

Maternal Antibody Transfer from Dams to Their Egg Yolks, Egg Whites, and Chicks in Meat Lines of Chickens

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ABSTRACT Maternal antibodies are transferred from hens to the chicks via the egg. To gain insight into maternal antibody transfer and endogenous production of antibodies in broiler chicks, total IgY, IgA, IgM, as well as anti-Newcastle disease virus (NDV) and anti-infectious bronchitis (IBV) antibody levels were examined in the dams' plasma, egg yolks, egg whites, and chicks' plasma on d 3, 7, 14, and 21. Blood was collected from 39-wk-old breeder hens (line 1, n = 17; line 2, n = 21). Fertile eggs were used for antibody extraction from the egg yolks and egg whites (4 to 5 eggs/dam) and for hatching. Unvaccinated chicks (4 to 5 chicks/dam) were reared in a HEPA-filtered room and were bled on d 3, 7, 14 and 21. Based on ELISA methods, plasma levels of IgY and IgM were higher ($P < 0.0001$), and those of IgA were similar ($P = 0.31$), in line 2 compared with line 1. Egg yolk IgY and IgA, as well as egg white IgY, IgA, and IgM levels were higher in line 2 compared with line 1 ($P < 0.0001$).

Independent of line of chicken, the percentage dam-to-chick (3 d) plasma transfer of IgY was estimated to be approximately 30%, with that for IgM and IgA less than 1%. Chicks synthesized IgM first, followed by IgA and IgY. Anti-NDV and anti-IBV antibodies were detected in the dams' plasma, egg yolks, and in the chicks' plasma on d 3 and 7, with line 2 having higher anti-IBV and lower anti-NDV levels than line 1 in all samples ($P < 0.0001$). In summary, IgY levels, total or antigen-specific, in the dams' plasma or eggs were found to be a direct indicator of maternal antibody transfer to the chicks' circulation, with an expected percentage transfer of approximately 30%. This knowledge, together with the observed time course of endogenous antibody production in broiler chicks, may find direct application in formulating strategies for protecting chicks, especially during the first few weeks of age when their immune system is not yet fully functional.

Key words: hen, chick, maternal antibody, meat line

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INTRODUCTION

Maternal antibody transfer can be defined as the transfer of antibodies by a female to her offspring either through the placenta, colostrum, milk, or egg (Grindstaff et al., 2003). Birds transmit maternal antibodies to their offspring by depositing the antibodies in the egg (Brambell, 1970). There are 3 classes of antibodies in chickens, namely IgY (IgG), IgA, and IgM. Chicken IgA and IgM are similar to mammalian IgA and IgM in terms of molecular weight, structure, and immunoelectrophoretic mobility (Leslie and Clem, 1969). Although structural differences exist between IgY and mammalian IgG, IgY is considered the avian equivalent to mammalian IgG. In eggs, IgY is present predominantly in the egg yolk (Leslie and Clem, 1969), whereas IgA and IgM are present in the egg white

as a result of mucosal secretion in the oviduct (Rose et al., 1974).

In chickens, the transfer of IgY from the dam to her 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 (Cutting and Roth, 1973; Loeken and Roth, 1983). In the second step, IgY is transferred from the egg yolk to the offspring via the embryonic circulation. Kramer and Cho (1970) reported that yolk IgY is transported at a low rate across the yolk sac into the embryonic circulation as early as embryonic d 7. The rate of transfer started to increase by embryonic d 14 and by embryonic d 19 to 21, there was a steep rise in the rate of transfer of IgY from the egg yolk to the embryonic circulation (Kowalczyk et al., 1985). The amount of IgY transferred to the egg yolk has been reported to be proportional to maternal serum IgY concentrations (Loeken and Roth, 1983; Al-Natour et al., 2004).

The pathway, amount, and kinetics of total IgA and IgM transferred to the egg yolk and egg white from the dam and, subsequently, to the chicks have not been stud-

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ied in detail. Although IgA and IgM are predominantly egg white Ig, Yamamoto et al. (1975) reported that there is transfer of IgA and IgM antibodies from the egg white into the nonincubated egg yolk in very low concentrations. As reported by Kaspers and coworkers (Kaspers et al., 1991, 1996), a significant amount of these Ig are present in the yolk sac 1 d prior to hatching, and they were found to originate from the egg white via transport rather than through synthesis by the embryo. Moreover, IgA or IgM are not transferred into the fetal circulation; instead, they are transferred to the embryonic gut as part of the egg white (Rose and Orlans, 1981; Kaspers et al., 1996).

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. There are many reports in the literature regarding the transfer of pathogen-specific antibodies from hens to their chicks via the egg and their role in the protection of newly hatched chicks from the pathogens (Sharma et al., 1989; Heller et al., 1990; Mondal and Naqi, 2001; Sahin et al., 2001; Rahman et al., 2002; Ahmed and Akhter, 2003). The time at which the newly hatched chicks start to synthesize antibodies endogenously depends on the type of antibody. Lawrence et al. (1981) reported that IgY-secreting B cells are not detectable in a chick's plasma until 6 d posthatch. Endogenously synthesized IgM and IgA antibodies have been detected in plasma of 3- to 4-d-old and 12-d-old chicks, respectively (Leslie and Martin, 1973; Martin and Leslie, 1973; Leslie, 1975).

