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Humoral Immunity in chicken lines developed by Embrapa Suínos e Aves: Natural and specific antibodies

Imunidade humoral em linhagens de frangos desenvolvidos pela Embrapa Suínos e Aves: anticorpos naturais e específicos

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Highlights

Difference between males and females in the production of antibodies.
Levels of production of natural antibodies and specific antibodies.
Difference of humoral immune response between lines.

Abstract

The antibodies produced in the first days of vertebrate life and are called natural antibodies (NAb). Other antibodies, produced in response to restricted contact with the antigen, are called specific antibodies (SpAb). To evaluate the production of NAb studies have used rabbit red blood cells (RRBC). On the other hand, evaluation of the production of specific antibodies can be performed with the use of sheep red blood cells (SRBC), a cell type that results in high production of SpAb. The aim of this study was to evaluate the production of NAb and SpAb in chicken lines developed by EMBRAPA Suínos e Aves. Animals of laying and poultry lines were inoculated intramuscularly with 5% of SRBC. The titers of NAb and SpAb were higher in laying hens than in broiler lines. The same results were obtained with anti-RRBC, the exception was males of the LLc lines, with lower antibody production compared to the other lines and females of the same line. These data show that the production of natural and specific antibodies is higher in laying hens compared to broilers, and that there is an effect of sex on antibody production.

Key words: IgY. IgM. Anti-α-Gal antibodies. Breeding. Immunity. B cells.

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Resumo

Os anticorpos produzidos nos primeiros dias de vida dos vertebrados são chamados de anticorpos naturais (NAb). Outros anticorpos são produzidos em resposta ao contato restrito com o antígeno e são chamados de anticorpos específicos (SpAb). Para avaliar a produção de NAb, foi utilizado glóbulos vermelhos de coelho (RRBC). Por outro lado, a avaliação da produção de anticorpos específicos foi realizada com o uso de hemácias de ovelha (SRBC), tipo celular que resulta em alta produção de SpAb. O objetivo deste estudo foi avaliar a produção de NAb e SpAb em linhagens de frango desenvolvidas pela EMBRAPA Suínos e Aves. Os animais das linhagens postura e de corte foram inoculados por via intramuscular com 5% de SRBC e RRBC. Os títulos de NAb foram maiores em fêmeas de linhagem poedeiras do que em linhagens de corte. Os mesmos resultados foram obtidos com SpAb, com exceção dos machos das linhagens LLc com menor produção de anticorpos em relação aos machos de outras linhagens e fêmeas da mesma linhagem. Esses dados mostram que a produção de anticorpos naturais e específicos é maior em galinhas poedeiras em comparação com linhagens de corte, e que há efeito do sexo na produção de anticorpos.

Palavras-chave: IgY. IgM. Anticorpos anti-α-Gal. Reprodução. Imunidade. Células B.

Introduction

Poultry farming is one of the most important agricultural activities in the world. In Brazil, EMBRAPA Suínos e Aves has been working on the development of laying and broiler hens. Laying hens from the CC line, which originate from a White Leghorn breed, are called the CC control line (CCc), MM is a brown egg laying hen line from the Rhode Island Red breed, the TT line is a broiler paternal line selected for higher weight gain, feed conversion, viability, fertility, hatching ability, and reduction of abdominal fat, and the LLc is a control line maintained without selection (Figueiredo et al., 2012a; Figueiredo et al. 2012b).

The mechanisms of adaptive immunity include cellular immunity, mediated by cells like T cytotoxic, neutrophils, and macrophage cells, and humoral immunity, mediated by antibodies produced by B cells (Wigley, 2013). In birds, three antibody classes are found: IgA, IgM, and IgY. IgA is predominant in egg white and secretions. IgM is found in serum and egg white, and IgY is the most abundant immunoglobulin in serum and egg yolk (Pereira et al., 2019).

After the first contact with the antigen, IgM is the predominant SpAb antibody class produced, while IgY is the main antibody class found in the secondary immune response (Eto et al., 2012; Pereira et al., 2019). Repeated exposure to an antigen results in faster antibody production and in antibodies with higher avidity (Muraille E & Goriely, 2017). In chickens, studies show that the antigen route and dose, the number and interval between inoculations, the use of immunostimulants, and even toxin contamination influence the production of specific antibodies (Darpossolo et al., 2010; Freitas et al., 2011; Andrade et al., 2013; Barbosa et al., 2017; Khan et al., 2019; Silva et al., 2020). In particular, genetic characteristics directly influence antibody production in chickens (Wijga et al., 2009; Lillie et al., 2017).

