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Comparative Study on Genetic Variations in Maternal Antibody (IgY) Transfer from Dam to Egg-yolk in Different Meat Lines of Chickens

Choudhury Nafisa Binte Hussain[a]*, Mohammad Chhiddikur Rahman[b]

[a]*MS Research Student, Bangladesh Agricultural University, Mymensingh-2002, Bangladesh*
[b]*Scientific Officer, Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh*
[a]*Email: nafisa.ps@gmail.com,* [b]*Email: siddiquer07@gmail.com*

Abstract

The amount of maternal antibody transferred to the egg and the amount taken up by the developing chicks are important parameters that may greatly influence the health and survival of the chicks. Thus, the study of these parameters might provide a new likelihood in improving the livability of chicks. Considering the short life span of broiler chicks from hatch to slaughter, the importance of maternal antibodies is highly relevant. Therefore, the study was conducted to compare genetic variations in transfer of maternal immunoglobulin Y (IgY) through egg yolk in different broiler sire and dam lines namely: Male Line White (MLW), Male Line Colour (MLC), Male Line White 2 (MLW2), Female Line White (FLW), Female Line Colour (FLC) and a two-way cross of MLW male and FLW female (MLW x FLW). In total, 42 freshly laid eggs were collected from apparently healthy hens; 7 each from six different lines of chicken. Egg yolk IgY of the collected eggs was isolated by polyethylene glycol (PEG) precipitation method with slight modifications. Furthermore, the purity of the isolated IgY was examined by the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The egg yolk IgY levels were significantly ($p<0.001$) varied among the studied lines of chicken. The total amount of IgY per milliliter of yolk was the highest in FLW (30.904 ± 7.621) and the lowest in MLW (16.753 ± 5.282). However, the calculated total amount of yolk IgY (mg/egg) contained by the entire egg yolk was the highest (392.030 ± 136.185) in MLW2 and was the lowest (206.015 ± 61.058) in FLC and it was varied between 206.015 and 392.030 in the experimental lines of chicken. This study concluded that genotype of chickens has significant effect on transferability of maternal IgY through egg yolk in different meat lines of chickens.

Keywords: maternal antibody; immunoglobulin Y; egg-yolk; broiler sire lines; broiler dam lines.

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* Corresponding author.

1. Introduction

In avian species, the transfer of maternal antibodies across the placenta, colostrum or eggs from mother to offspring play a key role to protect the newborns from diseases and might have a major impact on responses to infection and fitness. For the phenotypic variations in host phenotype, maternally transferred antibodies are considered as an important source. It also influences the host susceptibility and tolerance to infection across generations [1]. Maternal Immunoglobulin Y (IgY) is a major serum antibody in birds, reptiles and amphibians, and it is transferred from serum to egg yolk to confer passive immunity to their embryos and offspring [2-5]. In chicken, the IgY is found in high concentrations in egg yolk. Birds concentrate immunoglobulins into their egg yolks to protect the offspring. Very young chicks are susceptible to many pathogens during the first few weeks of age because their immune system is not fully developed; hence, maternal antibodies are the primary means of antigen-specific protection [6].Biologically active IgY antibodies are transmitted vertically from serum to egg yolk. The birds have a unique IgY transfer system from the maternal blood circulation to embryonic circulation. The transfer of IgY from the dam to the offspring takes place in a 2-step process. In the first step, IgY is taken up into the egg yolk by the IgY receptors on the ovarian follicle from the dam's blood [7-10]. In the second step, IgY are transferred from the egg yolk to the offspring via the embryonic circulation [11].

Maternal antibodies influence the immune system of developing chicks by enhancing the magnitude of antibody response to initial challenges [12]. In birds, innate immune mechanisms seem to be fully functional in the neonate but optimal adaptive immune responses only develop during the first few weeks after hatching [13]. In newly hatched chicks, which are relatively immune-incompetent, acquire an effective humoral immunity against some common avian pathogens by absorbing IgY from the egg yolk [14]. At the early post-hatching period of chicks, immunoglobulin (Ig)-secreting B cells have been detected in circulation after 6 days of post-hatching, meaning that during the first days of post-hatching period, humoral immunity is totally dependent on the maternally transferred Igs [15]. The percentage of total IgY transfer from dams to offspring was found to be 27 to 30% [6].

