

## Limited Day to Day Variation of IgY Levels in Eggs from Individual Laying Hens

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*Laying hens are highly efficient producers of polyclonal antibodies (PAb). These antibodies are transported to the egg yolk in large quantities from the blood of laying hens. The IgY concentration in the egg yolk is important to protect the newly hatched chick against infections and it is also an important factor for the production of yolk antibodies for commercial purposes. A single egg yolk contains approximately the same amount of IgY as 30 ml of blood. This is a significant loss of antibodies for an animal the size of a hen. We have studied the IgY levels in egg yolk. We found low day to day variability. There was no decrease in IgY levels at the end of the egg laying cycle and there was no correlation between IgY concentration and egg production.*

**Keywords:** Antibody, chicken, egg, IgY, immunoglobulin, yolk

### INTRODUCTION

Chicken IgY (Leslie & Clem, 1969), or chicken IgG as it is also called, is the functional equivalent of mammalian IgG. IgY is actively transported to the egg yolk in large quantities from the serum of laying hens during their production period. The maternally derived IgY protect the newly hatched chick until the young has developed its own immune system (Rose *et al.*, 1981). Without sufficient amounts of antibodies, the hatched chick may be infected by microorganisms in the environment resulting in a decreased growth rate and an increased mortality.

Chickens are regarded as very good producers of antibodies (Lösch *et al.*, 1986) as the amount produced is large and the collection is simple since IgY easily can be purified from the egg yolk. ECVAM (European Centre for the Validation of Alternative Methods), an

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organization within the European Community, recommends the use of yolk antibodies instead of mammalian antibodies because of animal welfare reasons (Schade *et al.*, 1996). IgY has biochemical properties that make it attractive for use in immunological assays in the laboratory as well as for use in immunotherapy: IgY neither activates the human complement system nor reacts with rheumatoid factors, human anti-mouse IgG antibodies (HAMA) or human Fc-receptors (Larsson *et al.*, 1993) which all are well known cell activators and mediators of inflammation.

There has been little published about IgY concentration in egg yolk from non-immunized chickens in contradiction to several studies giving figures of the IgY concentration in yolks from immunized chickens. We undertook a study where we followed 10 single comb white leghorn (SCWL) hens during a time period of 28 days and analysed the IgY concentration in each consecutive egg laid over the time period. Our hypothesis is that there would be an equal amount of IgY in each consecutive egg laid by a single individual as it is in the interest of the hen to give the same amount of protection to all of her offspring. The mechanism is a passive, maternally derived, immunization similar to the protection mammals give to their offspring through placental transfer or by colostrum. The day to day variability is also of interest when producing antibodies.

## MATERIALS AND METHODS

### Animals

Ten Single Comb White Leghorn (SCWL) hens, 50 weeks old at the start of the experiment were used. The experiment lasted 28 days. The hens were housed in single hen cages in ordinary batteries in a three-tier system. The temperature in the hen house during the test period was  $16 \pm 1^\circ\text{C}$ . The hens were fed water and all mash laying feed *ad libitum*.

### Yolk Preparation for IgY Measurement

Eggs were collected daily and labelled except during weekends and therefore the Monday collection contained two to three eggs. The eggs were stored in a cold room until antibody preparation. 1 ml of egg yolk from individual eggs were mixed with 4 ml 0.9% NaCl, 3.5% PEG 6000 (KEBO, Stockholm, Sweden), 0.2%  $\text{NaN}_3$  (Polson *et al.*, 1980). After incubation overnight at  $4^\circ\text{C}$ , the samples were centrifuged at  $2000 \times g$  for 10 min. The clear supernatant was used for the quantification of IgY.

### IgY Measurement

The IgY concentration was determined by rate nephelometry on a Beckman Array protein system (Beckman Instruments, Bream, CA, USA). Rabbit anti-IgY was obtained from Immunsystem AB (Uppsala, Sweden). The antibody was diluted 1:5 in 0.9% NaCl, 0.2%  $\text{NaN}_3$ . The samples were diluted in the same buffer. A standard curve was produced with a highly purified IgY sample from Immunsystem AB. The IgY concentrations in the samples were calculated against the standard curve.

### Statistical Analysis

Statistical analysis was performed with Statistica 4.5 (StatSoft, Tulsa, OK, USA).  $P < 0.05$  was considered significant.