There have been many studies regarding the isolation and purification of egg antibodies, especially considering the easy access to this source of antibodies and the high levels of specific antibodies present in the egg. Various chemicals have been used for the isolation of egg yolk antibodies. Polson et al. (1980, 1985) used polyethylene glycol (PEG); Bade and Stegemann (1984) used isopropanol and acetone; and Polson (1990) and Ntakarutimana et al. (1992) used chloroform for the extraction of the antibodies from the egg yolk. There is very limited information regarding the isolation and purification of antibodies from egg whites in the literature. Rose et al. (1974) reported the extraction of IgY from egg whites using 0.1 N sulfuric acid and ammonium sulfate.

Little information regarding maternal antibodies in the modern meat-type chicken is available. The current study was designed to examine the transfer of total IgY, IgA, and IgM and 2 pathogen-specific antibodies from broiler hens to their offspring by quantifying the levels of these antibodies in the dams' plasma, egg yolks, egg whites, and the chicks' plasma. Moreover, using a time-course approach, we aimed to explore the persistence of maternal antibodies in the young chicks and, simultaneously, gain insight into the chicks' endogenous antibody production.

MATERIALS AND METHODS

Experimental Design

Hens from 2 meat lines (line 1 and line 2) of chickens (17 hens from line 1 and 21 hens from line 2) from the same

breeder company were used for this study. Although the hens were housed on 2 different farms, they were hatched on the same day, were reared under the same management practices, and were immunized using the same vaccination protocols. Hens were vaccinated with Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) live vaccine at 13 and 18 wk (orally) and NDV- and IBV-killed vaccine at 15 wk (subcutaneously). When the hens were 39 wk old, blood samples were taken (see below), and collection of consecutively laid fertile eggs was initiated. The first 4 to 5 eggs from each hen were stored at 4°C for antibody extraction and analysis, and the remaining 7 to 10 eggs were kept for hatching. The eggs were hatched in the hatchery located at the University of Arkansas Department of Poultry Science poultry farm. Chicks (4 to 5/dam) were wing-banded and reared on wood shavings litter in a HEPA-filtered isolation room at the University of Arkansas Poultry Health Laboratory and were maintained on broiler starter feed with ad libitum access to feed and water. The light schedule was 23L:1D. Importantly, the chicks were neither vaccinated nor medicated throughout the 21-d study. Each chick was bled at 3, 7, 14, and 21 d (see below). Chicks were euthanized by CO₂ (70%) inhalation after the last blood sample was taken. All procedures involving birds were approved by the University of Arkansas Institutional Animal Care and Use Committee.

Collection of Blood from Dams and Chicks

All the hens were bled via the wing vein, and 2 mL of blood was collected using a 3-mL heparinized syringe with a 23-gauge needle (Becton, Dickinson and Co., Franklin Lakes, NJ). The blood for each of the hens was centrifuged at 5,000 × g for 2 min at room temperature in a Jouan A-14 micro centrifuge (Jouan Inc., Winchester, VA). The plasma samples were collected and stored at -20°C until analysis. The chicks were bled via the jugular vein (d 3 and 7) using a 0.5-mL heparinized insulin syringe with a 28.5-gauge needle (Becton, Dickinson and Co.). On d 14 and 21, the chicks were bled via the wing vein using a 1-mL heparinized syringe with a 25-gauge needle (Becton, Dickinson and Co.), and plasma samples were collected and stored at -20°C until analysis.

Extraction of Antibodies from Egg Yolk and Egg White

The antibodies were extracted individually from the 5 eggs/hen stored at 4°C. To minimize any differences that may arise due to early and late extraction of antibodies from the egg yolk and egg white, equal numbers of line 1 and line 2 eggs were used on an extraction day. Similarly, eggs were processed in the order they were laid by completing extraction of the first egg from all the hens before proceeding to the second egg.

To extract Ig from the egg white, a PEG-based Ig isolation method described by Polson et al. (1985) was modified for egg whites. Briefly, the eggshell from the nar-

Table 1. Working dilutions of the samples used for IgY, IgA, IgM, anti-Newcastle disease virus (NDV) and anti-infectious bronchitis virus (IBV) ELISA

Samples	Working dilution ¹			
	IgY	IgA	IgM	Anti-NDV and anti-IBV
Dam's plasma	1:150,000	1:5,000	1:10,000	1:400
Egg yolk	1:50,000	1:100	1:100	1:200
Egg white	1:100	1:10	1:10	—
Chick's plasma, 3 d	1:75,000	1:50	1:200	1:200
Chick's plasma, 7 d	1:75,000	1:350	1:500	1:100
Chick's plasma, 14 d	1:50,000	1:750	1:1,000	—
Chick's plasma, 21 d	1:50,000	1:1,500	1:1,500	—

¹The working dilution of the samples for each antibody examined was determined by carrying out preliminary ELISA using the various types of samples in our laboratory.

rowed end was broken, and the egg white was allowed to run into the measuring cylinder by gently inverting the egg to facilitate the flow of egg white. With the help of a Pasteur pipette, any remaining egg white was transferred into the measuring cylinder, and the volume of the egg white was noted. Twice the volume of Dulbecco's PBS (Sigma-Aldrich Inc., St. Louis, MO) was added into the cylinder containing egg white, and the contents were mixed thoroughly by shaking. Pulverized PEG-8000 (Mallinckrodt Baker Inc., Phillipsburg, NJ) was added to make a final concentration of 3.5% (wt/vol) and mixed thoroughly using a magnetic stirrer until the PEG was completely dissolved. The above mixture was then centrifuged at $14,000 \times g$ for 10 min at room temperature in an ultra high-speed centrifuge (Beckman Instruments Inc., Fullerton, CA). After centrifugation, the clear supernatant fluid containing the Ig was collected, leaving behind a semisolid pliable layer, aliquoted, and stored at -20°C until analysis.

