Effects of Egg and Yolk Weights on Yolk Antibody (IgY) Production in Laying Chickens

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ABSTRACT Twenty 35-wk-old chickens, including 10 Single Comb White Leghorn (SCWL) and 10 Rhode Island Red (RIR) hens, were used to examine the effects of egg and yolk weights on egg yolk antibody (IgY) production in the two strains of chickens immunized with BSA. The SCWL chickens had a greater ($P < 0.01$) percentage hen-day production and greater egg and yolk weights than did the RIR chickens. However, the anti-BSA antibody activities determined by ELISA in the serum and the egg yolk were similar ($P > 0.05$) between the SCWL and RIR chickens. Similarities between the two strains of hens were also observed in protein and total IgY contents (expressed as the percentage of wet weight of yolk) and the percentage of BSA-specific antibody in the total IgY. It was concluded that both the SCWL and RIR chickens immunized with BSA can produce egg yolk IgY containing similar proportions of BSA-specific antibodies. Therefore, the egg yolk weight and the percentage hen-day production, both of which are greater in the SCWL hens, are considered to be important factors for the efficient production of IgY.

(*Key words*: immune response, egg yolk, antibody, chicken, bovine serum albumin)

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INTRODUCTION

Immune responses by producing antibodies (immunoglobulins) against foreign materials (antigen) are important for chickens to protect themselves from infection. In laying hens, the immunoglobulin, IgG in the blood is efficiently transferred across the follicular epithelium of ovary and accumulated in the yolk during oogenesis (Rose and Orlans, 1981). This is important to protect their progeny. The concentration of IgG in the yolk, which is more commonly called IgY (Leslie and Clem, 1969), is higher than that in the serum (Rose *et al.*, 1974; Larsson *et al.*, 1993). The IgY is continuously absorbed by embryo during embryogenesis until the 2nd d after hatching, providing evidence of passive immunization protection acquired from hen.

Chickens store high contents of IgY in the yolk and are considered to be efficient antibody producers (Gottstein and Hemmeler, 1985). In a period of 6 wk, one immunized hen produces 298 g of IgY, which is much higher than the serum antibody (16.6 mg) obtained from one rabbit (Nakai *et al.*, 1994). Moreover, due to the phylogenetic distance between birds and mammals (Jensenius *et al.*, 1981), chickens produce more specific antibodies against mammalian antigens than do mammals. For example, BSA (Ermeling *et al.*, 1992) and human serum (Lösch *et al.*, 1986), are more antigenic to avian species than to mammals. The IgY is superior to serum antibody due to higher levels of specific antibodies (Orlans, 1967; Rose *et al.*, 1974) and relative ease of purification (Akita and Nakai, 1992) with low cost (Polson and Von Wechmar, 1980). The egg yolk antibody has also an advantage over the serum antibody because of its compatibility with modern animal protection regulations (Gottstein and Hemmeler, 1985).

Although chickens have been used as antibody producers for more than 10 yr and some antibodies have been obtained from egg yolk, information is still limited concerning the IgY production in relation to egg traits and hen productivity. The present study was undertaken 1) to examine the immune response against BSA as the antigen in the two strains (Single Comb White Leghorn, SCWL vs Rhode Island Red, RIR) of chickens and 2) to examine the effect of laying performance (e.g., rate of egg production, and egg and yolk size) on the efficiency of IgY production.

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Abbreviation Key: PCNA = proliferating cell nuclear antigen; RID = radial immunodiffusion; RIR = Rhode Island Red; SCWL = Single Comb White Leghorn; WSF = water-soluble fraction.

TABLE 1. Laying performance and egg yolk composition during the experimental period

Variables	White Leghorn	Road Island Red	*P*
Percentage hen-day production	85.1 ± 0.0	46.6 ± 11.8	<0.01
Egg weight, g[1]	50.9 ± 2.4	38.7 ± 2.3	<0.01
Yolk weight, g[1]	17.0 ± 1.2	13.2 ± 0.9	<0.01
Yolk weight/egg weight	0.33 ± 0.0	0.34 ± 0.0	>0.05
Moisture,[2] % of wet weight	50.5 ± 0.2	49.3 ± 0.6	>0.05
Fat,[2] % of wet weight	31.1 ± 0.7	31.9 ± 0.4	>0.05
Protein in yolk,[2] % of wet weight	14.5 ± 0.1	14.1 ± 0.0	>0.05
Protein per yolk, g	2.46 ± 0.1	1.85 ± 0.1	<0.01

[1]Both egg and yolk weights were derived from 200 eggs of Single Comb White Leghorn hens and 150 eggs of Rhode Island Red hens.