In broiler chicken industry, enteric diseases are an important concern due to loss of productivity, increased mortality, and the associated contamination of poultry products for human consumption (human food safety) [16]. The amount of maternal antibody transferred to the egg and the amount taken up by the developing chicks are important parameters that may greatly influence the health and survival of the chicks. Considering the short life span of broiler chicks from hatch to slaughter, the importance of maternal antibodies is highly relevant. Transmission of maternal antibody is influenced by genes expressed in both females and offspring and it would be necessary to understand the genetic correlations between dams and offspring in order to determine breed excellency [12]. The genotype of hen might have significant influence on its ability to transfer IgY into their eggs [17, 12]. With a view to develop locally adaptable broiler sire and dam lines, a long term selection program has been initiated by the Department of Poultry Science, Bangladesh Agricultural University, Mymensingh. Most of the developed sire and dam lines have passed four generations of selection and the line crossed broilers showed significantly lower mortality compared to their commercial counterparts. Therefore, we aimed to conduct this study to estimate and compare the genetic variations of maternal antibody (IgY) transfer in those dam lines namely: BAU Bro-white and BAU Bro-colour broilers (NB: BAU= Bangladesh Agricultural

University). Considering the performances of birds, we hypothesized that there was no significant differences on the transferability of IgY from dams to offspring in the studied lines of chicken.

2. Materials and Methods

2.1. Statement of the experiment

This study was conducted at Bangladesh Agricultural University Poultry Farm and Poultry Biotechnology and Genomics Laboratory, Department of Poultry Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.

2.2. Experimental populations

Apparently healthy hens (35-37 weeks of age) from previously developed lines namely: Male Line White (MLW), Male Line Color (MLC), Male Line White 2 (MLW2), Female Line White (FLW), Female Line Color (FLC), and a 2-way cross of MLW male and FLW female (MLW x FLW) were used in this study. These lines of chicken were developed at BAU Poultry farm by index selection for rapid early growth and better egg production. The MLW, MLC and MLW2 were selected for early rapid growth, use as a male line for producing broiler chicks, whereas FLW and FLC selected for increased egg production and moderate early growth. All experimental lines were reared in an open sided house. Feeding, lighting, medication, vaccination and other management were similar in the studied lines.

2.3. Collection of samples

About 42 freshly laid eggs were collected from six different lines of chicken (7 numbers from each line). Eggs were collected on the same day, same time from the different lines over the course of experiment. After collection, eggs were immediately transferred to the laboratory for further processing.

2.4. Isolation of IgY

There are several methods of IgY isolation depending on the type of starting materials and laboratory facilities [18]. In this study Polyethylene Glycol (PEG) precipitation method as described by Pauly and his colleagues [19] was used with slight modifications.

2.4.1. Yolk separation

At first, each egg was weighted and then eggshell was cracked carefully. The yolk was transferred to a modified "yolk spoon" in order to remove as much egg white as possible without piercing the vitelline membrane. Then the yolk was transferred to a filter paper and rolled to remove remaining egg white, and the vitelline membrane was punctured with a pipette tip. The yolk was poured into 50 mL tube and the yolk volume was recorded.

2.4.2. Separation of IgY

PBS (Phosphate Buffer Saline) taken twice of the egg yolk volume was mixed with the yolk, thereafter 3.5%

PEG6000 (in gram, pulverized) of the total volume was added and vortexed, followed by 10 min rolling on hand. This rolling process separates the suspension in two phases. One phase consists of "yolk solids and fatty substances" [20] and a watery phase containing IgY and other proteins. The tubes were centrifuged in a pre-cooled centrifuge (4°C) for 30 min at 4500 rpm (HARRIER 18/80, UK). The supernatant was poured through a folded filter paper and transferred to a new tube. Then 8.5 % PEG 6000 in gram (calculated according to the new volume) was added to the tube, vortexed and rolled for 10 min. The tubes were centrifuged again in a pre-cooled centrifuge (4°C) for 30 min at 4500 rpm. The supernatant was discarded and the resulting pellet was carefully dissolved in 1 ml PBS by means of a vortexer. PBS was added to a final volume of 10 ml. The solution was mixed with 12 % PEG 6000 (w/v, 1.2) and vortexed and rolled for 10 minutes. Again the tubes were centrifuged at 4°C for 30 min at 4500rpm and the supernatant was discarded. The pellet was carefully dissolved in 2 mL PBS. Finally, the isolated IgY samples were stored at -20°C until further processing.

2.5. SDS-PAGE of IgY

To determine the purity of IgY in the egg yolk final product, Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions was performed as described by Laemmeli [21]. SDS gel electrophoresis of IgY was done according to manufactures recommendations (Bio Craft, Ever Seiko Corporation, Tokyo, Japan). It was performed in a slab type vertical gel system. 10µL of each sample was placed in each well on one of the slab gels. Migration was performed at 20 mA per gel (or 40 mA totals) and it was taken approximately 1 hour to move down the slab. After migration, gels were stained with Coomassie Brilliant Blue R-250. It was used to visualize the protein bands. When protein bands were visualized, then the gels were carefully washed in distilled water and placed on the scanner to capture the image.