In addition to SpAb produced after exposure to an antigen, there are so-called natural antibodies, that are constitutively produced and are part of the so-called humoral innate immunity (Wijga et al., 2009). These antibodies are present in unimmunized individuals (Parmentier et al., 2004). High levels of NAb against rabbit red blood cells (RRBCs) are present in birds because RRBCs express high levels of the α-Gal epitope (Parmentier et al., 2008; Wijga et al., 2009). These epitopes are found in microorganisms present throughout the gastrointestinal tract, which stimulate B cells to produce anti-Gal antibodies (Parmentier, et al., 2008). On the other hand, in chickens, there are low levels of natural antibodies capable of agglutinating sheep red blood cells (SRBCs) (Bailey, 1923). Low levels of SRBC antibodies are probably due to the presence of few copies of the α-Gal epitope in SRBCs, less than 8,000 copies per cell, while RRBCs have approximately 2 million copies (Ogawa & Galili, 2006).

A genetic relationship between the levels of NAb against RRBCs and the production of SpAb against SRBCs has been suggested by the presence of high levels of natural antibodies against RRBCs in chickens selected for high production of antibodies against SRBCs (Cotter et al., 2005).

Considering the importance of poultry farming and the need for better understanding of the immune response in poultry and how it is affected by genetic improvement, the current study evaluated the production of NAb and SpAb in different chicken lines developed by EMBRAPA Suínos e Aves.

Material and Methods

Animals

Ninety animals (45 males and 45 females) were used, 18 animals from each of the MM, CC, CC control (CCc) laying lines, TT, and LLc control (LLc) broiler lines. The animals were supplied by EMBRAPA Suínos e Aves (Concórdia / Santa Catarina / Brazil) and kept at the premises of the School Farm of the State University of Londrina, with free access to water and feed. The use of the animals was submitted to and approved by the Animal Experimentation Ethics Committee of Londrina State University, n. 62/12.

Experimental procedure

The animals were inoculated with 5% SRBCs intramuscularly (200 μL / animal) in the pectoral muscle on the 1st and 30th days of the experiment. Blood samples were collected from wing brachial vein puncture, one day before the first immunization (pre-immune) and on the 7th and 37th days of the experiment. Sera were obtained and stored at -20ºC until analysis.

Natural and specific Antibodies titer determination

Natural antibodies to SRBCs and to RRBCs were determined using non-immune serum samples obtained one day before immunization. Total antibody titers (IgT) were determined by microhemagglutination reactions performed in U-bottom 96-well microplates, as described by Freitas et al. (2011). Serum samples were serially diluted 1:

2 to 1: 512 in 25 µl 1X PBS pH 7.4. Then 25 µL of 2% SRBC or RRBC solution was added to each well. The plates were incubated at room temperature for 1h. As a negative control, 25 µl 1X PBS pH 7.4 were added in place of serum samples. Results were expressed as the square root of the reciprocal of the last dilution that resulted in hemagglutination.

To determine the levels of specific antibodies against SRBCs, microhemagglutination reactions were performed using sera collected on the 7th and 37th days of the experiment, as described above. IgY anti-SRBC antibody titers were determined by incubating serum samples with an equal volume of 0.2 2-mercaptoethanol in 1X PBS pH 7.4, followed by the microhemagglutination reaction, as previously described. IgM anti-SRBC antibody titers were determined by subtracting the total antibody (IgT) titers from the IgY antibody titers.

Statistical analysis

Results were submitted to the Shapiro-Wilk normality test and are presented as mean and standard deviation, when variables demonstrate normal distribution, or as median and interquartile range. For comparisons between laying hens x broilers, males x females, and between males and females of the same line we used the t-test or the Mann-Whitney test depending on whether the variables presented normal distribution. For comparisons between lines, the Kruskal-Wallis test was used, followed by the Dunn test. P values <0.05 were considered significant. The software used for statistical analysis was GraphPad Prism 5.0 (GraphPad Software, San Diego, California, U.S.A.).

