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Effects of broiler breeder age on immune system development of progeny

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ABSTRACT - The objective of this study was to determine the effects that breeder age has on digestive and immune system development; the transfer of immunoglobulins to egg yolk, yolk sac, and neonate chicks; and the immune response of chicks up to 35 days old. Three ages (32, 42, and 52 weeks) of Hubbard breeders were studied with ages as treatments. A total of 425 eggs were weighed for each of the three treatments and incubated. After hatching, a total of 300 1-day-old chicks were used in each treatment. We studied the development of the gastrointestinal tract and immune system of progeny and IgY transfer from breeder to progeny. Chicks from 52-week-old breeders had greater gastrointestinal tract growth up to seven days of life and greater body weight at 14 days. Older breeders (52 weeks) had higher amounts of IgY in serum and egg yolk. Chicks from the youngest breeders (32-weeks-old) had a better immune response at two weeks post-vaccination. It can be concluded that the older breeders have a greater capacity to immunize progeny up to 14 days. Strategies can be developed to increase IgY in the serum of young breeders and, consequently, increase the innate immunity of the newly-hatched chicks.

Keywords: embryo development, hatchery, IgY, old breeders, young breeders

1. Introduction

The transfer of antibodies via egg yolk to a day-old chick is an important process that guarantees viability and improves broiler performance. It also works to passively protect chicks against common pathogens before their immune system matures (Leandro et al., 2011a).

Immunoglobulin Y (IgY) is the main type of immunoglobulin in birds. The transfer of IgY from hen to chick occurs in two stages. Circulating IgY must first be transferred from the bloodstream of the hen to the ovarian follicle, then it must be transferred from the egg yolk to the embryo (Ulmer-Franco et al., 2010; Murai et al., 2020). According to Apanius (1998), broiler chickens start to produce their own antibodies five days after hatching; however, they need the IgY that was transferred from the breeder during the first 13 days after hatching.

One of the factors that can change egg weight and the proportion of its constituents is breeder age; eggs from older breeders are heavier compared with those of young breeders (van der Wagt et al., 2020). Breeder age also influences chick quality (Attia et al., 1994, 1995). Chicks from older breeders

have higher body weight and develop faster due to the greater availability of nutrients in the egg (Nangsuay et al., 2016; Araújo et al., 2016). According to Machado et al. (2020) and Cardeal et al. (2022), chicks from eggs of older breeders have greater intestinal development. In addition, breeder age is positively correlated with newly-hatched chick, yolk sac, and bursa of Fabricius weight (Fernandes et al., 2014; Narinç and Aydemir, 2021).

On the other hand, little is known about how breeder age can influence the transfer of immunoglobulins to egg yolk and yolk sac as well as serum the concentration of IgY in progeny. Given the above, this study aimed to evaluate the effects of breeder age (32, 42, and 52 weeks) on digestive and immune system development and on the transfer of IgY from breeder to egg yolk, yolk sac, and chick serum from one to 35 days of age.

2. Material and Methods

The research was conducted in accordance with the institutional committee on the use of animals (001/2013). The experiment was carried out in Goiânia, state of Goiás, Brazil (16°35'33" S, 49°16'51", 710 m altitude).

2.1. Experimental design

Three ages (32, 42, and 52 weeks) of the same commercial flock of Hubbard breeders were studied. The three ages served as treatments and all three were evaluated by the same procedures. Feed used in the experiment was formulated according to Hubbard SAS Breeder Company (2011). Diets did not change during experiment, and breeders were maintained in same conditions. The experiment was between September (2013) and January (2014). The Hubbard broiler breeders were housed in open-sided houses with concrete floors covered with wood shavings with a depth 6 to 7 cm. The litter was stirred or turned to maintain its condition, and the wet litter spots were replaced with a fresh new litter, on daily basis. There were 24,800 hens and 2,480 rooters in the breeders' house. The stocking density used was 8.00 birds/m^2 following the Hubbard Technical Manual. Feed and water were provided *ad libitum*. The photoperiod was set to 16L:8D during the experimental period. All management practices were standardized across the three treatments.

2.2. IgY transfer

For each age (treatment), IgY was quantified in breeder serum, egg yolk, and yolk sac and progeny serum at 1, 7, 14, 21, 28, and 35 days. In three moments (32, 42, and 52 weeks) 5.0 mL blood were collected, via brachial vein in a tube for blood collection without anticoagulant, from 360 female breeders from the studied flock (120 samples per age). The samples were desorbed, and 1 mL of serum of each was placed in an individual plastic microtube, identified and stored in a cold chamber at −18 °C for subsequent quantification of serum IgY concentration. For this item, each individual serum was a replication. Immunoglobulin Y was quantified in breeder serum according to Sun et al. (2013) methodology.

At each of the ages (treatment), on the same day, 60 eggs were individually collected, weighed, and broken open for analysis of egg yolk. Yolks were separated from albumen, carefully rolled on scientific cleaning wipes to remove excess albumen and weighed. Equal volumes (8 mL) of three egg yolks per flock age were pooled into 50-mL plastic capped tubes (n = 20 pooled samples per flock age), mixed thoroughly, and stored at −18 °C. Immunoglobulin Y was quantified in egg yolk according to Ulmer-Franco et al. (2012) methodology.