To extract Ig from the egg yolk, a chloroform-based method described by Polson (1990) was used. The egg yolk was taken out of the eggshell and placed in a clean petri dish. The egg yolk membrane was washed with distilled water and then broken with the help of forceps. The yolk was allowed to run into a measuring cylinder, and its volume was noted after it settled down. Twice the volume of Dulbecco's PBS (Sigma-Aldrich Inc.) was added, and the contents were mixed thoroughly by shaking. Chloroform (Mallinckrodt Baker Inc.) equal to the volume of egg yolk and PBS was then added, and the contents were mixed vigorously, which resulted in the production of a thick emulsion. The emulsion was then centrifuged at $1,000 \times g$ for 30 min at room temperature in a Jouan CR-312 centrifuge (Jouan Inc.). After centrifugation, the mixture was separated into 3 distinct layers in the centrifuge tube: an orange-colored solution of lecithin at the bottom, a semisolid emulsion of yolk in chloroform in the middle, and a watery phase of chicken serum protein on top. The watery phase on the top containing the Ig was removed, aliquoted, and stored at -20°C until analysis.

Determination of Total IgY, IgA, and IgM Levels

The levels of the total IgY, IgA, and IgM in the dams' plasma, egg yolks, egg whites, and the chicks' plasma at 3, 7, 14, and 21 d were determined using quantitative ELISA kits (Bethyl Laboratories, Montgomery, TX) for IgY, IgA, and IgM, respectively, following the manufacturer's instruction with slight modification. The samples were analyzed in triplicate. Each plate had its own set of standards (3.12 to 200 ng/mL for IgY; 15.625 to 1,000 ng/mL for IgA; and 3.9 to 250 ng/mL for IgM). Predetermined working dilutions of the samples were used (Table 1). Reagents and buffers were prepared in our laboratory following the specifications of the manufacturer (Bethyl Laboratories). The working dilution of detection antibody used was 1:10,000 for IgA and IgM and 1:20,000 for IgY. The samples were incubated with tetramethylbenzidine (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) for 30 min (recommended range is 5 to 30 min), and the reaction was stopped using 2 M H_2SO_4 after 30 min. The plates were read at 450 nm of primary wavelength using an ELX 800 universal micro plate reader (Bio-Tek Instruments Inc., Winooski, VT) and KC junior software (Bio-Tek Instruments Inc.). The blank adjusted data were exported to an Excel file (Microsoft Corp., Redmond, WA). The standard curve describing the relation between the concentration of standards and their absorbance value was generated for each plate, and the concentration of antibody for each of the samples was expressed as micrograms per milliliter or milligrams per milliliter. Additional calculations were carried out for antibody levels in the egg yolk and egg white extracts to determine the concentration of these antibodies per milliliter of egg yolk or egg white as well as the total amount of these antibodies per egg yolk and per egg white.

Determination of Anti-NDV and Anti-IBV Antibodies

The levels of the anti-NDV and anti-IBV antibodies in the dams' plasma, egg yolks, egg whites, and the chicks'

Table 2. Levels of IgY, IgA, and IgM in the dams' plasma, egg yolks, and egg whites in 2 meat lines of chickens

	Line 1	Line 2	P-value
IgY levels ¹			
Dams' plasma ² (mg/mL)	3.26 ± 0.22 ^b	6.02 ± 0.40 ^a	<0.0001
Egg yolk total ^{3,4} (mg)	22.5 ± 0.70 ^b	43.9 ± 0.73 ^a	<0.0001
Egg yolk ^{3,4} (mg/mL)	1.15 ± 0.03 ^b	2.26 ± 0.03 ^a	<0.0001
Egg white total ^{3,4} (μg)	128 ± 9.44 ^b	354 ± 17.7 ^a	<0.0001
Egg white ^{3,4} (μg/mL)	3.57 ± 0.27 ^b	9.88 ± 0.50 ^a	<0.0001
IgA levels ¹			
Dams' plasma ² (μg/mL)	301 ± 30.8	346 ± 30.4	0.3050
Egg yolk total ^{3,4} (μg)	305 ± 13.7 ^b	428 ± 17.0 ^a	<0.0001
Egg yolk ^{3,4} (μg/mL)	15.5 ± 0.68 ^b	22.1 ± 0.85 ^a	<0.0001
Egg white total ^{3,4} (μg)	257 ± 11.5 ^b	493 ± 13.7 ^a	<0.0001
Egg white ^{3,4} (μg/mL)	7.06 ± 0.30 ^b	13.7 ± 0.35 ^a	<0.0001
IgM levels ¹			
Dams' plasma ² (μg/mL)	859 ± 30.4 ^b	975 ± 35.6 ^a	<0.0210
Egg yolk total ^{3,4} (μg)	387 ± 14.4	413 ± 14.8	0.2157
Egg yolk ^{3,4} (μg/mL)	19.7 ± 0.68	21.3 ± 0.750	0.1248
Egg white total ^{3,4} (μg)	207 ± 6.65 ^b	249 ± 6.55 ^a	<0.0001
Egg white ^{3,4} (μg/mL)	5.67 ± 0.16 ^b	6.87 ± 0.16 ^a	<0.0001

^{a,b}Mean values within a row that have different letters are different at $P \leq 0.05$.

