, E. T., Silva, M. E. M., Kappel, L. A., Cruz, P. B., & Pinto, M. A. 2011. Applied biotechnology for production of immunoglobulin Y specific to hepatitis A virus. Journal of Virological Methods, 1711, 102–106.

Eto, S. F., Andrade, F. G., Pinheiro, J. W., Balarin, M. R., Ramos, S. P., & Venancio, E. J. 2012. Effect of inoculation route on the production of antibodies and histological characteristics of the spleen in laying hens. In Revista Brasileira de Ciência Avícola Vol. 14, Issue 1, pp. 63–66. https://doi.org/10.1590/s1516-635x2012000100011

Ferreira Júnior, A., Santiago, F. M., Silva, M. V., Ferreira, F. B., Macêdo Júnior, A. G., Mota, C. M., Faria, M. S., Silva Filho, H. H., Silva, D. A. O., Cunha-Júnior, J. P., Mineo, J. R., & Mineo, T. W. P. 2012. Production, characterization and applications for Toxoplasma gondii-specific polyclonal chicken egg yolk immunoglobulins. PloS One, 77, e40391.

Forthal, D. N. 2014. Functions of Antibodies. Microbiology Spectrum, 24, 1–17.

Hatta, H., Tsuda, K., Ozeki, M., Kim, M., Yamamoto, T., Otake, S., Hirasawa, M., Katz, J., Childers, N. K., & Michalek, S. M. 1997. Passive immunization against dental plaque formation in humans: effect of a mouth rinse containing egg yolk antibodies IgY specific to Streptococcus mutans. Caries Research, 314, 268–274.

Holanda, E., Arruda, F. V. S., do Nascimento, K. S., Alves, V., Shiniti, C., da Silva, B. R., Holanda, A., & Sousa, B. 2012. Biological Applications of Plants and Algae Lectins: An Overview. In Carbohydrates - Comprehensive Studies on Glycobiology and Glycotechnology. https://doi.org/10.5772/50632

Horton, J. J., Holden, C. A., Ward, P. J., MacDonald, D. M., & Sanderson, A. R. 1985. Exploitation of Phylogenetic Distance in Cell Surface Immune Labeling: Studies with Beta2-Microglobulin. In Journal of Investigative Dermatology Vol. 84, Issue 2, pp. 96–99. https://doi.org/10.1111/1523-1747.ep12274979

Korekane, H., Hasegawa, T., Matsumoto, A., Kinoshita, N., Miyoshi, E., & Taniguchi, N. 2012. Development of an antibody-lectin enzyme immunoassay for fucosylated α-fetoprotein. Biochimica et Biophysica Acta, 18209, 1405–1411.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 2275259, 680–685.

Lagarda-Diaz, I., Guzman-Partida, A. M., & Vazquez-Moreno, L. 2017. Legume Lectins: Proteins with Diverse Applications. International Journal of Molecular Sciences, 186. https://doi.org/10.3390/ijms18061242

Lesley, S. A., & Groskreutz, D. J. 1997. Simple affinity purification of antibodies using in vivo biotinylation of a fusion protein. Journal of Immunological Methods, 2072, 147–155.

Li, X., Nakano, T., Sunwoo, H. H., Paek, B. H., Chae, H. S., & Sim, J. S. 1998. Effects of egg and yolk weights on yolk antibody IgY production in laying chickens. Poultry Science, 772, 266–270.

Mahmood, T., & Yang, P.-C. 2012. Western blot: technique, theory, and trouble shooting. North American Journal of Medical Sciences, 49, 429–434.

Marques, G. F. O., Osterne, V. J. S., Almeida, L. M., Oliveira, M. V., Brizeno, L. A. C., Pinto-Junior, V. R., Santiago, M. Q., Neco, A. H. B., Mota, M. R. L., Souza, L. A. G., Nascimento, K. S., Pires, A. F., Cavada, B. S., & Assreuy, A. M. S. 2017. Contribution of the carbohydrate-binding ability of Vatairea guianensis lectin to induce edematogenic activity. Biochimie, 140, 58–65.

Matheis, W., & Schade, R. 2011. Development of an IgY-based rocket-immunoelectrophoresis for identity monitoring of Pertussis vaccines. Journal of Immunological Methods, 3691-2, 125–132.

McLaren, R. D., Prosser, C. G., Grieve, R. C. J., & Borissenko, M. 1994. The use of caprylic acid for the extraction of the immunoglobulin fraction from egg yolk of chickens immunised with ovine α-lactalbumin. In Journal of Immunological Methods Vol. 177, Issues 1-2, pp. 175–184. https://doi.org/10.1016/0022-17599490154-6

Mohammed, S. M., Morrison, S., Wims, L., Trinh, K. R., Wildeman, A. G., Bonselaar, J., & Etches, R. J. 1998. Deposition of genetically engineered human antibodies into the egg yolk of hens. Immunotechnology: An International Journal of Immunological Engineering, 42, 115–125.

Munhoz, L. S., Vargas, G. D., Fischer, G., de Lima, M., Esteves, P. A., & de Oliveira Hübner, S. 2014. Avian IgY antibodies: characteristics and applications in immunodiagnostic. In Ciência Rural Vol. 44, Issue 1, pp. 153–160. https://doi.org/10.1590/s0103-84782014000100025

Patterson, R., Youngner, J. S., Weigle, W. O., & Dixon, F. J. 1962. Antibody production and transfer to egg yolk in chickens. Journal of Immunology , 89, 272–278.

Peumans, W. J., & Van Damme, E. J. 1995. Lectins as plant defense proteins. Plant Physiology, 1092, 347–352.

Polson, A., von Wechmar, M. B., & van Regenmortel, M. H. 1980. Isolation of viral IgY antibodies from yolks of immunized hens. Immunological Communications, 95, 475–493.

Schade, R., Behn, I., Erhard, M., Hlinak, A., & Staak, C. 2014. Chicken Egg Yolk Antibodies, Production and Application: IgY-Technology. Springer.

Schroeder, H. W., Jr, & Cavacini, L. 2010. Structure and function of immunoglobulins. The Journal of Allergy and Clinical Immunology, 1252 Suppl 2, S41–S52.

Schwarzkopf, C., & Thiele, B. 1996. Effectivity of Alternative Adjuvants in Comparison to Freund's Complete Adjuvant. ALTEX, 135, 22–25.

Silva, H. C., Nagano, C. S., Souza, L. A. G., Nascimento, K. S., Isídro, R., Delatorre, P., Rocha, B. A. M., Sampaio, A. H., Assreuy, A. M. S., Pires, A. F., Damasceno, L. E. A., Marques-Domingos, G. F. O., & Cavada, B. S. 2012. Purification and primary structure determination of a galactose-specific lectin from Vatairea guianensis Aublet seeds that exhibits vasorelaxant effect. In Process Biochemistry Vol. 47, Issue 12, pp. 2347–2355. https://doi.org/10.1016/j.procbio.2012.09.014

Woolley, J. A., & Landon, J. 1995. Comparison of antibody production to human interleukin-6 IL-6 by sheep and chickens. In Journal of Immunological Methods Vol. 178, Issue 2, pp. 253–265. https://doi.org/10.1016/0022-17599400263-v

Wu, Y.-M., & Haab, B. B. 2010. Applications of Antibody-Lectin Sandwich Arrays ALSA to Pancreatic Cancer Diagnostics and Drug Discovery. In Drug Discovery in Pancreatic Cancer pp. 243–269. https://doi.org/10.1007/978-1-4419-1160-5_13

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