2.6. Determination of IgY concentration in egg yolk

The IgY content (mg/mL) of the samples is measured photometrically at 280 nm (1:40 dilution with phosphate buffer saline) and calculated according to the Lamber-Beer law with and extinction coefficient of 1.33 for IgY.

2.7. Statistical analysis

Collected data were analyzed by using a linear mixed model implemented in R (R Core Team, 2014) [22]. Effects of line, egg weight, yolk volume, yolk weight and batch of IgY separation were treated as fixed effects. The covariates and their interactions that had significant effects at the nominal 5% level were included in the final model for comparisons of IgY levels among the lines of chickens. Line differences of IgY levels in egg yolk were determined by Tukey HSD post hoc test.

3. Results

3.1. Egg and yolk parameters of different lines of chickens

Egg and yolk parameters of different lines of chickens in between 35 to 37 weeks of age (Experimental period) are shown in Table 1. The weights of egg, yolk, yolk-egg ratio, yolk volume and yolk % were significantly

different among the studied lines of chicken. The egg weight of MLW (65.97 ± 4.53) was the highest among the lines, whereas it was the lowest in FLC (47.31 ± 2.84). The egg weight was almost similar between MLW, MLW2 and MLW × FLW (P>0.05). On the other hand, the egg weight of FLW and MLC were similar (P>0.05) with FLC as determined by Tukey HSD. In contrary, the yolk weight and yolk volume were significantly different among the lines. The yolk weight was the highest in MLW2 (17.40 ± 3.11) and the lowest in FLW (10.72 ± 1.15). Similar trend was also observed in yolk volume of different lines of chicken. MLW2 showed the highest yolk volume (17.25 ± 2.77) and FLW showed the lowest yolk volume (10.50 ± 0.89) among the lines. Since the egg and yolk weights were significantly different among the lines and the trend was dissimilar, the ratio of yolk weight to egg weight was of MLW, MLC, MLW2, FLW, FLC and MLW × FLW calculated. The highest yolk to egg weight ratio was found in MLW2 (0.29 ± 0.02) and the lowest in FLW (0.21 ± 0.03), whereas MLW and MLC showed intermediate values. The findings of the study also suggests that there is a significant correlation among the yolk weight(g), yolk volume (mL) and yolk-egg weight ratio in the studied lines of chicken.

Table 1: Egg and yolk parameters of different lines of chicken during experimental period

Chicken lines	Number of samples	Egg weight (g)	Yolk weight (g)	Yolk and egg weight ratio	Yolk volume (mL)	Yolk(%, w/w)
MLW	7	65.97 ± 4.53^{a}	16.25 ± 1.03^{ab}	0.25 ± 0.01^{bc}	16.13 ± 1.14^{ab}	24.65 ± 1.02^{bc}
MLC	7	47.44 ± 0.91^{b}	12.83 ± 0.33^{cd}	0.27 ± 0.01^{ab}	12.63 ± 0.22^{cd}	27.04 ± 0.57^{ab}
MLW2	7	59.78 ± 7.93^{a}	17.40 ± 3.11^{a}	0.29 ± 0.02^{a}	17.25 ± 2.77^{a}	28.93 ± 1.63^{a}
FLW	7	48.94 ± 1.49^{b}	10.72 ± 1.15^{d}	0.21 ± 0.03^{c}	10.50 ± 0.89^{d}	21.91 ± 2.30^{c}
FLC	7	47.31 ± 2.84^{b}	11.10 ± 0.42^{d}	0.24 ± 0.02^{c}	11.13 ± 0.49^{d}	23.55 ± 2.08^{c}
MLW × FLW	7	62.55 ± 3.07^{a}	14.13 ± 1.27^{bc}	0.23 ± 0.01^{c}	14.20 ± 1.60^{bc}	22.55 ± 1.14^{c}
P value		4.383×10^{-05}	1.504×10^{-05}	5.78×10^{-05}	1.038×10^{-05}	5.775×10^{-05}

Data are mean ± standard deviation. Values with common superscript within a column do not differ significantly ($P \geq 0.05$)

3.2. Isolation of immunoglobulin Y (IgY)

In this study, the yolk IgY was separated by Polyethylene Glycol (PEG) precipitation method. It is an excellent method to precipitate a specific protein from a complex mixture of proteins [19].The purity of the isolated IgY was examined by the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). A representative SDS-PAGE of individual IgY preparation from different eggs under reducing conditions is shown in Figure 1. It is evident that both heavy and light chain IgY fragments is present in the isolated total IgY without the existence of other protein impurities. The presence of heavy chain in the gel electrophoresis pattern of antibody is an

indication of appropriate extraction method [23]. A particularly efficient method consists of two successive precipitations in PEG, using 3.5% PEG to remove fatty substances, and then 12% PEG to precipitate the IgY. An improvement of this method incorporates an emulsification step, adding one volume of chloroform to one volume of egg yolk, rather than using the 3.5% PEG precipitation step [20, 24].Therefore, the purity of IgY samples obtained by PEG-precipitation in this study was acceptable and may be worked well in different immunological assays.