## RESULTS

### Day to Day Variation of IgY

The IgY concentrations among individuals varied from  $2.8 \pm 0.3$  (mean  $\pm$  SD) to  $7.0 \pm 0.5$  (Figure 1). The day to day variation in IgY concentration in a single individual was low in

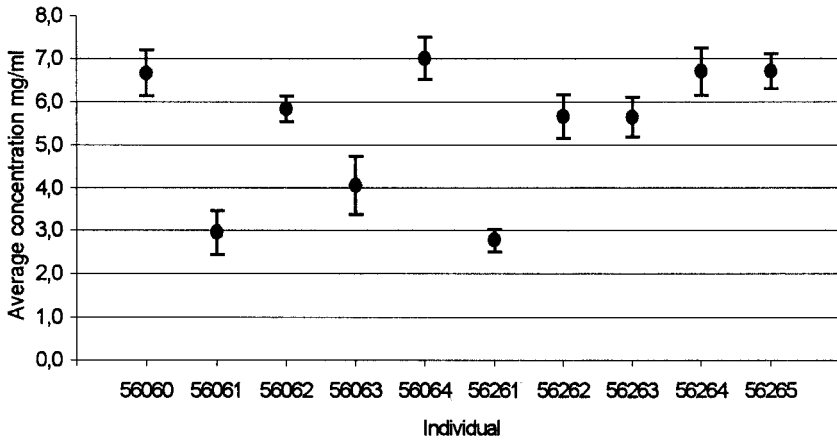


FIG. 1. IgY concentration in egg yolk (mean  $\pm$  SD) in 10 Single Comb White Leghorn hens over a time period of 28 days.

relation to the inter-individual variation. There was no decrease in IgY concentration at the end of the egg-laying cycle (results not shown).

#### Correlation Between IgY Concentration and Egg Production

The number of eggs produced per hen varied between 8 and 25 eggs. There was no correlation between the number of eggs produced and the concentration of IgY in the yolk. The  $r^2$ , coefficient of determination was 0.0051 (Figure 2).

#### DISCUSSION

We have studied the IgY concentrations in egg yolk over a period of 4 weeks in 10 randomly chosen SCWL hens. The animals were not immunized to avoid changes in IgY

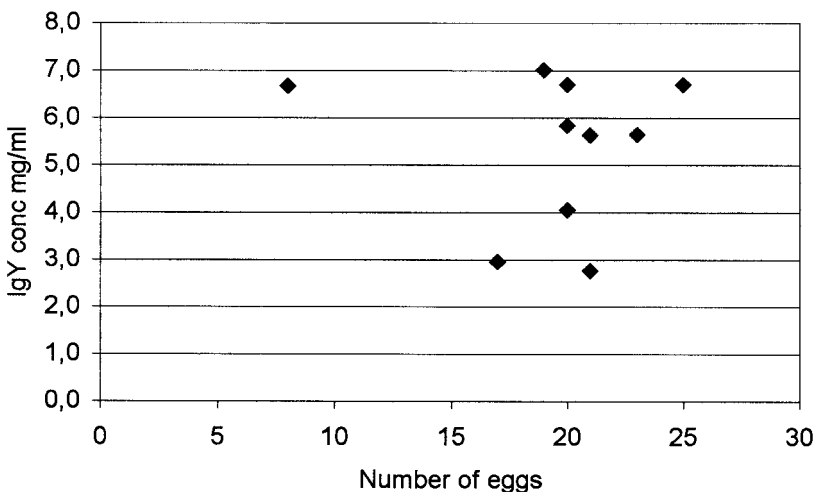


FIG. 2. IgY concentration versus number of eggs produced by 10 Single Comb White Leghorn hens

concentrations due to the formation of specific antibodies (Patterson *et al.*, 1962). There was a more than twofold difference between individuals with high and low IgY concentrations and this difference was stable over the time period studied (Figure 1). The same amount of protective antibodies is transported to each consecutive egg and the chance of survival to each one of her progeny would not be affected by different starting opportunities. We did not see a decreased transport of IgY to the yolk when the hen broke the egg laying to set herself back in phase or a higher concentration in the first egg after the break.

Life-history theory assumes that the reproductive effort is costly and is achieved at the expense of other resources such as a reduced immune response (Nordling *et al.*, 1998). A hen that lays many eggs will lose a lot of IgY, which is costly to replace. This could result in an increased susceptibility to infections and a decreased IgY transfer to the yolk. In this experiment we found no correlation between yolk IgY concentration and egg production. This is opposed to the life-history theory but in a situation with unlimited food supply and modern hygienic production environment, the hen has the possibility to increase the protein intake to balance the loss. With a limited food supply the reproductive effort would be achieved at the expense of other resources.