¹The IgY, IgA, and IgM levels were determined using a chicken IgY, IgA, and IgM quantitative ELISA kit.

²For dams' plasma, antibody levels are means ± SEM; means are based on 17 hens from line 1 and 21 hens from line 2.

³For egg yolk and egg white, antibody levels are means ± SEM; means are based on 84 eggs from line 1 and 96 eggs from line 2.

⁴These antibodies were extracted from egg yolk and egg white (4 to 5 eggs/dam) using chloroform and polyethylene glycol methods, respectively. The antibody levels were determined per milliliter of egg yolk or egg white extract, which then was used to calculate total antibody per milliliter of egg yolk or egg white and total antibody per egg yolk or egg white per egg.

plasma at 3, 7, 14, and 21 d were determined using an NDV antibody test kit and an IBV antibody test kit (Affinotech Ltd., Bentonville, AR), respectively. The ELISA plates included in the test kits were precoated with the inactivated viral antigen (NDV or IBV). All the samples were analyzed in triplicate, with each plate containing negative and positive controls. The ELISA was carried out for NDV and IBV as per the protocol outlined by the ELISA manufacturing company (Affinotech Ltd.), with slight modification. Different predetermined working dilutions of the samples were used (Table 1). The plates were read at a primary wavelength of 405 nm and a reference wavelength of 630 nm using a PowerWave × 340 ELISA plate reader (Bio-Tek Instruments Inc.) and KC junior software (Bio-Tek Instruments Inc.). The levels of anti-NDV and anti-IBV antibody for each sample were determined by calculating the sample to positive ratio. The sample to positive ratio was calculated as average absorbance of the sample minus the average absorbance of the negative control, divided by the average absorbance of the positive control minus the average absorbance of the negative control. This value was then multiplied by 100, because the positive control was preset at 100 ELISA units (EU). The values were expressed as ELISA units.

Statistical Analyses

One-way ANOVA was carried out to determine line difference in levels of total IgY, IgA, IgM, anti-NDV, and anti-IBV antibodies in the dams' plasma, egg yolks, and egg whites using SYSTAT statistical analysis computer software (Systat Inc., Evanston, IL). For the chicks' plasma

levels of total IgY, IgA, IgM, anti-NDV, and anti-IBV antibodies, a repeated measure analysis was carried out in which hen was the experimental unit and chick was the sampling unit. Additionally, *F*-tests were conducted for the main effect and interaction of line (line 1 and line 2) and time (d 3, 7, 14, and 21). Multiple *t*-tests were used for pairwise comparison of means when appropriate based on the significance of the *F*-test. Computing was done using the MIXED procedure in SAS (Version 9; SAS Institute Inc., Cary, NC). Data were reported as mean ± SEM, and the differences were considered significant at $P \leq 0.05$.

RESULTS

Total IgY, IgA, and IgM Levels in the Dams' Plasma, Egg Yolks, and Egg Whites

Line 2 dams had higher plasma IgY levels (6.02 ± 0.40 mg/mL) than line 1 dams (3.26 ± 0.22 mg/mL; Table 2). The levels of IgY per milliliter of egg yolk were higher in line 2 than line 1, and so were the total amounts of IgY per egg yolk (Table 2). Similar to blood and egg yolks, IgY levels per milliliter of egg white were also higher in line 2 compared with line 1, as were the total amounts of IgY in the egg white (Table 2). IgY was present at higher levels in egg yolk (milligram range) compared with egg white (microgram range; Table 2).

Plasma IgA levels in the dams were not different. The dams' plasma IgA levels were 301 ± 30.8 μg/mL for line 1 and 346 ± 30.4 μg/mL for line 2 (Table 2). The IgA

levels per milliliter of egg yolk or egg white, as well as total amounts per egg yolk or egg white, were higher in line 2 than line 1 (Table 2).

Line 2 dams had higher plasma IgM levels ($975 \pm 35.6 \mu\text{g/mL}$) than line 1 dams ($859 \pm 30.4 \mu\text{g/mL}$; Table 2). There were no line differences in the levels of IgM per milliliter of egg yolk, as well as in the total amounts of IgM per egg yolk, but the IgM levels per milliliter of egg white, as well as the total amounts per egg white, were higher in line 2 than line 1 (Table 2).

Total IgY, IgA, and IgM Levels in the Chicks' Plasma

The levels of IgY, IgA, and IgM in the chicks' plasma were examined at 4 time points (3, 7, 14, and 21 d). The plasma levels of IgY were higher in line 2 chicks compared with line 1 chicks at all time points examined (Figure 1, panel A). The IgY levels were highest on d 3, decreased by d 7, were lowest on d 14, and increased again by d 21 for both the lines (Figure 1, panel A). The plasma levels of IgA were higher in line 2 chicks than line 1 chicks on d 3 (2.51 ± 0.21 vs. 1.57 ± 0.18) and 7 (19.1 ± 1.37 vs. 11.4 ± 1.11) but did not differ between the lines on d 14 and 21 (Figure 1, panel B). Plasma IgA levels were lowest on d 3, increased from d 3 to 7 (8-fold increase compared with d 3), and continued to increase until d 21 (Figure 1, panel B) for both lines of chicken. The plasma IgM levels were higher in line 2 chicks compared with line 1 chicks on d 3 (8.98 ± 0.57 vs. 6.38 ± 0.37), whereas, on d 21, levels were higher in line 1 chicks than line 2 chicks (Figure 1, panel C). There were no line differences in IgM levels on d 7 and 14. Plasma IgM levels were lowest on d 3, increased from d 3 to 7 (15-fold increase compared with d 3), and continued to increase until d 21 (Figure 1, panel C).