[2]Six eggs from each strain were analyzed.

MATERIALS AND METHODS

Experimental Birds

Twenty 35-wk-old chickens, including 10 SCWL and 10 RIR hens, averaging in body weights 1.53 ± 0.20 (SD) and 2.06 ± 0.06 kg, respectively, were kept in the University of Alberta Poultry Unit. The SCWL hens used had high laying performance, whereas the RIR hens had low laying performance. All chickens were cared for in accordance with the Canadian Council on Animal Care guidelines of animal welfare.

Immunization of Chickens with BSA

Procedures of chicken immunization followed those described by Sunwoo *et al.* (1996). For the first injection, 2 mg BSA[2] was dissolved in PBS (0.14 *M* NaCl, 0.0015 *M* KH_2PO_4, 0.0081 *M* Na_2HPO_4, and 0.0027 *M* KCl, pH 7.2), and emulsified with an equal volume of complete Freund's adjuvant to obtain the final concentration of 1 mg BSA / mL. Each hen was intramuscularly injected with 1 mg BSA (Shimizu *et al.*, 1992) at four different sites (0.25 mg per site) of breast muscles (two sites per left or right breast muscle). A booster injection was given intramuscularly 2 wk after the first injection with the same dose emulsified with Freund's incomplete adjuvant. Blood samples were collected from the wing vein on 0, 14, 35, 56, 63, and 70 d after the initial injection. Eggs were collected daily and stored at 4 C until analyzed.

Isolation of the Water-Soluble Fraction

Isolation of the water-soluble fraction (WSF) was carried out by the method described by Akita and Nakai (1992) with small modification. Egg yolk was separated from the white using egg yolk separator and then rolled on paper towels to remove adhering egg white. The yolk membrane was punched and the yolk without membrane was transferred into a graduated cylinder. The yolk samples was mixed with six volumes of cold acidified water, pH 2.5 adjusted with 0.1 *N* HCl. The mixture, which had a pH of 5.0, was kept at 4 C for 6 h, and then centrifuged at 12,100 × *g* and 4 C for 15 min. After centrifugation, supernatant was collected as WSF. The WSF samples obtained were used for determinations of the anti-BSA antibody activity by ELISA, the total IgY content by the radial immunodiffusion (RID) technique, and the content of IgY specific to BSA by BSA-agarose affinity column chromatography.

ELISA

Antibody activities in sera and WSF were determined using microtiter plates with 96 wells,[3] which were coated by adding 150 μL of a BSA solution (25 μg / mL 0.05 *M* carbonate buffer, pH 9.6) to each well and incubating at 22 C for 2 h. Plates were washed with PBS containing 0.05% Tween (PBST). Samples (150 μL) of sera or WSF were then prepared for ELISA. Sera were diluted 1,000× and the WSF, obtained by six times dilution of yolk (see above) was diluted 167 times to obtain 1,000× dilution. Diluted samples and PBS as control were added to the well, and incubated at 22 C for 3 h subsequent to incubation, plates were washed with PBST, and incubated with the second antibody (150 μL of a 1:1000 dilution of peroxidase conjugated rabbit anti-chicken IgG[2]) at 37 C for 1 h. Plates were washed with PBST, and 100 μL of substrate solution, 2, 2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)[2] in 0.05 *M* phosphate-citrate buffer, pH 5.0 containing 0.03% sodium perborate,[2] was added to the well. After 30 min, the absorbance of reaction mixture was read at 405 nm using an ELISA reader[4] against the reaction mixture prepared with PBS.

An ELISA was also carried out to examine the reactivity of chicken anti-BSA antibody to sera from elk and rabbit, which were chosen as species representing ruminant and nonruminant species, respectively. If a positive reaction is observed, the anti-BSA IgY produced may be useful to purify serum albumin from those species by preparing an anti-BSA IgY conjugated affinity column. Elk serum was a

[2]Sigma Chemical Co., St. Louis, MO 63178-9916.
[3]Costar Corp., Cambridge, MA 02139.
[4]Bio-Tek Instruments, Inc. Winooski, VT 05404.