A total of 425 eggs were weighed for each of the three treatments (63.66, 65.49, and 70.12 g for 32, 42, and 52 weeks, respectively). The eggs were collected on the same day. The eggs were subsequently fumigated with formaldehyde gas at temperature of 24 °C and 75% relative humidity (RH) for 20 min and stored for four days at 17 °C and 75% RH. Eggs were incubated in a single stage incubator (CAPS Ug 62 HT,

capacity of 61,920 eggs) at an initial temperature of 37.8 °C and 60% RH with turning from day 1 to day 18 of incubation. The setter was equipped with infrared sensors, which constantly monitored eggshell temperature, coupled with setter control to provide the ideal temperature for each embryo development stage. Thus, the air temperature changed to maintain the eggshell temperature at 37.8 °C. The five trays containing the experimental eggs (85 per tray) were identified and equally distributed at three different positions inside the trolleys (upper (one), middle (tree), and lower (one) positions). The other spaces of incubation trolleys were occupied with other trays containing eggs from the same broiler breeder batches to maintain the environmental conditions of the incubator within required technical standards. On day 18 of incubation (432 h), the eggs were transferred to a hatcher (CASP 108 HR, capacity of 19,264 eggs) set to maintain a temperature of 36.3 °C and 70.0% RH, with no vaccination being performed. Incubation was completed at 508 hurs, when hatched chicks were removed from the hatch baskets.

After incubation process, 90 hatched chicks (30 per treatment) were individually weighed, slaughtered by cervical dislocation, and dissected. Yolk sacs were individually weighed, pooled into groups of three, mixed thoroughly and stored at −18 °C for analysis of yolk sac at pulling; for this analysis each pool of three yolk sacs were a replication. Immunoglobulin Y was quantified in yolk sac according to Ulmer-Franco et al. (2012) methodology.

Breeder serum samples, yolk, and yolk sac contents were subjected to analysis by ELISA Kit (Enzyme-Linked® Immunosorbent Assay) for detection of antibodies (chicken IgY) against ND (Biochek®), being processed according to the manufacturer's recommendations. The microplate containing the sampled material was read immediately after the process was completed using a Polaris ELISA microplate reader (Celer®), with absorbance measured at a wavelength of 405 nm, and the results obtained using Biochek II Software (2015 version), which correlates the reading of samples to the reading of controls and establishes, based on this correlation, the concentration of immunoglobulins. The average values of absorbance or optical density (OD) at a wavelength of 650 nm per sample were used for statistical analyses (Leandro et al. 2011a, 2011b; Ulmer-Franco et al., 2012).

The breeders are isogenic individuals, so the test was performed on all serum in duplicate in the antigen concentration of 5 µm/mL with the dilution of serum set at 1:40 for breeders and 1:10 for chicks.

2.3. Development of the gastrointestinal tract and immune system of progeny

At pulling, all the chicks were sexed and then weighed (41.32, 44.19, and 48.82 g for ages 32, 42, and 52 weeks, respectively). A total of 300 1-day-old chicks were used for each age with 30 replicates of 10 birds each (five males and five females). Each cage was considered a replicate. Birds were housed in galvanized steel battery cages (0.5×0.4×0.4 m), equipped with trough drinkers and feeders. Water and feed were provided *ad libitum*. A lighting program of 23L:1D of darkness was adopted throughout the experimental period (days 1 to 35). Birds were fed diets based on corn and soybean meal formulated to supply their nutritional requirements during pre-starter and starter phases, according to Rostagno et al. (2011).

At pulling, one chick per repetition, for a total of 30 birds per treatment, was weighted, and 2.0 mL of blood were collected in blood collection tubes without anticoagulant by cardiac puncture. The chicks were then euthanized by cervical dislocation (Cardeal et al., 2022). Individual weights of liver, gizzard + proventriculus, pancreas, bursa of Fabricius, spleen, and yolk sac were obtained using a precision scale (0.0001 g); after that, relative organ weight (%) was calculated (Araújo et al., 2020). Blood samples and necropsies were repeated at day 7 and 14 post-hatch for 30 chicks of each treatment. For these items, each chick was a replication for each breeder age.

At five days post-hatch, all chicks of all treatments were vaccinated with a vaccine consisting of a sample of the La-Sota Newcastle Disease (ND) virus from Biovet Laboratory®. The ocular route was used for application to ensure that all birds would receive the vaccine dose in the ideal concentration. Blood samples were subsequently collected from the chicks at 21, 28, and 35 days of age to quantify serum IgY (Cardeal et al., 2022).