Pathogen-Specific Antibody Levels in the Dams' Plasma, Egg Yolks, and Egg Whites

The levels of 2 pathogen-specific antibodies (anti-NDV and anti-IBV) were determined. Line 1 dams had higher plasma levels of anti-NDV antibody (130 ± 7.06 EU) compared with line 2 dams (45.70 ± 3.40 EU; Table 3). The levels of anti-NDV antibody in egg yolks also were higher in line 1 than line 2 (Table 3). For anti-IBV antibody, line 2 dams had higher plasma levels (87.5 ± 6.09 EU) compared with line 1 dams (39.3 ± 3.96 EU; Table 3). The levels of anti-IBV antibody in egg yolk also were higher in line 2 than line 1 (Table 3). Both anti-NDV and anti-IBV antibody could not be detected, even in undiluted egg white extracts, by the ELISA kit used for the detection of these antibodies.

Pathogen-Specific Antibody Levels in the Chicks' Plasma

The levels of anti-NDV and anti-IBV antibody in the chicks' plasma were determined at 2 time points (d 3 and 7). For both anti-NDV and anti-IBV, there were line-by-

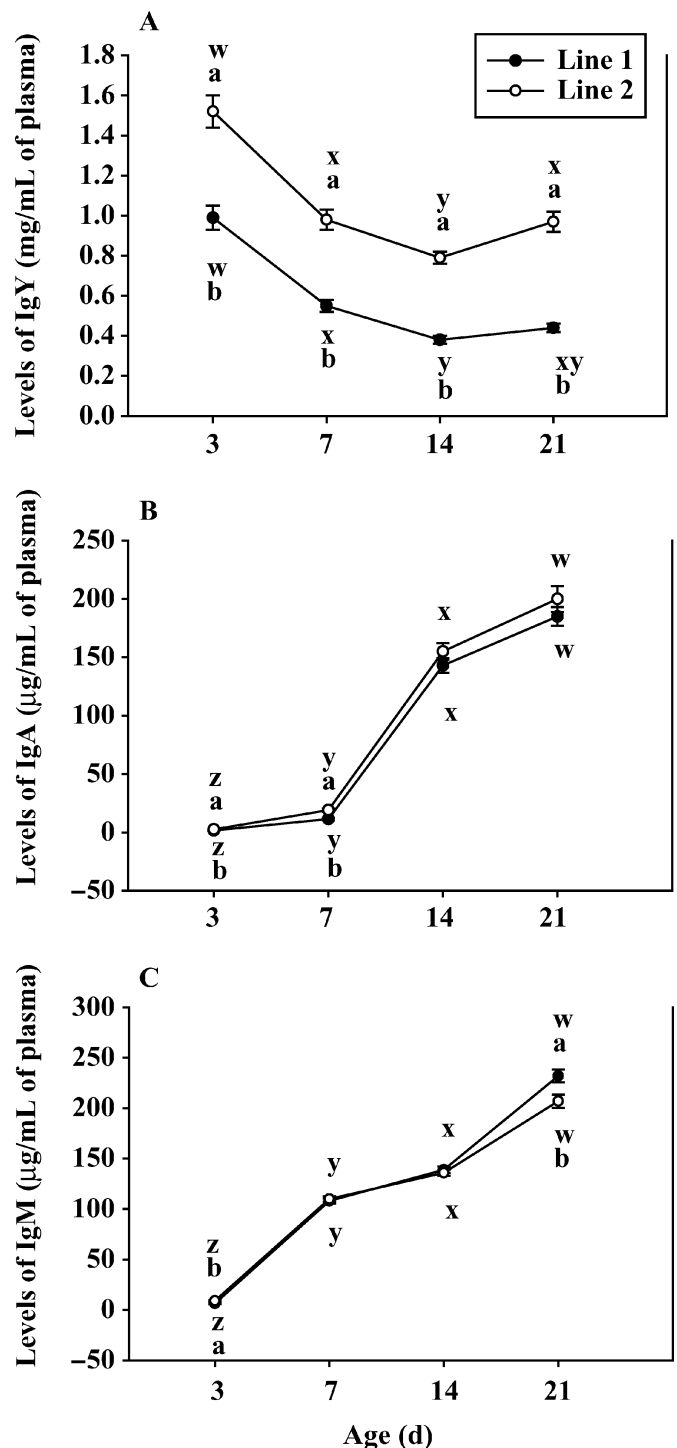


Figure 1. Plasma levels of IgY (A), IgA (B), and IgM (C) in 3-, 7-, 14- and 21-d-old chicks from 2 meat lines of chickens. The chicks were hatched from consecutively laid fertile eggs collected when the hens were 39 wk of age. The chicks were vaccinated for bacterial and viral poultry pathogens following standard vaccination protocol used by the breeding company. The chicks (4 to 5/dam; line 1, 17 hens; line 2, 21 hens) were raised on wood shavings litter in a HEPA-filtered isolation room. The chicks were not vaccinated, and no medication was given during the 21-d study period. Plasma levels of IgY, IgA, and IgM were determined using chicken IgY, IgA, and IgM quantitative ELISA kits. There was no line \times time interaction for IgY ($P = 0.69$) and IgM ($P = 0.13$), but there was significant line \times time interaction for IgA ($P = 0.01$). For each time point, antibody levels are expressed as means \pm SEM, and the means are based on 72 chicks from line 1 and 72 chicks from line 2. Letters a and b indicate line differences and letters w, x, y, and z indicate differences among the different time points within a line; for each analysis, means without a common letter are different ($P \leq 0.05$).