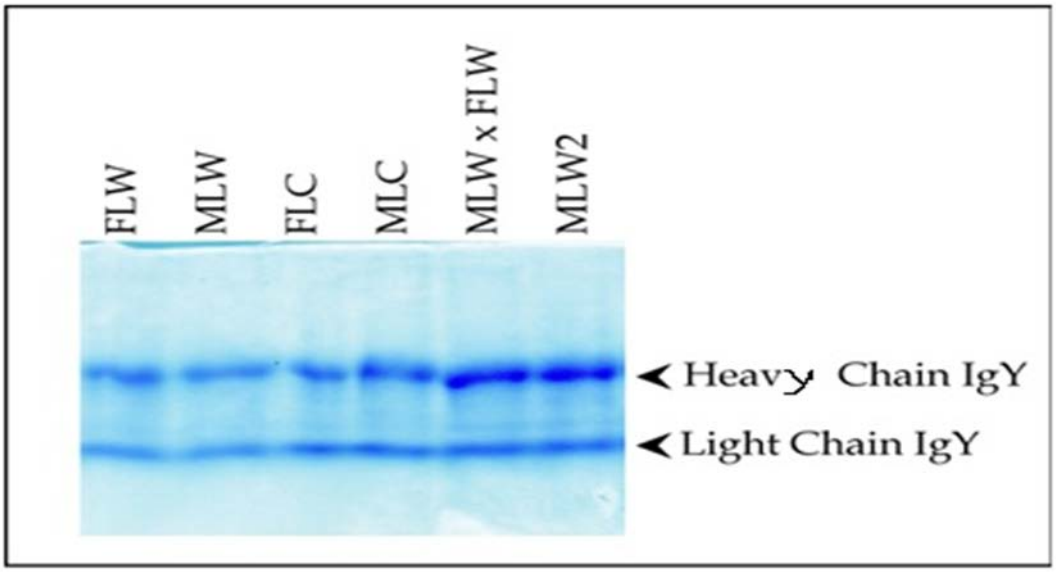

Figure 1: SDS-PAGE of IgY of different lines of chicken. FLW: Female Line White, MLW: Male Line White, FLC: Female Line Color, MLC: Male Line Color, MLW2: Male Line White 2, MLW x FLW: Two way cross of MLW male and FLW female.

3.3. Effect of genotype on egg yolk IgY level

Since the egg weight, yolk weight and volume were significantly different among lines (Table 1), egg yolk IgY content was expressed in three different ways such as: milligrams of IgY per gram of egg (mg/g egg), milligrams of IgY per milliliter of yolk (mg/mL yolk) and milligrams of IgY per egg (mg/egg) to know the effect of initial variations of egg weight and yolk weight on yolk IgY content among lines (Table 3). There was a significant correlation among the egg parameters (egg weight, yolk weight and total IgY) of studied lines of chickens (Table 2). Here, MLC showed the highest (6.822 ± 2.309) yolk IgY (mg/g egg), and MLW showed the lowest (4.034 ± 1.065) among the lines. The egg weight (g) of MLC (47.44 ± 0.91) was lower than the egg weight of MLW (65.97 ± 4.53). The yolk-egg ratio and yolk % of MLC was higher than MLW (table1). Therefore, there was no significant correlation between the weight of egg and the amount of IgY per gram of egg in the studied lines, may be it was related with the yolk-egg ratio and yolk % of the chickens. The total amount of IgY per milliliter of yolk was the highest in FLW (30.904 ± 7.621) and the lowest in MLW (16.753 ± 5.282), whereas, FLC and MLW × FLW showed intermediate values. In the studied lines, the lowest yolk volume (mL) was found in FLW and MLW showed the higher yolk volume (mL) (table1).There was no significant correlation between the yolk volume (mL) and the amount of yolk IgY (mg/mL yolk) in the studied

lines of chickens. However, the total amount of yolk IgY (mg/egg) contained by the entire egg yolk was the highest in MLW2 (392.030 ± 136.185) and lowest in FLC (206.015 ± 61.058) and it was varied from 206.015 to 392.030 in the studied lines. The egg and yolk parameters (egg weight, yolk weight, yolk volume, yolk-egg ratio, yolk %) of MLW2 was comparatively higher within the lines, whereas FLC showed lower values of these parameters (table1). The findings of this study suggest that the egg and yolk parameters of the studied lines have significant influence on the total amount of IgY (mg/egg) of the entire egg and it was varied among the lines of chickens.