To investigate intestinal development, histological slides were made from small intestine (duodenum and jejunum). Segments of approximately 2.0 cm were collected and fixed in 10% buffered formaldehyde solution for 24 h. After fixation, the samples were stored in 70% alcohol and processed according to the methodology described by Luna (1968) and stained by the hematoxylin-eosin (HE) method. Intestinal histomorphometry (villus height and crypt depth) was investigated in chicks at 7 and 14 days of age by making 10 measurements on each intestinal segment per bird for a total of 300 measurements per treatment per chick age. Measurements were made using an optical microscope (5X) coupled to an image analyzer system (Axio Vision 3.0; Zeiss) according to Moreira Filho et al. (2019) by employing image analysis software (UTHSCSA ImageTool software, version 3.0).

Spleen and bursa were used to compare the quantification of B-lymphocytes (%) at 7 and 14 days post-hatch with 20 chicks per treatment. Immediately following euthanasia, the spleen and bursa of Fabricius of each chick was weighted and placed into a Petri dish with frost tamponed saline solution without Ca^{2+} and Mg^{2+} (CMF). The cells from the spleen and from the bursa were collected according to Peralta et al. (2017). Briefly, 1.5 mL with cells collected were layered on 1.5 mL of Histopaque-1077 in a plastic tube and centrifuged at 2,000 × *g* for 30 min; this was implemented further two times. Viable lymphocyte counts were made using the trypan blue exclusion method, and the cells were diluted into a working concentration from 1×10^7 cells/mL to a final volume of 2 mL.

2.4. Statistical analysis

All data were analyzed using the following fixed effect model:

$$Y_{ijk} = \mu + T_i + e_{ijk},$$

in which Y_{ijk} is the response variable, μ is the overall mean, T_i is the fixed effect of treatment (i = 32, 42, 52), and e is the residual error. A normality test based on the Shapiro-Wilk statistics was applied for the residuals. When the normality assumption was not met (P<0.01), a log-transformation was applied. Normal and homoscedastic data were subjected to analysis of variance with means compared by Tukey's test (5%) and non-normal comparison by Kruskal-Wallis test (5%). Data analyses were performed using R software (R Core Team, 2016).

3. Results

Breeder age had an effect on chick weight at pulling (P<0.05), with it being greatest for chicks from 52-wk-old breeders followed by those from the 42-week-old breeders, and then from 32-wk-old breeders (P<0.05). Lower liver weight and higher gizzard + proventriculus weight were observed for newly-hatched chicks from 52-wk-old breeders than for newly-hatched chicks from breeders of the other ages studied (P<0.05). Pancreas weight and the weight of the lymphoid organs were not affected by breeder age (P>0.05). Breeder age had an effect on yolk sac weight (P<0.05), with the highest being observed in chicks from the oldest (52 weeks) breeders (Table 1).

Breeder age had an effect on chick weight at seven days (P<0.05), with it being greatest for chicks from 52-wk-old breeders followed by those from the 42-wk-old breeders and then from 32-wk-old breeders (Table 1). Also, at seven days of age, chicks from 52-wk-old breeders had lower liver relative weight and greater relative weight of gizzard + proventriculus compared with chicks from breeders of the other ages studied (P<0.05). At seven days of age, the relative weight of the pancreas, lymphoid organs, and yolk sac did not differ among chicks from breeders of the different ages (P>0.05). At 14 days of age, only chick body weight was affected by breeder age, with the same differences as observed at seven days (P<0.05).

At pulling, villi height for the duodenum was higher for chicks from 32-wk-old than for those from 52-wk-old breeders (P<0.05), although the difference was not maintained thereafter. Breeder age had no effect on villi height for the duodenum and the jejunum at seven and 14 days (P>0.05) (Table 2).

Table 1 - Effect of breeder age on the growth of organs of the digestive and immune systems

Breeder age (weeks)	Chick weight (g)	Liver (%)	Giz + Pro (%)	Spleen (%)	Bursa (%)	Pancreas (%)	Yolk sac (%)
			Newly-hatched chicks at pulling				
32	41.32c	2.98a	7.47b	0.03	0.1	0.19	8.52c
42	44.19b	2.96a	8.21b	0.02	0.1	0.18	11.38b
52	48.82a	2.66b	9.70a	0.01	0.1	0.17	12.65a
SEM	1.55	0.22	0.56	0.001	0.001	0.001	1.18
P-value	0.019	<0.001	<0.001	0.891	0.911	0.877	<0.001
			7 days				
32	111.33c	4.47a	7.85b	0.07	0.19	0.47	0.19
42	128.76b	4.33a	8.10b	0.07	0.18	0.50	0.15
52	144.90a	3.97b	9.39a	0.07	0.18	0.49	0.30
SEM	8.90	0.55	1.00	0.01	0.02	0.10	0.01
P-value	<0.001	0.004	<0.001	0.238	0.155	0.871	0.344
			14 days				
32	357.23b	3.38	4.98	0.07	0.28	0.43	-
42	360.26b	3.07	4.87	0.07	0.22	0.42	-
52	389.03a	3.41	5.57	0.08	0.22	0.47	-
SEM	11.14	0.90	0.99	0.01	0.01	0.10	-
P-value	<0.001	0.072	0.111	0.899	0.911	0.678	-

Giz + Pro - proventriculus + gizzard; SEM - standard error of means.
a-c - Means followed by different letters in the column differ between each other by Tukey's test (P<0.05).