Table 3. Levels of anti-Newcastle disease virus (NDV) and anti-infectious bronchitis virus (IBV) antibodies in the dams' plasma, egg yolks, and egg whites in 2 meat lines of chickens

	Line 1	Line 2	P-value
Anti-NDV levels (EU) ¹			
Dams' plasma ²	130 ± 7.06 ^a	45.7 ± 3.40 ^b	0.0001
Egg yolk ³	54.0 ± 1.73 ^a	21.8 ± 1.03 ^b	<0.0001
Egg white ³	Not detected	Not detected	—
Anti-IBV levels (EU) ¹			
Dams' plasma ²	39.3 ± 3.96 ^b	87.5 ± 6.09 ^a	<0.0001
Egg yolk ³	17.9 ± 1.07 ^b	48.2 ± 2.09 ^a	<0.0001
Egg white ³	Not detected	Not detected	—

^{a,b}Mean values within a row that have different letters are different at $P \leq 0.05$.

¹The hens were vaccinated for NDV and IBV at 13 and 18 wk using live vaccine (orally) and at 15 wk using killed vaccine (subcutaneously). The anti-NDV and anti-IBV antibody levels were determined using NDV test ELISA kit and IBV test ELISA kit, respectively. The values were expressed as ELISA unit (EU).

²For dams' plasma, antibody levels are means ± SEM; means are based on 17 hens from line 1 and 21 hens from line 2.

³These antibodies were extracted from egg yolk and egg white (4 to 5 eggs/dam) using chloroform and polyethylene glycol methods, respectively. For egg yolk, antibody levels are expressed as means ± SEM; means are based on 84 eggs from line 1 hens and 96 eggs from line 2 hens. These antibodies could not be detected in the egg white.

time interactions ($P < 0.0001$ and $P = 0.0006$, respectively). The plasma levels of anti-NDV antibody in line 1 chicks were higher both on d 3 and 7 compared with line 2 chicks (Figure 2, panel A). The plasma levels of anti-IBV antibody were higher in line 2 chicks than in line 1 chicks, both on d 3 and 7 (Figure 2, panel B). The anti-NDV antibody, as well as the anti-IBV antibody levels in the chicks' plasma, were highest on d 3, decreased considerably by d 7, and could not be detected by d 14 onward in chicks from both lines (Figure 2).

Percentage Transfer of Antibodies from the Dams' Plasma to the Chick's Plasma

The percentage transfer of antibody from the dams to their chicks was calculated by dividing the plasma antibody levels in 3-d-old chicks (earliest time point examined) by their dam's antibody levels and multiplying this value by 100 for each of the antibodies examined (IgY, IgA, IgM, anti-NDV antibody, anti-IBV antibody). There were no line differences in the percentage of transfer of antibodies from dams to their offspring (Table 4). The percentage of transfer for total IgY was found to be

27 to 30 %; for total IgA and IgM, it was <1%, and for anti-NDV and anti-IBV antibodies, it ranged from 31 to 41%.

DISCUSSION

Maternal IgY

In chickens, as in mammals, it has been well established that IgG (IgY) is the antibody isotype that is transferred from the dam to her offspring (Brambell, 1970). The current study reaffirmed this concept, establishing IgY as the predominant Ig isotype transferred to the egg as well as to the newly hatched broiler chick. Moreover, using 2 genetic meat lines of chickens, that differed significantly in circulating total IgY levels (line 2 > line 1), it was found that the amount of IgY deposited in the egg and the levels transferred to the offspring were directly related to the circulating levels of IgY in the dam (Table 2, Figure 1). However, the percentage of transfer, expressed as the percentage of the dam's plasma IgY levels circulating in the blood of 3-d-old chicks (approximately 30%), was similar in the 2 lines of chickens. This observation suggests that the amount of maternal antibody present in

Table 4. Progeny plasma Ig concentration as a percentage of the maternal plasma Ig concentration¹

Antibody type	Line 1	Line 2	P-value
IgY ²	31.7 ± 3.79	26.2 ± 3.15	0.262
IgA ²	0.66 ± 0.13	0.90 ± 0.11	0.173
IgM ²	0.74 ± 0.06	0.96 ± 0.09	0.065
Anti-NDV antibody (IgY isotype) ³	31.3 ± 4.52	36.0 ± 4.73	0.479
Anti-IBV antibody (IgY isotype) ³	40.7 ± 3.98	35.5 ± 3.93	0.365

¹The percentage transfer of Ig from the dams' plasma to the plasma of 3-d-old chicks was calculated by dividing the 3-d-old chicks' plasma antibody levels by the dams' plasma antibody levels and multiplying this value by 100. The percentage of transfers are expressed as means ± SEM. Means are based on 17 hens and 72 chicks from line 1 and 21 hens and 72 chicks from line 2.