An egg contains the same amount of IgY as 30 ml of blood. This is a considerable loss for a hen with a blood volume of approximately 100–125 ml. Without a highly efficient synthesis and transport of IgY this loss would cause a significant drop in IgY concentration. The rate of IgY transfer from serum is correlated to the size of the developing oocyte and the IgY concentration in the oocyte is essentially constant throughout the entire oocyte maturation (Kowalczyk *et al.*, 1985). There is an active transport of IgY from the hen to the egg (Rose *et al.*, 1981) resulting in a higher IgY concentration in the egg yolk than in serum (Rose *et al.*, 1974).

Earlier studies have shown that SCWL have a greater hen-day production as well as greater egg and yolk weights giving an overall rise in specific antibody production compared to Rhode Island Red (Li *et al.*, 1998). This was shown in an immunization trial made with bovine serum albumen (BSA) as the antigen. Using an average yolk volume of 15 ml our data gives the amount of IgY in the yolk to be between 42 and 105 mg/yolk which corresponds well to earlier reported figures of 105 mg/yolk in immunized chickens. The volume of an ordinary egg yolk is approximately 15 ml, which contains around 100 mg of antibodies ( $7 \text{ mg ml}^{-1}$ ). The average concentration in this experiment is  $5.4 \text{ mg ml}^{-1}$  ( $2.7 \text{ mg ml}^{-1}$  up to  $7.0 \text{ mg ml}^{-1}$ ). Löscher *et al.* (1986) reported the yolk concentration to be  $3.4 \text{ mg ml}^{-1}$  in non-immunized eggs. Kowalczyk *et al.* (1985) reported the average concentration in non-immunized yolks to be about  $7.9 \text{ mg ml}^{-1}$ , by a radioimmunoassay. The discrepancy between our value and other reported values is probably due to differences between strains studied and genetic variation among individuals and losses during the initial PEG precipitation step used in this study. This step was performed to obtain a clear supernatant without high molecular weight molecules that could interfere with the nephelometric assay. The average egg production in this experiment was 19.4 eggs/28 days, and this represents an egg yolk volume of approximately 300 ml egg yolk per month. This is much more than the serum volume that can be obtained by bleeding chickens or rabbits.

Egg yolk is a convenient source for large-scale production of polyclonal antibodies (PABs), and it avoids the traditional bleeding of animals (Löscher *et al.*, 1986). Eggs have been used as a source for the production of PABs specific to a variety of different infectious agents such as bacteria (Sugita-Konishi *et al.*, 1996; Hatta *et al.*, 1997), virus (Bartz *et al.*, 1980; Kuroki *et al.*, 1993; Kuroki *et al.*, 1994) and parasites (Gottstein & Hemmeler, 1985). The exact molecular mechanism behind the effect of egg yolk antibodies on bacterial growth is not known, but the antibodies have been shown to inhibit bacterial adhesion, bacterial growth and toxin production (Sugita-Konishi, *et al.*, 1996).

In summary, yolk antibodies offer several biochemical advantages compared to mammalian antibodies when used for detection of mammalian proteins. Yolk antibodies

recognize other epitopes than mammalian antibodies (Song *et al.*, 1985) and raising antibodies in laying hens is therefore a suitable alternative to mammalian antibodies, especially for the production of antibodies against antigens that are weakly immunogenic in mammalian species. In contrast to mammalian antibodies, immune complexes containing IgY will not cause immune complex mediated effects in the human gastrointestinal tract such as cell activation or inflammation. IgY also seems, in our opinion, to be the best alternative for peroral immunotherapy when large amounts of antibody is needed. It is easy to scale up the production of yolk antibodies, as hens are inexpensive to maintain in comparison to rabbits. An important property regarding peroral immunotherapy is that eggs are also normal dietary components and there is practically no risk of toxic side effects of IgY.

The day to day variation in this experiment was small in comparison to the differences between individuals. Thus, genetic differences will have a strong influence on the IgY concentration in egg yolk, and by breeding it will most likely be possible to raise the IgY concentration.