Table 2 - Effect of breeder age on intestinal mucosal morphometry of newly hatched chicks and chicks of 7 and 14 days

Breeder age (weeks)	Chick age					
	At pulling		7 days		14 days	
	Villus (µm)	Crypt (µm)	Villus (µm)	Crypt (µm)	Villus (µm)	Crypt (µm)
			Duodenum			
32	672.0a	86.2	1,164.2	333.3	1,813.8	518.8
42	569.0b	86.2	1,176.0	324.9	1,759.9	527.8
52	574.0ab	91.5	1,164.1	315.4	1,828.9	515.2
SEM	29.0	3.0	49.0	13.0	48.0	18.4
P-value	0.021	0.098	0.124	0.432	0.872	0.287
			Jejunum			
32	259.0	58.3	417.1	170.3	618.0	271.0
42	245.0	58.3	404.3	161.0	667.9	265.4
52	258.0	64.1	465.0	166.7	645.4	227.3
SEM	29.0	5.0	50.1	6.6	33.3	25.0
P-value	0.199	0.981	0.651	0.221	0.220	0.233

SEM - standard error of means.
a-b - Means followed by different letters in the column differ between each other by Tukey's test (P<0.05).

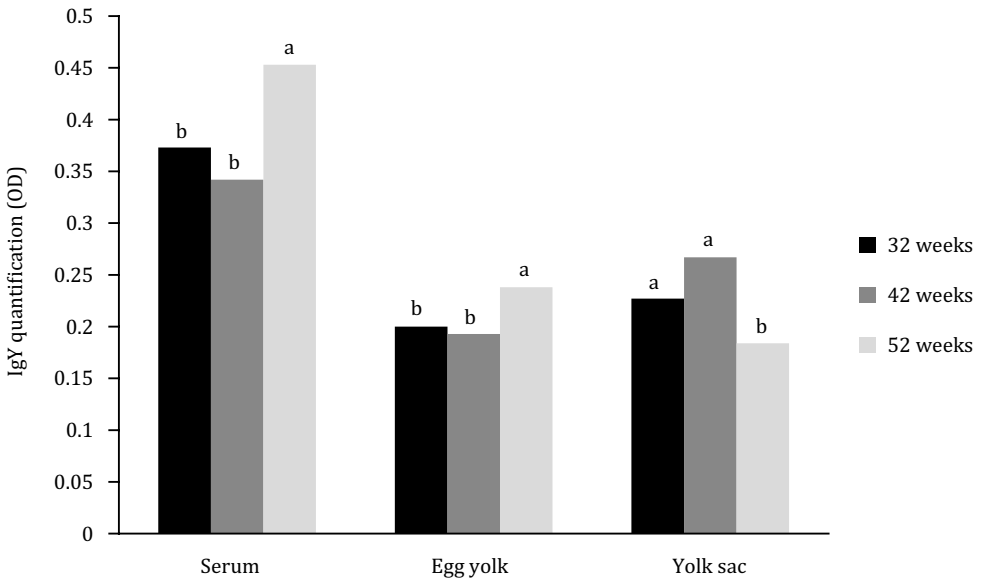
At pulling, there was less B-lymphocytes (%) present in the bursa of Fabricius (P<0.05) for chicks from 32-wk-old breeders compared with chicks from 42 and 52-wk-old breeders, which was also the case for chicks at seven and 14 days (P<0.05) (Table 3). Breeder age did not affect the amount of B-lymphocytes (%) present in the spleen of the chicks (P>0.05).

Table 3 - Effect of breeder age on on B-lymphocyte count of chick lymphoid organs of newly hatched chicks and chicks of 7 and 14 days

Breeder age (weeks)	Bursa of Fabricius			Spleen		
	At pulling	7 days	14 days	At pulling	7 days	14 days
32	8.85b	13.22b	16.13b	7.13	11.60	12.33
42	11.00a	16.52a	18.30a	7.80	11.31	12.00
52	10.00a	15.40a	19.46a	7.99	11.42	11.00
SEM	0.5	0.09	0.29	0.91	0.55	1.00
P-value	<0.001	0.003	<0.001	0.222	0.956	0.871

SEM - standard error of means.
a-b - Means followed by different letters in the column differ between each other by Tukey's test (P<0.05).