Table 2: Correlation between different egg parameters of studied lines of chicken

Parameters	Egg weight	Yolk weight	Total IgY
Egg weight	-	***	*
Yolk weight	0.83	-	*
Total IgY	0.46	0.47	-

Data in the below diagonal: correlation between parameters, above diagonal: Significance level of respective correlation. *: $p < 0.05$ to $p = 0.01$; ***: $p < 0.001$.

Table 3: Egg yolk IgY content of different lines of chicken

Chicken lines	Absorbance[1]	IgY (mg/g egg)	IgY(mg/mL yolk)	Total IgY (mg/egg)
MLW	0.448 ± 0.149^{ab}	4.034 ± 1.065^{b}	16.753 ± 5.282^{c}	269.624 ± 89.606^{a}
MLC	0.539 ± 0.188^{b}	6.822 ± 2.309^{a}	25.651 ± 8.819^{abc}	324.511 ± 113.572^{a}
MLW2	0.625 ± 0.226^{a}	6.454 ± 1.594^{a}	22.532 ± 5.875^{abc}	392.030 ± 136.185^{a}
FLW	0.535 ± 0.126^{a}	6.580 ± 1.538^{a}	30.904 ± 7.621^{a}	322.165 ± 75.966^{a}
FLC	0.343 ± 0.102^{a}	4.345 ± 1.206^{b}	18.590 ± 5.710^{bc}	206.015 ± 61.058^{b}
MLW × FLW	0.652 ± 0.040^{a}	5.891 ± 0.359^{ab}	26.197 ± 2.998^{ab}	368.120 ± 24.071^{a}
P value	1.174×10^{-03}	2.596×10^{-03}	9.403×10^{-04}	1.174×10^{-03}

Note: [1]280 nm, 40 times diluted. Data are mean ± standard deviation. Values with common superscript within a column do not differ significantly ($P \geq 0.05$).

4. Discussion

In chickens, it has been well established that IgY is the antibody isotype which is transferred from dam to the offspring through the egg [6]. It is predominantly systemic rather than a secretory antibody but can also be found in duodenal contents, tracheal washings and seminal plasma [25], besides the egg yolk. Since, yolk antibody levels are known to be related to serum levels, the use of egg yolk instead of blood serum becomes an attractive alternative for monitoring the flock's susceptibility to infectious diseases. By using egg yolk instead of blood serum for maternal antibody determination, more hens can be sampled and monitoring of flocks becomes more economically feasible [12].

For the separation of high purity and intact IgY from egg yolk, lipids and lipoproteins are the major barrier. Different researchers have proposed several methods for efficient IgY separation [18]. Chang and his colleagues [26] reported that addition of 0.1% of λ-carrageenan was effective in removing lipoproteins from the water extract of egg yolk at pH 5. Asemota and his colleagues [27] purified IgY from the yolk of avian egg using trichloroacetic acid (TCA) to separate egg yolk proteins, mainly IgY. Almeida and his colleagues [28] used PEG 6000 (w/v) and ammonium sulfate to extract IgY from yolk. Commonly and most frequently used procedures involve protein precipitation with ammonium sulphate, dextran sulphate or polyethyleneglycol (PEG); separation by ion exchange chromatography is also used. Akita and Nakai [29] compared four different methods of IgY separation, namely polyethylene glycol (PEG), dextran sulphate (DS), xanthan gum (Xan) and water dilution (WD) in terms of yield, purity, ease of use, potential scaling up and immune activity of IgY. They have shown that purification methods had no adverse effect on the immune activities of IgY; however, the yield of IgY may vary depending on the methods. In some cases, depending on the final application, a simple water extract of IgY is sufficient to achieve good results [30]. There is, in fact, a surplus of effective extraction methods [30], which could be problematic in that potential users of IgY technology may have no rational basis for choosing one method rather than another. In practice, the choice of a specific extraction procedure is usually influenced by the intended application of the antibody, as well as by the experience of the laboratory concerned [31]. Pauly and his colleagues [19] described a protocol of total IgY extraction from egg yolk by means of polyethylene glycol (PEG) precipitation procedure. The method involves two important steps. The first one is the removal of lipids and the second one is the precipitation of total IgY from the supernatant of step one. After dialysis against a buffer Phosphate Buffer Solution (PBS), the IgY-extract can be stored at -20°C for more than a year. The purity of the extracted IgY by this method is around 80%, the total IgY per egg varies from 40-80 mg, depending on the age of the laying hen.