TABLE 2. Immunoglobulin Y content in the yolk and the total IgY produced during the experimental period[1]

Variable	SCWL	RIR	*P*
IgY in yolk,[2] % of wet weight	0.64 ± 0.5	0.63 ± 0.5	>0.05
IgY per yolk,[3] mg	105.4 ± 0.7	83.2 ± 0.5	<0.01
Total IgY production,[4] g	61.1 ± 0.6	23.4 ± 0.5	<0.01

[1]SCWL = Single Comb WHite Leghorn; RIR = Rhode Island Red.

[1]Thirty eggs collected from each strain on Days 0, 14, 21, 28, 35, 42, 49, 56, 63, and 70 were analyzed by Radial immunodiffusion.

[2]Calculated from the concentration of IgY in the yolk and the yolk weight (Table 1).

[3]Calculated based on the content of IgY produced during the 18-wk experimental period.

gift from S. L. Kuryo,[5] and rabbit serum was obtained from Gibco BRL.[6] Bovine serum used as control was obtained from a local abattoir. Chicken sera containing anti-BSA antibody diluted at 1 to 1,000 were added to the plates coated with diluted serum (0.5 μL/mL 0.05 *M* carbonate buffer, pH 9.6) from different species, and ELISA was performed as above.

Radial Immunodiffusion

Radial immunodiffusion of WSF was performed by the method previously described (Sunwoo *et al.*, 1996). The solution A was prepared by mixing 0.3 mL rabbit anti-chicken IgG[4] with 1.7 mL barbital buffer[2] (50 m*M* sodium barbital and 10 m*M* barbital, pH 8.6) and incubating in a 56 C water bath. Solution B was prepared by mixing 70 mg of agarose[2] with 4.6 mL barbital buffer and 0.4 mL of 0.35% (wt/vol) sodium azide, and holding the mixture in a boiling water bath until the agarose dissolved. Solutions A and B were mixed well, equilibrated at 56 C, and poured into RID plate. Serum and WSF (both 6 μL) and standard IgG from chicken[2] (0 to 1.0 mg in 6 μL of PBS) were then added to 2.5 mm diameter wells, and incubated at room temperature for 3 d. The diameter of precipitation ring formed was measured, and the concentration of IgG in sample was calculated by referring to the standard curve prepared by using known amounts of IgG (see above).

BSA Agarose Affinity Chromatography

Affinity column of BSA-agarose (15 mg BSA immobilized/mL of 4% beaded agarose)[2] was used to isolate specific anti-BSA IgY from WSF. The WSF sample containing approximately 18 mg of total IgY was applied to a 1 × 1.3 cm column of BSA-agarose equilibrated with 0.01 *M* sodium phosphate buffer pH 7.2 containing 0.5 *M* NaCl and 0.02% sodium azide. After loading the sample, the column was washed with 3 mL of this buffer to remove unbound materials. The column was then eluted with 0.5 *M* acetic acid containing 0.5 *M* NaCl, pH 2.4. Fractions (1 mL) were collected into tubes containing 0.81 mL of 0.5 *M* Tris to neutralize acidic eluates. All fractions collected were determined for protein contents by measuring absorbance at 280 nm, antibody activities by ELISA, and the content of IgY by the RID method.

Analytical Methods

The content of moisture in egg yolk was measured by weighing before and after heating at 110 C overnight. The content of crude fat was determined by measuring the content of petroleum ether (boiling range 37.8 to 56.9 C) extractable material using a Goldfisch extraction apparatus.[7] The content of nitrogen in yolk was determined by the Kjeldahl method (AOAC, 1980), and the content of protein was calculated using a factor of 6.25 to convert from nitrogen content. All data were calculated as the mean ± SD. A *t* test was used to evaluate the difference between means.

RESULTS AND DISCUSSION

Percentage hen-day production in chickens and their egg and yolk weights during the experimental periods are given in Table 1. The percentage hen-day production was approximately two times higher ($P < 0.01$) in the SCWL than in the RIR hens, and both the egg and yolk weights were 1.3 times greater ($P < 0.01$) in the SCWL