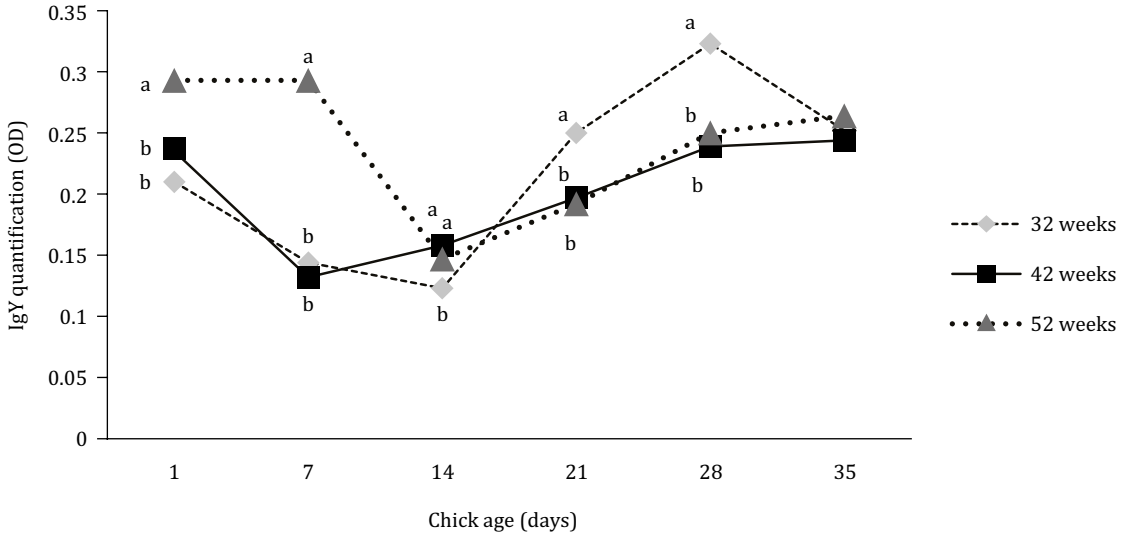
The 52-wk-old breeders had higher OD of serum (i.e., IgY immunoglobulin concentration; Figure 1), obtained from the ELISA, than did the breeders of other ages (P<0.05) and the amount of IgY present in egg yolk was also higher for eggs from 52-wk-old breeders (P<0.05). The amount of IgY present in the yolk sac of chicks was lower for chicks from 52-wk-old breeders in relation to the other breeder ages studied (P<0.05).



Letters denote significant (P<0.05) differences between treatments.

Figure 1 - Effect of breeder age on immunoglobulin Y (IgY) concentration (optic density, OD) obtained from ELISA of the serum of the breeders, egg yolk, and chick yolk sac.

At pulling, chicks from 52-wk-old breeders had higher serum IgY immunoglobulin concentration compared with chicks from 32- and 42-wk-old breeders (P<0.05). Despite the reduction in the amount of IgY up to 28 days, chicks from 52-wk-old breeders continued to have a higher serum IgY immunoglobulin concentration than chicks from breeders of the other ages studied (Figure 2). Breeder age stopped having an effect on the amount of IgY present in chick serum at 35 days (P>0.05).



Letters denote significant (P<0.05) differences among treatments within each chick age.

Figure 2 - Effect of breeder age on immunoglobulin Y (IgY) concentration (optic density, OD) obtained from the ELISA of chick serum.

4. Discussion

The transfer of immunoglobulin from breeders to progeny is a very important process in poultry production since the initial immune status of chicks determines performance during the initial phase. Literature on how breeder age affects this process is scarce. Thus, the results presented here contribute to a better understanding of how immunity is transferred from hens to eggs and, consequently, to chicks. These results also show how breeder age affects the initial development of organs of the digestive and immune systems.

Breeder age was expected to affect the weight of the newly-hatched chicks. Breeder age influences egg size and these two factors are positively correlated such that larger eggs produce heavier and longer chicks (Attia et al., 1994, 1995; O'Dea et al., 2004; Araújo et al., 2016; Iqbal et al., 2017).

The relative weight of gizzard + proventriculus was greater for chicks of 52-wk-old breeders until seven days, after which the effect was lost. According to Fernandes et al. (2014), the weight organs of the digestive system of neonatal chicks are proportional to the age of the breeders and weight of the chicks. However, in the present study, liver weight of chicks from 52-wk-old breeders was lower than that of chicks from the breeders of the other ages, corroborating Johnson-Dahl et al. (2017). This may be explained by the lower demands of the organ during the initial phase of life of larger chicks, since the organ is likely less required during the embryonic phase due to greater beta-oxidation caused by the greater oxygen supply, a result that extends to 14 days of age, perhaps because there is compensation for the liver of younger breeders that develop more. In general, the intestinal villi of chicks were not affected by breeder age, which is in agreement with the findings of El-Sabry et al. (2015); however, Fernandes et al. (2014) found breeder age to have a positive linear effect on chick intestinal development.

Breeder age did not affect the weight of lymphoid organs, which is consistent with reports in the literature (El-Sabry et al., 2013; Fernandes et al., 2014; El-Sabry et al., 2015). However, at pulling, there was less B-lymphocytes in the bursa of Fabricius of chicks from 32-wk-old breeders than for chicks from older breeders, which was a pattern maintained up to 14 days. This result indicates that there is less transfer of immunity by younger breeders, resulting in one-day-old chicks that are more susceptible to challenges during the first days of life. Thus, it can be inferred that older breeders have the ability to more efficiently immunize their progeny up to 14 days. These results are confirmed by the analysis of

the amount of IgY present in the serum of chicks up to 14 days of age, during which the birds basically had only maternal immunoglobulin since they were not vaccinated. It was expected (Leandro et al., 2011b) that ND antibody would be practically undetectable (mean OD = 0.123, 0.147, and 0.150 for 32, 42, and 52-wk-old breeders, respectively) at 14 days of age (Figure 2). Broiler progeny of 32-wk-old breeders had lower IgY.