Results and Discussion

Natural antibodies (NAb)

The titers of NAb RRBCs and SRBCs are shown in figure 1. The production of natural antibodies against RRBCs (Figure 1A) was higher in females of the CC and CCc lines, even when compared to males of the same line. LLc male animals produced the fewest NAb RRBC antibodies. These results demonstrate the influence of sex and line on the production of immunoglobulins. There was no significant difference in NAb SRBC levels (Figure 1B).

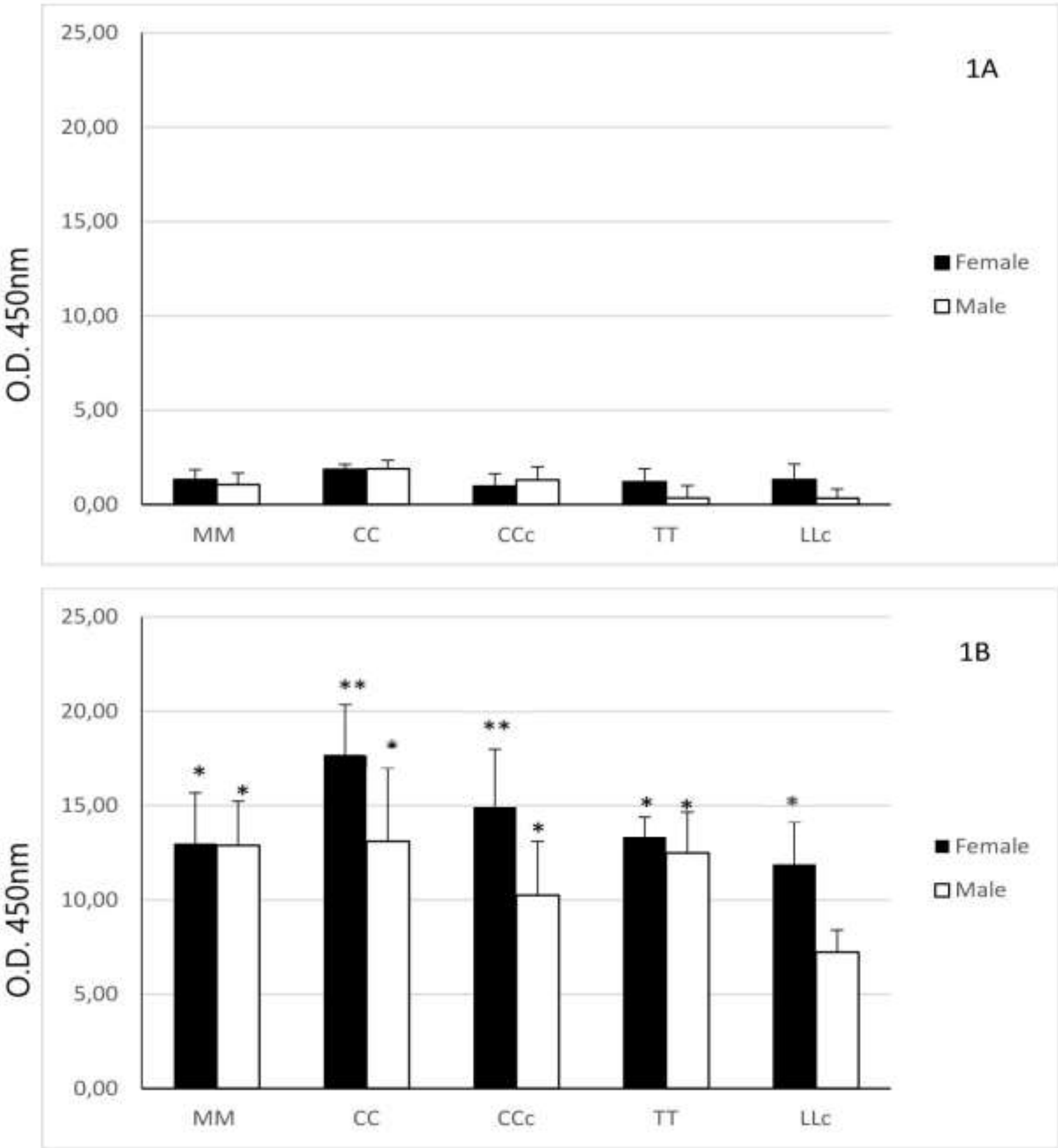

Figure 1. The Natural antibody (NAb) titers against Sheep red blood cells Fig. 1 A, and natural antibody (NAb) titers against Rabbit red blood cells Fig. 1 B, * p≥0,05; ** p≥0,01.