²The IgY, IgA, and IgM antibody levels were determined using quantitative ELISA kits.

³The anti-Newcastle disease virus (NDV) and anti-infectious bronchitis virus (IBV) antibody levels were determined using ELISA kits.

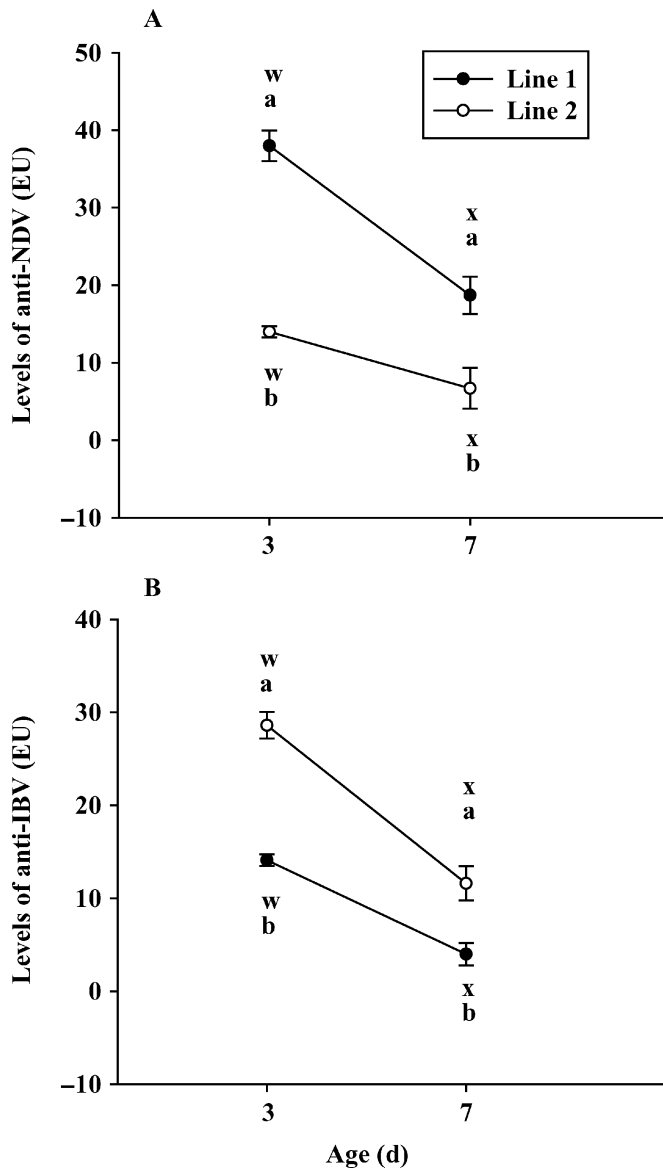


Figure 2. Plasma levels of anti-Newcastle disease virus (NDV; A) and anti-infectious bronchitis virus (IBV; B) antibody in 3- and 7-d-old chicks from 2 meat lines of chickens. The chicks were hatched from consecutively laid fertile eggs collected when the hens were 39 wk of age. The hens were vaccinated for NDV and IBV at 13 and 18 wk using live vaccine (orally) and at 15 wk using killed vaccine (subcutaneously). The chicks (4 to 5/dam; line 1, 17 hens; line 2, 21 hens) were raised on wood shavings litter in a HEPA-filtered isolation room. The chicks were not vaccinated, and no medication was given during the 21-d study period. The anti-NDV and anti-IBV antibody levels were determined using a NDV and IBV ELISA test kit, respectively. There was significant line \times time interaction for anti-NDV ($P < 0.0001$) and anti-IBV ($P = 0.0006$). For each time point, antibody levels are expressed as mean \pm SEM based on the 72 chicks from line 1 and 72 chicks from line 2. Letters a and b indicate line differences and letters w and x indicate differences among the different time points within a line; for each analysis, means without a common letter are different ($P \leq 0.05$). EU = ELISA unit.

the chicks is ultimately decided by the levels in the dam and argues against line differences in the transport of IgY to the egg or from the egg to the offspring's circulation. A similar trend that antibody levels in the egg yolk are directly proportional to the antibody levels in the dams' serum was also observed by Loeken and Roth (1983) and

by Al-Natour et al. (2004), who based this conclusion on antibodies isolated from the egg yolks of 3 flocks differing in levels of antibody against infectious bursal disease virus (no antibody flock, medium level of antibody flock, and high level of antibody flock). The examination of antigen-specific IgY levels to NDV and IBV in our study further emphasizes this relationship. In this case, line 1 had higher antibody levels to NDV than line 2, and the reverse was observed for antibodies to IBV (line 2 > line 1). In concurrence with the transfer concept established above, line differences for anti-NDV and anti-IBV in the eggs and offspring paralleled those observed in the dams (i.e., anti-NDV:line 1 > line 2; anti-IBV:line 2 > line 1) (Table 3, Figure 2). Considering that total IgY levels were higher in line 2 than line 1, but the reverse was true for anti-NDV IgY levels, one can also conclude that differences in total IgY levels do not necessarily predict the antibody response to a given antigen.

The higher levels of IgY in the egg yolk (milligram range) compared with egg white (microgram range) emphasize that IgY is mainly an egg yolk Ig. Nevertheless, the direct relationship of egg yolk IgY levels to the dams' IgY plasma levels is maintained for IgY in the egg white as well. Considering the nature of the transfer mechanism of IgY from the dam's plasma into her growing ovarian follicle and the concept that egg white Ig are primarily contributed by mucosal secretion in the form of IgA and IgM (Rose et al., 1974), it is likely that the IgY found in the egg white has been transported from the egg yolk.