The reduction in the amount of IgY in broiler progeny observed for all breeder ages up to the 14 days of life is a result of the aging of proteins and their consumption by metabolic processes carried out by chicks during the first weeks of life (Hamal et al., 2006). However, immune system effectiveness depends not only on the possibility of recognizing antigens, but also on the amplification of this response through the induction of lymphocyte proliferation (Pompéia et al., 2000) and maternal nutrition and age (Attia et al., 1994, 1995).

The present study reaffirms the concept of the predominance of IgY immunoglobulin in the bloodstream of breeders, in the vertical transmission of the immunoglobulin to the egg yolk and yolk sac and in the transfer to newly hatched chicks. Younger breeders were expected to have higher serum concentrations of IgY and, consequently, to deposit a greater amount of IgY in egg yolk because, according to Murai et al. (2020), vaccination programs for breeders are more intense up to about 20 weeks and, thus, higher concentrations of these immunoglobulins were expected in 32-wk-old breeders. The last vaccine applied to breeders of the flock used in the present study occurred when the birds were 18 weeks old and was against ND. Still, the fact that the reduced number of follicles and the greater interval between ovulations may explain the greater accumulation of IgY in yolks of eggs from older breeders, it is still possible to raise the hypothesis that the physiology of older breeders attempts to increase nutritional support that will be offered to embryos for development (Attia et al., 1995).

Yolk sac weight was greater for chicks from 52-wk-old breeders, possibly due to the weight of the chick itself (Araújo et al., 2016). Ulmer-Franco et al. (2012) argued that the variation found in the literature regarding the use of the yolk sac is due to the influence of mechanical incubation as well as the waiting time for chicks in hatchers. The greater supply of IgY in the yolk sac of embryos from younger breeders may be a reflection of the greater absorption of these proteins by the larger embryos of the older breeders. According to Moran Jr. (2007), the greater IgY absorption by yolk for embryos from older breeders can be explained by the larger vitellin membrane and greater development of the yolk vascular system. Thus, it is possible to suggest future studies that measure development of the yolk vascular system.

With the knowledge that the egg yolk of young breeders has a lower concentration of IgY, strategies can be developed to increase IgY in the serum of these breeders and, consequently, increase the innate immunity of the newly hatched chicks. According to Araújo et al. (2019), broilers are slaughtered earlier, which increases the importance of the pre-initial rearing phase, and so it would be interesting to promote an improvement in their initial immunity. Interestingly, when observing the response of chicks to the ND vaccine received at 15 days of age, chicks from 32-wk-old breeders were noticed to have a greater capacity for IgY production compared with chicks from 42- and 52-wk-old breeders.

Chicks at 21 days of age from the flock of 32-wk-old breeders were found to obtain a higher concentration of IgY after vaccination. This indicates that chicks from younger breeders are able to react more effectively in the production of antibodies, which, in an infectious focus, would be extremely useful in preventing the proliferation of pathogens. However, despite responding more quickly, at 28 days of age, chicks from the 42-wk-old breeders were able to maintain serum IgY immunoglobulin concentrations more persistently, while at 35 days there was no significant difference among treatments. Thus, it can be said that chicks that have a lower amount of innate IgY are capable of producing a greater amount of endogenous IgY in the bloodstream. With the intensification of the poultry industry and the continuous need to control pathogens, there is a critical need to extend

our understanding of the avian immune system and interventions in the development of immune competence in neonatal chicks (Taha-Abdelaziz et al., 2018).

5. Conclusions

Fifty-two-week-old breeders have a greater amount of circulating immunoglobulin Y and a greater capacity for depositing immunoglobulin Y in the yolk of fertile eggs. Embryos from 52-wk-old breeders absorb more immunoglobulin Y present in the yolk and have greater innate immunity for up to 14 days. Upon receiving a vaccine, chicks from 32-wk-old breeders present a greater vaccine response with a higher concentration of immunoglobulin Y for up to two weeks after the vaccine.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: J.H. Stringhini. Data curation: J.S. Santos. Formal analysis: N.S.M. Leandro and J.H. Stringhini. Funding acquisition: J.H. Stringhini. Investigation: J.S. Santos, M.J.R. Lacerda, M.A. Andrade and J.H. Stringhini. Project administration: J.H. Stringhini. Resources: M.B. Café, N.S.M. Leandro and J.H. Stringhini. Supervision: M.A. Andrade and J.H. Stringhini. Visualization: I.C.S. Araújo, M.B. Café and N.S.M. Leandro. Writing – original draft: I.C.S. Araújo, M.A. Andrade, N.S.M. Leandro and J.H. Stringhini. Writing – review & editing: I.C.S. Araújo and J.H. Stringhini.

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References

Apanius, V. 1998. Ontogeny of immune function. p.203-222. In: Avian growth and development: Evolution within the altricial-precocial spectrum. Starck, J. M. and Ricklefs, R. E., eds. Oxford University Press, New York, NY.