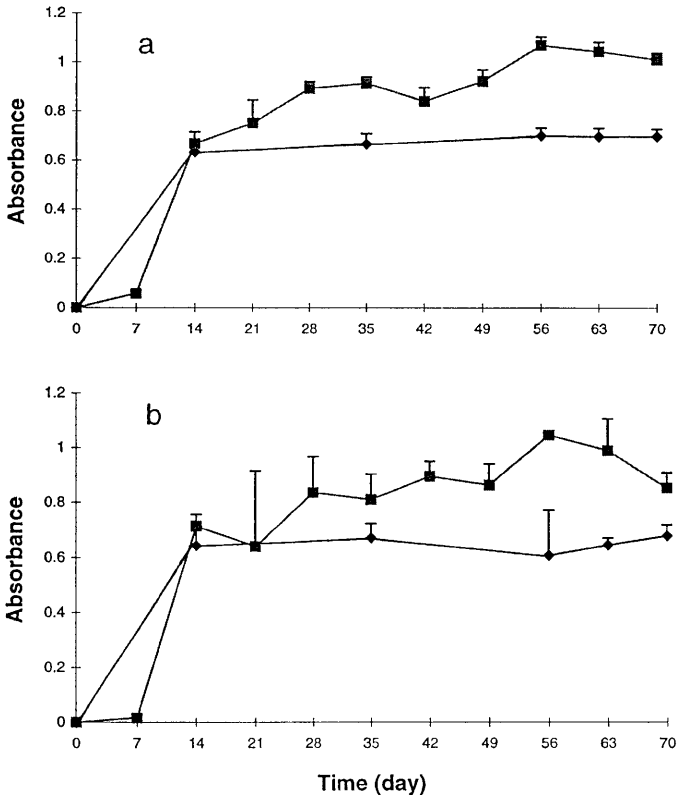

FIGURE 1. Changes of antibody activities in serum and egg yolk during the immunization period. Antibody activities in sera (♦) and egg yolk (■) (both at 1:1000 dilution) from Single Comb White Leghorn (a) and Rhode Island Red (b) hens were measured by ELISA, and were expressed as ELISA absorbance at 405 nm. Vertical bars indicate SD.

[5]InnerSense International Inc., Sherwood Park, AB Canada, T8A 2A6.
[6]Burlington, ON Canada, L7P 1A1.
[7]Perstorp Analytical/Tecator Inc., Herndon, VA 22071.

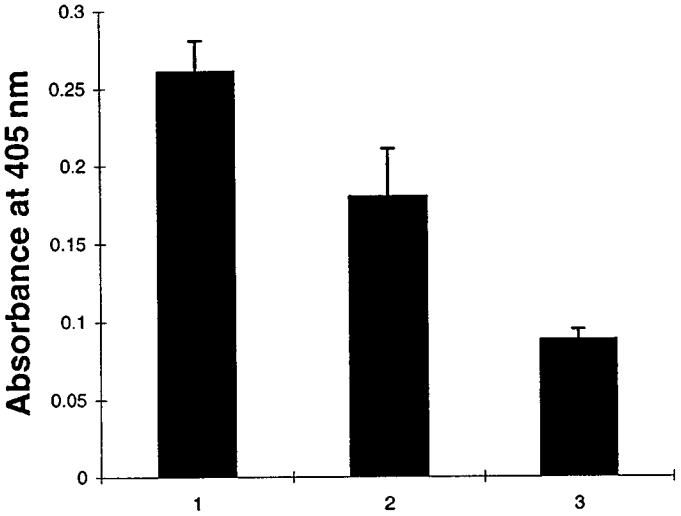

FIGURE 2. Reactivity of chicken anti-BSA antibody to cattle, elk, and rabbit sera 1) bovine serum, 2) elk serum, 3) rabbit serum. Vertical bars indicate SD based on six observations from the same serum sample.

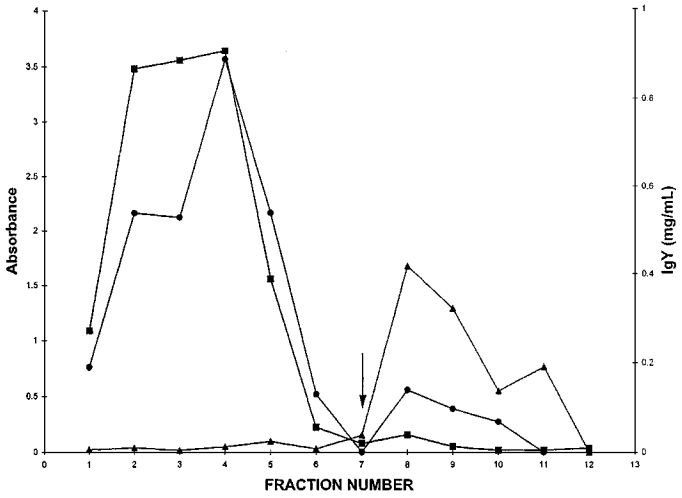

FIGURE 3. Purification of anti-BSA antibody by affinity chromatography. Fractions (1 mL) collected were monitored for anti-BSA antibody activity (absorbance at 405 nm, ▲), and contents of IgY (●) and protein (absorbance at 280 nm, ■). Arrow indicates the application of 0.5 *M* acetic acid containing 0.5 *M* NaCl, pH 2.4.