The IgY levels in the chicks' plasma on d 3 ranged between 0.99 to 1.52 mg/mL (Figure 1, panel A), which agrees with the levels (1 to 2 mg/mL) reported by Kowalczyk et al. (1985). The initial decrease in the plasma IgY levels up to d 14 may be due to the catabolism of maternal IgY in chicks as the half-life of IgY in the plasma of 1-d-old chicks was reported to be 3 d (Patterson et al., 1962). The increase in IgY levels in the chicks' plasma on d 21 compared with d 14 suggests that, by d 21, the IgY synthesized by the chicks starts to appear in the circulation. This time course of the presence of maternal antibody (IgY) is further supported by our analysis of antigen-specific IgY provided by the mother in the form of anti-NDV and anti-IBV antibodies, which were found to be highest on d 3, decreased substantially on d 7, and were no longer detectable on d 14 onwards (Figure 2). These observations are in agreement with the reports of Mondal and Naqi (2001) and Rahman et al. (2002), suggesting that these antibodies are purely maternal in nature because only the dams were vaccinated for NDV and IBV and the chicks were not vaccinated or exposed to these pathogens.

Maternal IgA

Examination of IgA levels in the various samples revealed that plasma levels of total IgA were not different in dams from the 2 lines, although total IgA levels in the egg yolk and egg white were (line 2 > line 1; Table 2). Because IgA is a major mucosal Ig and its presence in the egg is due to mucosal secretions into the egg white (Rose

et al., 1974), this would suggest that the lines differed in the secretion of mucosal IgA. As observed here, IgA can also be found in the egg yolk, which is in agreement with reports by Yamamoto et al. (1975). Moreover, in embryonic development, IgA detected in the yolk sac appears to have been transported from the egg white (Kaspers et al., 1991, 1996). Although present in the egg at 0.5- to nearly 1-mg levels, maternal IgA does not appear to be taken up into the circulation of the offspring (Table 4, Figure 1) and may have its major function in the newly hatched chick as a protective Ig in the alimentary tract or as an additional source of protein (Rose and Orleans, 1981; Kaspers et al., 1996). Plasma IgA levels in the chicks were lowest on d 3, increased by d 7, and were approaching adult levels by d 21. The observed increase in plasma IgA levels in very young chicks hence precedes that of IgY, suggesting that endogenous antibody production and mechanisms for isotype switching are functional at a very young age, and immune responses were likely initiated by antigens encountered at mucosal surfaces. Although we did not examine mucosal secretions of IgA in the chicks, IgA levels in mucosal secretions are also expected to have increased concurrently with plasma IgA levels. Lastly, it is interesting to note that line differences in plasma IgA levels similar to those observed for egg IgA (line 2 > line 1) existed on d 3 and 7. However, as in the dams, plasma IgA levels did not differ thereafter in chicks from the 2 lines. The line difference in plasma IgA on d 3 and 7 may be suggestive of some transfer of maternal IgA into the chicks' circulation (estimated to be less than 1%).

Maternal IgM

Plasma IgM levels in dams from line 2 were higher than those in line 1. This line difference was also detected in egg whites but not egg yolks. It is known that IgM is primarily an egg white Ig (Rose et al., 1974), although the concentration of IgM ($\mu\text{g}/\text{mL}$) observed here was higher in the egg yolks than egg whites. Similar to IgA, the source and transport mechanisms for IgM into the egg, either from the dam's blood into the egg yolk, from the egg white into the egg yolk, or both, is not understood and cannot be discerned from this study. In the chicks, circulating levels of IgM were very low on d 3, with maternal transfer estimated at less than 1%, if any. There was a significant rise in the plasma levels of IgM by d 7, suggesting active production of IgM by the chicks. By d 21, chicks from line 1 had higher plasma IgM levels than those from line 2, which is contrary to the levels observed in the dams. Additionally, the chick plasma IgM levels at that age were only a quarter of the levels observed in dams.

Overall, IgY was the principal antibody transferred to the chick's plasma. With the half-life of maternal IgY antibody estimated to be 3 d (Patterson et al., 1962), our estimate of a 30 to 40% maternal IgY transfer based on circulating levels in 3-d-old chicks is likely a conservative estimate (Table 4). The observed direct relationship

among IgY levels to a given antigen in the dam and those in her offspring implies that individual differences in the dams' antibody titers are a good indicator of expected transfer of these antibodies to the offspring. Considering that this direct relationship is also consistent with IgY levels in the egg yolk, comparison of individual birds based on egg yolk IgY antibody levels to specific antigens also appears to be a good indicator of both antibody titers in the dams and maternal antibody transfer to the offspring. We found IgA and IgM in both compartments of the egg, although at substantially lower levels than IgY. Further studies are needed to examine the source, transfer, and role of maternal IgA and IgM in chickens. Based on the chronological changes in circulating levels of IgY, IgA, and IgM in chicks, in posthatch chicks IgM appears to be synthesized earliest, followed in turn by IgA and IgY. Understanding the interplay between maternal antibody transfer and endogenous antibody production in broiler chicks may find direct application in formulating strategies for protecting chicks, especially during the first few weeks of age when their own immune systems are not yet fully functional.

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