Araújo, I. C. S.; Leandro, N. S. M.; Mesquita, M. A.; Café, M. B.; Mello, H. H. C. and Gonzales, E. 2016. Effect of incubator type and broiler breeder age on hatchability and chick quality. Brazilian Journal Poultry Science 18(Spe):17-25. https://doi.org/10.1590/1806-9061-2015-0146

Araújo, I. C. S.; Café, M. B.; Noleto, R. A.; Martins, J. M. S.; Ulhoa, C. J.; Guareshi, G. C.; Reis, M. M. and Leandro, N. S. M. 2019. Effect of vitamin E in ovo feeding to broiler embryos on hatchability, chick quality, oxidative state, and performance. Poultry Science 98:3652-3661. https://doi.org/10.3382/ps/pey439

Araújo, I. C.; Café, M. B.; Mesquita, M. A.; Caiado, B. N.; Faria, A. M.; Mello, H. H. C.; Stringhini, J. H. and Leandro, N. S. M. 2020. Effect of a commercial product containing canthaxanthin for in ovo feeding to broiler embryos on hatchability, chick quality, oxidation status, and performance. Poultry Science 99:5598-5606. https://doi.org/10.1016/j.psj.2020.08.044

Attia, Y. A.; Burke, W. H. and Yamani, K. A. 1994. Response of broiler breeder hens to forced molting by hormonal and dietary manipulations. Poultry Science 73:245-258. https://doi.org/10.3382/ps.0730245

Attia, Y. A.; Burke, W. H.; Yamani, K. A. and Jensen, L. S. 1995. Daily energy allotments and performance of broiler breeders. 2. Females. Poultry Science 74:261-270. https://doi.org/10.3382/ps.0740261

Cardeal, P. C.; Araújo, I. C. S.; Vaz, D. P.; Abreu, A. R. C.; Melo, E. F.; Saldanha, M. M.; Pompeu, M. A. and Lara, L. J. C. 2022. Short communication: Effects of breeder age and pre-placement feed on IgY concentration in egg yolk and chick serum. Journal of Animal Physiology and Animal Nutrition 106:561-565. https://doi.org/10.1111/jpn.13604

El-Sabry, M. I.; Yalçin, S. and Turgay-İzzetoğlu, G. 2013. Interaction between breeder age and hatching time affects intestine development and broiler performance. Livestock Science 157:612-617. https://doi.org/10.1016/j.livsci.2013.07.012

El-Sabry, M. I.; Yalçin, S. and Turgay-İzzetoğlu, G. 2015. Effect of breeder age and lighting regimen on growth performance, organ weights, villus development, and *bursa of fabricius* histological structure in broiler chickens. Czech Journal of Animal Science 60:116-122. https://doi.org/10.17221/8076-CJAS

Fernandes, J. I. M.; Contini, J. P.; Scapini, L. B.; Gurski, T. J.; Esser, A. F. G. and Santos, A. L. 2014. Influência da idade da matriz sobre a biometria de órgãos e a morfometria da mucosa do intestino delgado dos pintos à eclosão. Semina: Ciências Agrárias 35:1083-1090. https://doi.org/10.5433/1679-0359.2014v35n2p1083

Hamal, K. R.; Burgess, S. C.; Pevzner, I. Y. and Erf, G. F. 2006. Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. Poultry Science 85:1364-1372. https://doi.org/10.1093/ps/85.8.1364

Hubbard SAS Breeder Company. 2011. Hubbard JV Management guide (manuals).

Iqbal, J.; Mukhtar, N.; Rehman, Z. U.; Khan, S. H.; Ahmad, T.; Anjum, M. S.; Pasha, R. H. and Umar, S. 2017. Effects of egg weight on the egg quality, chick quality, and broiler performance at the later stages of production (week 60) in broiler breeders. Journal of Applied Poultry Research 26:183-191. https://doi.org/10.3382/japr/pfw061

Johnson-Dahl, M. L.; Zuidhof, M. J. and Korver, D. R. 2017. The effect of maternal canthaxanthin supplementation and hen age on breeder performance, early chick traits, and indices of innate immune function. Poultry Science 96:634-646. https://doi.org/10.3382/ps/pew293

Leandro, N. M.; Ali, R.; Koci, M.; Moraes, V.; Malheiros, R. D.; Wineland, M. J. and Oviedo-Rondón, E. O. 2011a. Effects of broiler breeder genetic, diet type, and feeding program on maternal antibody transfer and development of lymphoid tissues in chicken progeny. Journal Applied Poultry Research 20:474-484. https://doi.org/10.3382/japr.2010-00268

Leandro, N. M.; Ali, R.; Koci, M.; Moraes, V.; Eusebio-Balcazar, P. E.; Jornigan, J.; Malheiros, R. D.; Wineland, M. J.; Brake, J. and Oviedo-Rondón, E. O. 2011b. Maternal antibody transfer to broiler progeny varies among strains and is affected by grain source and cage density. Poultry Science 90:2730-2739. https://doi.org/10.3382/ps.2011-01573

Luna, L. G. 1968. Manual of histological staining methods of the Armed Forces Institute of Pathology. 3rd ed. McGraw-Hill Book Co., New York, NY.