than in the RIR hens, as expected. The ratio of yolk weight to egg weight was similar (average 0.3, $P > 0.05$) between the two strains of chickens. Moisture, fat, and protein contents in the yolk (Table 1) were similar ($P > 0.05$) between the strains of chickens averaging 49.9, 31.5, and 14.3% of wet weight, respectively. These are close to those reported previously (Stadelmen and Cotteril, 1986; Burley and Vadehra, 1989). The total protein content per yolk was accordingly lower in the RIR hens.

The changes of anti-BSA antibody activity, determined by ELISA in the serum and egg yolk from either SCWL or RIR birds are shown in Figure 1. In both strains, the antibody activity in serum rapidly increased and reached plateau on Day 14. The antibody activity in egg yolk from either strain was very weak on Day 7, rapidly increased on Day 14, and gradually increased thereafter to reach the peak on Day 56. There was no significant ($P > 0.05$) difference in the antibody activity between the two strains of chickens in either the serum and egg yolk throughout the experimental period. These results indicated that BSA is highly antigenic to both strains of chickens. The chicken anti-BSA antibody from either strain was found to react with sera from cattle, elk, and rabbit (Figure 2).

The content of total IgY in the egg yolk was relatively constant (average 0.6%, wt/wt, Table 2) among the chickens regardless of the strains, egg weight, egg production or days of the experimental period. A similar trend has been reported previously in the concentration of egg yolk IgY during immunization of chickens (Shimizu *et al.*, 1988; Sunwoo *et al.*, 1996). However, the total content of IgY in the yolk was approximately 1.3 times greater, and the total IgY produced during the 18 wk experimental period was three times greater in the SCWL than in the RIR hens (Table 2). This is expected as both the rate of egg production and yolk weight were less in the latter (Table 1).

The foregoing results showed that both the antibody activity and total IgY concentration in the egg yolk, were similar between the two strains of chickens, suggesting that the concentration of IgY, which is specific to BSA, is similar between the two strains of hens. However, the concentration of specific IgY in the egg yolk is unknown. To examine the content of BSA-specific IgY, we used a BSA-agarose affinity column. The elution pattern of WSF on BSA-agarose was similar between the two strains of hens. A representative chromatogram is shown in Figure 3. A majority (94%) of anti-BSA antibody activity was retained on the column, and eluted with 0.5 *M* acetic acid in fractions 7 to 11. The ratio of anti-BSA titer to protein was 50 times greater in the BSA-specific IgY than in the WSF. The proportion of BSA-specific IgY in the total egg yolk IgY was similar between the two strains of hens as suggested above, and averaged 9.0%.

There is limited information available on determination of the content of antigen-specific immunoglobulin. Affinity chromatography with antigen as a ligand appears to be an appropriate technique to be used. Gassmann *et al.* (1990) raised IgY against the proliferating cell nuclear antigen (PCNA), and purified specific antibodies by using affinity chromatography on PCNA-agarose. These authors reported that 3.2% of IgY was specific to the antigen. Available information from a chemical supplier[8] showed that loading the BSA-cellulofine affinity column with 19 mL of immune rabbit serum resulted in elution of 26.85 mg of anti-BSA antibodies from the column. Assuming that the concen-

[8]Cellulofine affinity chromatography (in Japanese). Seikagaku Kogyo Co., Ltd. Tokyo Yakugyo Bldg., 1-5, Nihonbashi-honcho 2-chome, Chuo-ku, Tokyo, 103 Japan.

tration of immunoglobulin in rabbit serum is 13 mg / mL, the proportion of BSA-specific antibody in total immunoglobulin, which is 10.9% by calculation, is apparently close to the proportion of BSA-specific antibody found in this study (see above).

The purified anti-BSA IgY may be useful for preparing anti-BSA IgY coupled affinity column for small or large scale purification of BSA. The anti-BSA IgY affinity column may also be useful for purifying serum albumin from nonbovine species (e.g., elk) to which the anti-BSA IgY can bind.

In conclusion, the present results suggest that both the SCWL and RIR hens immunized with BSA can produce egg yolk IgY with similar proportions of BSA-specific antibodies. Thus, the egg yolk weight and the percent hen-day production, both of which were greater in the SCWL hens, appear to be important factors for the efficient production of anti-BSA IgY.

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