The amount of IgY transported is independent of egg size and known to be proportional to the maternal serum IgY concentration [8]. Carlander and his colleagues [32] did not see any correlation between IgY concentration and egg laying. This study shows that the IgY concentration in the egg yolk is correlated with genotype of birds and by genetic selection it would be possible to increase the IgY content. The total egg yolk IgY determined in this study is greater than the 5.2 mg/mL and 42 to 105 mg/yolk reported by Carlander and his colleagues [32] in 50-wk-old Single-Comb White Leghorn (SCWL) hens. It was also greater than the 1.15 and 2.26 mg/mL and 22.5 and 43.9 mg/yolk reported by Hamal and his colleagues [6] in 39-wk-old hens of 2 meat lines. Ulmer-Franco and his colleagues [33] studied with 3 different (32, 40 and 55 weeks) ages of birds. They reported that

total calculated amount of IgY contained by the entire egg yolk increased with flock age ($P < 0.0001$) accordingly with the increase of egg yolk weight. They found 134.4 mg of IgY in 32-wk-old breeders, 178.0 mg at 40 wk of age, and 248.1 mg of IgY per egg yolk at 55 wk of age (SEM ± 7.75). In this study, calculated amount of total egg yolk IgY (mg/egg) is greater than the reported value by Ulmer-Franco and his colleagues [33] in 32-wk-old breeders. The differences observed between the results could be a consequence of the different IgY extraction and quantification methods used in each study [34]. Furthermore, IgY is the main antibody which is transferred to the egg yolk with IgA and IgM. IgA and IgM are present in minimal quantities with IgY [35]. It is therefore considered that the small amounts of IgA and IgM that have been present in the egg yolk don't have any influence of the results obtain in an experiment [33].

In a study comparing 3 strains of laying-type birds (Single Comb White Leghorn (SCWL), Rhode Island Red, and their cross), Carlander and his colleagues [17] reported different levels of IgY/mL of egg yolk among strains, although egg yolk weight and bird age were not indicated in that study. Li and his colleagues [35] compared egg yolk IgY levels after immunization of 35-wk-old SCWL and Rhode Island Red hens. They reported no differences in the percentage of IgY/mL of yolk between strains, concluding that egg yolk weight was a deciding factor on the total egg yolk IgY content [35]. In the present study, we found that there was a significant correlation between the weight of egg yolk and the total egg yolk IgY content in the studied six lines of chicken. The weight of egg yolk (g) was the highest in MLW2 (17.40 ± 3.11) and the amount of total egg yolk IgY (mg/egg) was also highest in MLW2 (392.030 ± 136.185). The yolk weight (g) of FLC showed the lower value (11.10 ± 0.42) as compared to other lines and the total amount of IgY (mg/egg) was also lowest (206.015 ± 61.058) in FLC among the studied lines of chicken. Results from the present experiment in different meat-type of birds are in agreement with that the weight of egg yolk influences the total egg yolk IgY content.

Gadde and his colleagues [36] studied about the production, structure and properties of IgY and reported that the concentration of IgY in egg yolk is always higher (~8– 25 mg mL^{-1}) and selectively transported from the maternal circulation to the yolk in the ovarian follicle. The findings of the current study are agreed well with Gadde and his colleagues [35]. The amount of IgY (mg/mL yolk) was the lowest in MLW (16.753 ± 5.282) and the highest in FLW (30.904 ± 7.621), other lines of chicken also showed the intermediate values. The amount of IgY (mg/mL yolk) was higher than the reported value due to the genotypic variation and the age of the birds of this experiment. Genetic variations in number of egg, egg weight and yolk weight in the studied lines of chicken could also explain the differences observed with Carlander and his colleagues [32]. In this study, the egg weight, yolk weight and the total IgY concentration per egg, per gram of egg and per mL of yolk were found to be significantly different due to their different selection strategies and origin of the lines. The base populations of male lines (MLW, MLC, MLW2) were originated from highly heterozygous synthetic birds and were selected for rapid early growth. Here, MLW, MLC and MLW2 showed the highest egg weight, IgY (mg/g egg) and total IgY (mg/egg) respectively. Whereas FLW was originated from dual purpose pure breed, FLC was a cross between Rhode Island Red and Aseel (local breed) chicken and they were selected for higher egg number and size with moderate growth, meaning that egg production is higher in female lines than in male lines. FLC showed the lowest egg weight (g) and total egg yolk IgY (mg/egg) among the studied lines of chickens. MLW × FLW is a 2-way cross of male and female lines and it showed intermediate values in total IgY per gram of egg and per mL of yolk. In addition, there is a possibility that the differences observed in IgY contents in the present

experiment were a consequence of differences in natural antibodies of hens that had been exposed to the environment for a longer time.