Machado, J. P.; Mesquita, M. A.; Café, M. B.; Assis, S. D.; Veríssimo, S.; Santos, R. R.; Leandro, N. S. M. and Araújo, I. C. S. 2020. Effects of breeder age on embryonic development, hatching results, chick quality, and growing performance of the slow-growing genotype. Poultry Science 99:6697-6704. https://doi.org/10.1016/j.psj.2020.09.008

Moran Jr., E. T. 2007 Nutrition of the developing embryo and hatchling. Poultry Science 86:1043-1049. https://doi.org/10.1093/ps/86.5.1043

Moreira Filho, A. L. B.; Ferket, P. R.; Malheiros, R. D.; Oliveira, C. J. B.; Aristimunha, P. C.; Wilsmann, D. E. and Givisiez, P. E. N. 2019. Enrichment of the amnion with threonine in chicken embryos affects the small intestine development, ileal gene expression and performance of broilers between 1 and 21 days of age. Poultry Science 98:1363-1370. https://doi.org/10.3382/ps/pey461

Murai, A.; Hamano, T.; Kakiuchi, M.; Kobayashi, M. and Horio, F. 2020. Evaluation of a receptor gene responsible for maternal blood IgY transfer into egg yolks using bursectomized IgY-depleted chickens. Poultry Science 99:1914-1920. https://doi.org/10.1016/j.psj.2019.11.045

Narinç, D. and Aydemir, E. 2021. Chick quality: an overview of measurement techniques and influencing factors. World's Poultry Science Journal 77:313-329. https://doi.org/10.1080/00439339.2021.1892469

Nangsuay, A.; Meijerhof, R.; van den Anker, I.; Heetkamp, M. J. W.; Morita, V. S.; Kemp, B. and van den Brand, H. 2016. Effects of breeder age, broiler strain, and eggshell temperature on development and physiological status of embryos and hatchlings. Poultry Science 95:1666-1679. https://doi.org/10.3382/ps/pew080

O'Dea, E. E.; Fasenko, G. M.; Feddes, J. J. R.; Robinson, F. E.; Segura, J. C.; Ouellette, C. A. and van Middelkoop, J. H. 2004. Investigating the eggshell conductance and embryonic metabolism of modern and unselected domestic avian genetic strains at two flock ages. Poultry Science 83:2059-2070. https://doi.org/10.1093/ps/83.12.2059

Peralta, M. F.; Magnoli, A.; Alustiza, F.; Nilson, A.; Miazzo, R. and Vivas, A. 2017. Gut-associated lymphoid tissue: A key tissue inside the mucosal immune system of hens immunized with *Escherichia coli* F_4. Frontiers in Immunology 8:568. https://doi.org/10.3389/fimmu.2017.00568

Pompéia, C.; Lopes, L. R.; Miyasaka, C. K.; Procópio, J.; Sannomiya, P. and Curi, R. 2000. Effect of fatty acids on leukocyte function. Brazilian Journal of Medical and Biological Research 33:1255-1268. https://doi.org/10.1590/S0100-879X2000001100001

R Core Team. 2016. A language and environment for statistical computing. Version 3.4.4. R Foundation for Statistical Computing, Vienna.

Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T. and Euclides, R. F. 2011. Tabelas brasileiras para aves e suínos - Composição de alimentos e exigências nutricionais. 3.ed. Imprensa Universitária - Universidade Federal de Viçosa, Viçosa, MG.

Sun, H.; Chen, S.; Cai, X.; Xu, G. and Qu, L. 2013. Correlation analysis of the total IgY level in hen serum, egg yolk and offspring serum. Journal of Animal Science and Biotechnology 4:10. https://doi.org/10.1186/2049-1891-4-10

Taha-Abdelaziz, K.; Hodgins, D. C.; Lammer, A.; Alkie, T. N. and Sharif, S. 2018. Effects of early feeding and dietary interventions on development of lymphoid organs and immune competence in neonatal chickens: A review. Veterinary Immunology and Immunopathology 201:1-11. https://doi.org/10.1016/j.vetimm.2018.05.001

Ulmer-Franco, A. M.; Fasenko, G. M. and Christopher, E. E. O. 2010. Hatching egg characteristics, chick quality, and broiler performance at 2 breeder flock ages and from 3 egg weights. Poultry Science 89:2735-2742. https://doi.org/10.3382/ps.2009-00403

Ulmer-Franco, A. M.; Cherian, G.; Quezada, N.; Fasenko, G. M. and McMullen, L. M. 2012. Hatching egg and newly hatched chick yolk sac total IgY content at 3 broiler breeder flock ages. Poultry Science 91:758-764.

van der Wagt, I.; de Jong, I. C.; Mitchell, M. A.; Molenaar, R. and van den Brand, H. 2020. A review on yolk sac utilization in poultry. Poultry Science 99:2161-2175. https://doi.org/10.1016/j.psj.2019.11.041