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## REFERENCES

- BARTZ, C. R., CONKLIN, R. H., TUNSTALL, C. B. & STEELE, J. H. (1980) Prevention of murine rotavirus infection with chicken egg yolk immunoglobulins, *Journal of Infectious Disease*, **3**, 439–441.
- GOTTSTEIN, B. & HEMMELER, E. (1985) Egg yolk immunoglobulin Y as an alternative antibody in the serology of echinococcosis, *Zeitschrift für Parasitenkunde*, **71**, 273–276.
- HATTA, H., TSUDA, K., OZEKI, M., KIM, M., YAMAMOTO, T., OTAKE, S., HIRASAWA, M., KATZ, J., CHILDERS, N. K. & MICHALEK, S. M. (1997) Passive immunization against dental plaque formation in humans, effect of a mouth rinse containing egg yolk antibodies (IgY) specific to *Streptococcus mutans*, *Caries Research*, **4**, 268–274.
- KOWALCZYK, K., DAISS, J., HALPERN, J. & ROTH, T. F. (1985) Quantitation of maternal-fetal IgG transport in the chicken, *Immunology*, **54**, 755–762.
- KUROKI, M., IKEMORI, Y., YOKOYAMA, H., PERALTA, R. C., ICATLO, F. C. & KODAMA, Y. (1993) Passive protection against bovine rotavirus-induced diarrhea in murine model by specific immunoglobulins from chicken egg yolk, *Veterinarian Microbiology*, **37**, 135–146.
- KUROKI, M., OHTA, M., IKEMORI, Y., PERALTA, R. C., YOKOYAMA, H. & KODAMA, Y. (1994) Passive protection against bovine rotavirus in calves by specific immunoglobulins from chicken egg yolk, *Archives of Virology*, **138**, 143–148.
- LARSSON, A., BÄLÖW, R. M., LINDAHL, T. L. & FORSBERG, P. O. (1993) Chicken antibodies, taking advantage of evolution – a review, *Poultry Science*, **72**, 1807–1812.
- LESLIE, G. A. & CLEM, W. L. (1969) Phylogeny of immunoglobulin structure and function. III. Immunoglobulin of the chicken, *Journal of Experimental Medicine*, **130**, 1337–1352.
- LI, X., NAKANO, T., SUNWOO, H. H., PAK, B. H., CHAE, H. S. & SIM, J. S. (1998) Effects of egg and yolk weights on yolk antibody (IgY) production in laying chickens, *Poultry Science*, **77**, 299–270.
- LÖSCH, U., SCHRANNER, I., WANKE, R. & JURGENS, L. (1986) The chicken egg, an antibody source, *Zentralblatt für Veterinärmedizin [B]*, **8**, 609–619.
- NORDLING, D., ANDERSSON, M., ZOHARI, S. & GUSTAVSSON, L. (1998) Reproductive effort reduces specific immune response and parasite resistance, *Proceedings of the Royal Society of London B*, **265**, 1291–1298.
- PATTERSON, R., YOUNGER, J. S., WEIGLE, W. O. & DIXON, F. J. (1962) Antibody production and transfer to egg yolk in chickens, *Journal of Immunology*, **89**, 272–278.
- POLSON, A., VON WECHMAR, M. B. & VAN REGENMORTEL, M. H. V. (1980) Isolation of viral IgY antibodies from yolk of immunized hens, *Immunological Communications*, **9**, 475–493.
- ROSE, M. E. & ORLANS, E. (1981) Immunoglobulins in the egg, embryo and young chick, *Developmental and Comparative Immunology*, **5**, 15–20.
- ROSE, M. E., ORLANS, E. & BUTTRESS, N. (1974) Immunoglobulin classes in the hen's egg, their segregation in yolk and white, *European Journal of Immunology*, **4**, 521–523.

- SCHADE, R., STAAK, C., HENDRIKSEN, C., ERHARD, M., HUGL, H., KOCH, G., LARSSON, A., POLLMANN, W., REGENMORTEL, M., RIJKE, E., SPIELMANN, H., STEINBUSCH, H. & STRAUGHAN, D. (1996) The production of avian (egg yolk) antibodies, IgY, *Alternatives to Laboratory Animals*, **24**, 925–934.
- SONG, C. S. YU, J. H., BAI, D. H., HESTER, P. Y. & KIM, K. H. (1985) Antibodies to the alpha-subunit of insulin receptor from eggs of immunized hens, *Journal of Immunology*, **5**, 3354–3359.
- SUGITA-KONISHI, Y., SHIBATA, K., YUN, S. S., HARA-KUDO, Y., YAMAGUCHI, K. & KUMAGAI, S. (1996) Immune functions of immunoglobulin Y isolated from egg yolk of hens immunized with various infectious bacteria, *Bioscience, Biotechnology and Biochemistry*, **5**, 886–888.