5. Conclusion

The present study has shown that there is a substantial variation among the studied lines in the proportion of their own antibodies they transfer to their eggs and this is related to the overall genotypic variations of lines. The genotype has significant contribution into total amount of IgY of the entire egg and the egg – yolk parameters of the studied lines of chickens.

6. Recommendations

The findings of this study recommended that through genetic selection, it's possible to develop more locally adaptable dam and sire lines of crossed broilers with increased immunity and lower mortality rate as compared to their commercial counterparts.

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References

[1] C. M. Coakley,V. Staszewski, K. A. Herborn, E. J. Cunningham. "Factors affecting the levels of protection transferred from mother to offspring following immune challenge." Frontiers in Zoology, 11; 46. 2014.

[2] N. M. Nemeth, P. T. Oesterle, R. A. Bowen. "Passive immunity to west nile virus provides limited protection in a common passerine species". The American journal of tropical medicine and hygiene, 79; 283-290. 2008.

[3] R. Chalghoumi, Y. Beckers, D. Portetelle, A.Théwis. "Hen egg yolk antibodies (IgY), production and use for passive immunization against bacterial enteric infections in chicken: a review." Biotechnology, Agronomy, Society and Environment, 13; 295-308. 2009.

[4] J. Kovacs-Nolan, Y. Mine. "Egg yolk antibodies for passive immunity." Annual review of food science and technology, 3; 163-182. 2012.

[5] T. Diraviyam, B. Zhao, Y. Wang, R. Schade, A. Michael, X. Zhang. "Effect of chicken egg yolk antibodies (IgY) against diarrhea in domesticated animals: A systematic review and meta-analysis." PloS one, 9; e97716. 2014.

[6] K. R. Hamal, S. C. Burgess, I. Y. Pevzner, G. F. Erf. "Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens." Poultry Science, 85; 1364–1372. 2006.

[7] J. A. Cutting,T. F. Roth. "Changes in specific sequestration of protein during transport into the developing oocyte of the chicken." Biochimica et Biophysica Acta, 298; 951-955. 1973.

[8] M. R. Loeken, T. R. Roth. "Analysis of maternal IgG subpopulations which are transported into the chicken oocyte." Immunology, 49; 21-28. 1983.

[9] A. M. Ulmer-Franco. "Transfer of chicken immunoglobulin Y (IgY) from the hen to the chick." Avian Biology Research, 5; 81-87. 2012.

[10] A. Lardinois, T. Van den Berg, B. Lambrecht, M. Steensels. "A model for the transfer of passive immunity against Newcastle disease and avian influenza in specific pathogen free chickens." Avian Pathology, 43; 118-124. 2014.

[11] K. Kowalczyk, J. Daiss, J. Halpern, T. F. Roth. "Quantitation of maternal-fetal IgG transport in the chicken." Immunology, 54; 755–762. 1985.

[12] A. S. Abdel-Moneim, M. Abdel-Gawad. "Genetic variations in maternal transfer and immune responsiveness to infectious bursal disease virus." Veterinary Microbiology,114; 16-24. 2006.

[13] F. Davison, K. Magor, B. Kaspers. "Structure and evolution of avian immunoglobulins." Avian Immunology. In: Davison F, Kaspers B, Schat KA, editors.. Elsevier: Academic press; 2008 .p.107-127.

[14] R. Schade, E. G. Calzado, R. Sarmiento, P. A. Chacana, J. Porankiewicz-Asplund, H. R. Terzolo. "Chicken egg yolk antibodies (IgY-technology): A review of progress in production and use in research and human and veterinary medicine." ATLA, 33; 129-154. 2005.

[15] R. Agrawal, S. D. Hirpurkar, C. Sannat, A. K. Gupta. "Comparative study on immunoglobulin Y transfer from breeding hens to egg yolk and progeny chicks in different breeds of poultry." Veterinary World, 9; 425-31. 2016.

[16] T. Diraviyam, T. Jeevitha, P. Saravanan, A. Michael, S. Meenatchisundaram."Preparation of chicken (IgY) antibodies consortium for the prevention of enteric infections in poultry." Journal of Microbiology and Biotechnology Research,1; 95-103. 2011.

[17] D. Carlander, M. Wilhelmson, A. Larsson. "Immunoglobulin Y levels in egg yolk from three chicken genotypes." Food and Agricultural Immunology, 15 ; 35-40. 2003.