Specific Antibodies (SpAb)

In the primary immune response, the total antibody titers (Figure 2A) showed no significant differences between lines or sex of the animals. In the secondary response (Figure 2B), we observed significantly higher production of SpAb to SRBCs in the females of the CC and CCc lines than males of the same lines. These differences were not related to the production of IgM specific antibodies to SRBCs (Figure 2C and 2D), however, a relation with production of IgY anti-SRBC antibody titers was detected. The results show significantly higher production of IgY anti-SRBC antibody titers in sera of females in comparison to sera of males in secondary immune response (Figure 2E, and 2F). Furthermore, we detected similar results in females and males from the LLc line (Figure 2F).

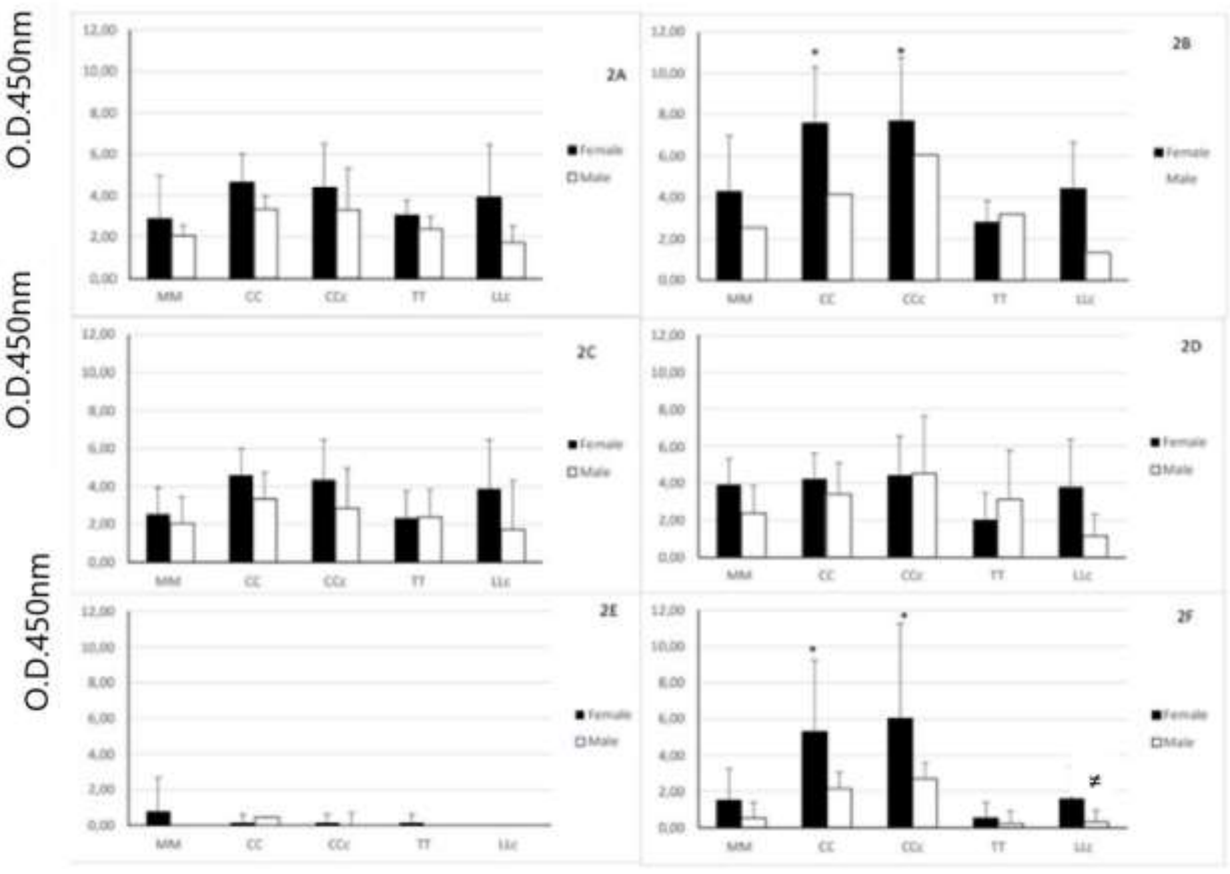

Figure 2. Dynamics of SpAb response per line. Analysed were total antibody (IgM and IgY) for Sheep red blood cell, primary imune response (Fig. 2 A) and secondary immune response (Fig. 2 B). Dosage of SpAb IgM for Sheep red blood cell in primary response (Fig. 2 C), and secondary immune response (Fig. 2 D). IgY levels in primary imune response (Fig. E) and secondary imune response (Fig. 2 F). Significances of * p≤0,05, significances de ≠ for p≤0,05 between males and females of LLC lines.