[18] R. Schade, C. Staak, C. Hendriksen, M. H. Erhard, H. Hugl, G. Koch, A. Larsson, W. Pollmann, M. Regenmortel, E. Rijke, H. Spielmann, H. Steinbusch, D. Straughan. "The production of avian (egg

yolk) anitibodies: IgY." ATLA. 24; 925-934. 1996.

[19] D. Pauly, P. A. Chacana, E. G. Calzado, B. Brembs, R. Schade. "IgY technology: ex-traction of chicken antibodies from egg yolk by polyethylene glycol (PEG) precipitation." Journal of Visualized experiments, 51; 3084. 2011.

[20] A. Polson, M. B. von Wechmar, M. H. V. van Regenmortel. "Isolation of viral IgY antibodies from yolks of immunized hens." Immunological Communications , 9; 475-493. 1980.

[21] U. K. Laemmeli. "Cleavage of structural proteins during the assembly of the head of bacteriophage T4." Nature, 227; 680-685. 1970.

[22] R Core Team. R: "A language and environment for statistical computing." Vienna: R Foundation for Statistical Computing. Available: http://www.R-project.org/. 2014.

[23] M. M. S. Dallal, E. Kalantar, L. K. Khorasani, M. H. H. Tehrani, M. Z. Zamani. "Identification and Extraction of Chicken Egg Yolk Immunoglobulin from Egg by Polyethylene Glycol (PEG) Precipitation." Journal of Medical Bacteriology. 4;13-18. 2015.

[24] A. Polson. "Isolation of IgY from the yolk of eggs by a chloroform polyethylene glycol procedure." Immunological Investigations, 19; 253-258. 1990.

[25] G. A. Leslie, L. W. Clem. "Phylogeny of immunoglobulin structure and function iii. Immunoglobulins of the chicken." The Journal of Experimental Medicine, 130; 1337–1352. 1969.

[26] H. M. Chang, T. C. Lu, C. C. Chen, Y. Y. Tu, J. Y. Hwang. "Isolation of immunoglobulin from egg yolk by anionic polysaccharides." Journal of Agriculture and Food Chemistry, 48; 995-999. 2000.

[27] H. Asemota, S. Curtello, A. A. J. Vaillant, W. Mohammed, S. Vuma, A. V. C. Rao, A. Kurhade, S. Kissoon, M. Smikle, B. Wisdom, G. Kurhadeet. "Purification of avian IgY with Trichloroacetic acid (TCA)." Journal of Chromatography & Separation Techniques, 4; 205. 2013.

[28] C. M. Almeida, C. L. Silva, H. P. Couto, R. C. Escocard, D. G. Rocha, L. P. Sentinelli, T. L. Kipnis, W. D. Silva. "Development of process to produce polyvalent IgY antibodies anti-african snake venom." Toxicon, 52; 293-301. 2008.

[29] E. M. Akita, S. Nakai. "Immunoglobulins from egg yolk: isolation and purification." Journal of Food Science, 57; 629-634. 1992.

[30] E. M. Akita, S. Nakai. "Production and purification of Fab' fragments from chicken egg yolk immunoglobulin Y (IgY)." Journal of Immunological Methods, 162; 155-164. 1993.

[31] C. Schwarzkopf, B. Thiele. "Effectivity of different methods for the extraction and purification of

IgY." Altex, 1; 35-39. 1996.

[32] D. Carlander, M. Wilhelmson, A. Larsson. "Limited day-to-day variation of IgY levels in eggs from individual laying hens." Food and Agricultural Immunology , 13; 87–92. 2001.

[33] A. M. Ulmer-Franco, G. Cherian, N. Quezada, G. M. Fasenco, L. M. MacMullen."Hatching egg and newly hatched chick yolk sac total IgY content at 3 broiler breeder flock ages." Poltry Science, 91; 758-764. 2012.

[34] T. Deignan, J. Kelly, A. Alwan, H. J. Gert. "Comparative analysis of methods of purification of egg yolk immunoglobulin." Food and Agricultural Immunology, 12 ; 77–85. 2000.

[35] X. Li, T. Nakano, H. H. Sunwoo, B. H. Paek, H. S. Chae, J. S. Sim. "Effects of egg and yolk weights on yolk antibody (IgY) production in laying chickens." Poultry Science, 7; 266–270. 1998.

[36] U. Gadde, T. Rathinam, H. S. Lillehoj. "Passive immunization with hyperimmune egg-yolk IgY as prophylaxis and therapy for poultry diseases--A review." Animal Health Research Reviews, 16; 163-176. 2015.