In the current study, the results show that female laying hens of CC and CCc lines have higher levels of natural antibodies than males from the same lines. This variation between antibody levels between different lines is consistent with other studies. Sun et al. (2011) evaluated natural antibody levels against keyhole limpet hemocyanin (KLH) in six lines of Rhode Island Red laying hens and six lines of White Leghorn origin. In the current study, it was observed that antibody titers were higher in White Leghorn than in Rhode Island Red lines. In addition, there was great variability in anti-KLH antibody titers between Rhode Island Red and White Leghorn lines, while Kjærup et al. (2017) observed that Hevellad and Hisex animals have higher levels of natural anti-KLH antibodies than Bovan chickens.

This variation in natural antibody production is probably due to differences in humoral immunity-related genes, as studies have shown a relationship between specific antibody levels and natural antibody levels. Hangalapura et al. (2003) found higher natural antibody titers in laying hens selected for higher antibody production against SRBCs than in animals selected for low antibody production and in non-selected animals. Similar results were observed in chickens selected for higher production of antibodies against SRBCs that have higher levels of natural antibodies against chicken egg white protein, myoglobin, thyroglobulin, transferrin, bovine albumin, KLH, and ovalbumin compared to chickens selected for lower antibody production (Parmentier et al., 2004).

In addition, Minozzi et al. (2008) found no significant difference in the production of natural antibodies against KLH and lipopolysaccharide (LPS) between the laying hen lines selected for high Newcastle disease antibody production (ND3-L), high cell-mediated immune response (PHA-L), high phagocytic activity (CC-L), or control. However, ND3-L and PHA-L birds had higher levels of natural antibodies against lipoteichoic acid (LTA) and RRBCs (anti-Gal). These results suggest that natural antibody titers depend on the genetic selection made and may affect natural antibody production against a specific antigen, while not representing an increase in total antibody titers.

It is important to note that there is probably a relationship between NAb levels and SpAb production. In a study of natural antibody levels against KLH and LPS and Newcastle disease virus specific antibodies in 12 pure laying hen lines, it was observed that lines showing the highest natural antibody levels also demonstrated the highest Newcastle specific antibody levels (Star et al., 2007). In the work of Parmentier et al. (2004) similar results were also observed, with high levels of natural antibodies observed in laying hens selected for high production of antibodies against SRBCs. Wijga et al. (2009) found a positive correlation between anti-SRBC antibody levels and anti-RRBC natural antibodies. These studies suggest a genetic relationship between natural antibody levels and subsequent production of specific antibodies.

Furthermore, it was observed that females produced more natural and specific antibodies than males of the same line, thus, the findings showed that the production of natural antibodies is also influenced by sex. Females from CC and CCc lines produced more natural antibodies against RRBCs and anti-SRBCs antibodies than males of this line.

These results show that sex can influence the production of natural antibodies and specific antibodies, as described by other authors (Wils-Plotz & Klasing, 2017; Hu et al., 2016). An effect of sex on IgA production has been observed in laying hens as well as an interaction between sex and immunomodulatory treatment, where lutein-treated males have a higher plasma IgA concentration than vitamin E-fed males, while treatment did not affect IgA levels in females (Wils-Plotz & Klasing, 2017). Higher production of natural antibodies in females than in males of the same line has been observed previously (Minozzi et al., 2007). It is probable that this effect is related to differences in gene expression related to immune response between males and females (Voigt et al., 2019; Kumar et al., 2011).

From the data obtained in the present study, we can conclude that the humoral immune response is higher in laying birds, since they produced more natural and specific antibodies than broilers, demonstrating an important effect of sex. These results may contribute to the development of genetic improvement projects for more pathogen resistant birds.

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