Developmental and Comparative Immunology 52 (2015) 10–16

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

The influence of year, laying date, egg fertility and incubation, individual hen, hen age and mass and clutch size on maternal immunoglobulin Y concentration in captive Steller's and spectacled eider egg yolk

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ARTICLE INFO

Article history:
Received 16 January 2015
Revised 28 March 2015
Accepted 12 April 2015
Available online 16 April 2015

Keywords:
Steller's eider
Spectacled eider
Immunoglobulin Y
Maternal transfer
Egg yolk
Passive immunity

ABSTRACT

Steller's eiders and spectacled eiders are sea duck species whose populations have declined significantly and infectious diseases could influence offspring survival. Therefore, the maternal transfer of immunoglobulin Y (IgY) into yolk was investigated in captive Steller's and spectacled eiders during the 2007–2013 breeding seasons. This project had two objectives: establish baseline IgY levels in Steller's and spectacled eider yolk under controlled captive conditions and evaluate the effect of year, laying date, egg fertility, egg incubation duration, individual hen, hen age and mass, and laying order to determine which variables influenced IgY levels. Average IgY concentrations were 0.03–0.48 mg ml^{-1} in Steller's eider yolk and 0.10–0.51 mg ml^{-1} in spectacled eider yolk. The year and individual hen influenced IgY concentration in Steller's and spectacled eider yolk. The laying date was negatively correlated with egg IgY levels for most Steller's eider hens, but laying order was positively correlated with egg IgY concentration for spectacled eiders.

1. Introduction

Steller's eiders (*Polysticta stelleri*) and spectacled eiders (*Somateria fischeri*) breed in coastal regions of western and northern Alaska, but both species have experienced significant population declines (Quakenbush et al., 2004; Stehn et al., 1993). The Alaska breeding population of Steller's eiders was listed as threatened under the Endangered Species Act in 1997 due to reductions in their numbers and nesting habitat (Federal Register, 1997). Spectacled eiders were listed as threatened in 1993 because of population decline, especially in western Alaska (Federal Register, 1993). The reason for the precipitous decline in the eider populations is unknown, although predation, climate change, contaminants and disease may be factors (U.S. Fish and Wildlife Service, 1996, 2002). Brood survival can be highly variable in eiders, and understanding factors affecting duckling survival is important (Flint et al., 2006; Safine, 2013).

Maternal transfer of antibodies via yolk is important for the health and survival of ducklings. Ducklings hatch with a functional innate immune system, but their adaptive immune system is still developing (Davison et al., 2008). Newly hatched ducklings depend on maternal immunoglobulin Y (IgY), which is the primary antibody in serum and the principal antibody involved in defense against systemic infections (Davison et al., 2008; Lundqvist et al., 2006). The passive immunity provided by the mother to the hatchling protects the offspring against disease agents present in the environment, and can be especially important in dense breeding populations where disease transmission may be enhanced (Addison et al., 2009; Garnier et al., 2012). Maternal IgY will form complexes with antigens to protect the duckling and alleviate pressure on the duckling's developing immune system (Addison et al., 2009). Besides providing protection against pathogens, maternal antibodies can have additional benefits for the duckling. The presence of maternal IgY can stimulate B cell development and augment or modulate responses to antigens that will result in optimization of future reactions to the same antigen (Fink et al., 2008; Lemke and Lange, 1999). Idiotype alterations could also arise that modify the specificity of

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http://dx.doi.org/10.1016/j.dci.2015.04.005
0145-305X/

immunoglobulins for antigens or even suppress particular antibody types (Lemke and Lange, 1999). The availability of maternal antibodies to defend the hatchling against parasites can decrease demands on its growth and development by reducing the need for an innate immune response which allows the duckling to focus resources on musculature and feather development (Grindstaff, 2008).

Maternal IgY is transferred into yolk through the ovarian follicle from the serum of the hen and the quantity transferred is proportional to IgY concentrations in hen serum (Hamal et al., 2006; Kowalczyk et al., 1985). The IgY is transported to the circulation of the developing embryo across the yolk sac (Hamal et al., 2006; Kowalczyk et al., 1985). Immunoglobulin Y is the last antibody to be synthesized by newly hatched birds and generally begins around 2 weeks post-hatch in chickens and 3 weeks post-hatch in mallards (Hamal et al., 2006; Liu and Higgins, 1990; Mast and Goddeeris, 1999). Mallard ducklings have the highest concentration of maternal IgY in their serum after hatching and levels decline to their lowest point 2 weeks post-hatch (Liu and Higgins, 1990). The bursa of Fabricius fully matures when the hatchling is 5–7 weeks old and chickens are capable of the full range of B lymphocyte and antibody production at this point, but mallards do not synthesize adult levels of IgY until they are 10 weeks old (Davison et al., 2008; Liu and Higgins, 1990).

The quantity of IgY a hen allocates into her eggs depends on several factors. First, the hen is only capable of transferring antibodies that are present in her serum (Kowalczyk et al., 1985). Hens have been shown to transfer specific antibodies into yolk due to vaccination (Al-Natour et al., 2004; Rollier et al., 2000) or because of an active infection prior to egg laying (Buechler et al., 2002; Gasparini et al., 2001). Therefore, differences in immunological experience among females could be reflected in the yolks of their eggs. The age of the female could also influence IgY deposition in yolk because humoral immunosenescence can occur in older females resulting in decreased antibody availability for disease resistance and distribution into yolk (Barua et al., 1998a, 1998b; Cichon et al., 2003; Haussmann et al., 2005). Environmental factors can impact yolk IgY concentrations, also. Higher breeding densities of birds have been linked to increased IgY concentrations potentially due to elevated pathogen prevalence (Muller et al., 2004), while a sufficient food supply results in lower IgY concentrations (Blount et al., 2002; Gasparini et al., 2007). Egg production can be biologically demanding, so the amount of IgY transferred into yolk is likely influenced by individual and environmental factors that vary each breeding season (Lochmiller and Deerenberg, 2000).

The distribution of maternal antibodies within a clutch may also vary. Black-headed gulls provide the first laid egg with the most IgY (Groothuis et al., 2006), while collared flycatchers allocate the most IgY to the last egg in the clutch (Hargitai et al., 2006). Both species are asynchronously hatching species and earlier laid eggs usually have a higher chance of survival (Groothuis et al., 2006; Hargitai et al., 2006). Providing the first laid eggs with higher concentrations of IgY furthers their advantage, while distributing more IgY to later laid eggs may be an attempt to help them survive (Groothuis et al., 2006; Hargitai et al., 2006).

The level of IgY provided by Steller's and spectacled eider hens could be important for duckling survival in the event of disease exposure. Therefore, the maternal investment of IgY in yolk was investigated using samples collected from eggs laid by captive Steller's and spectacled eiders during the breeding seasons of 2007–2013. This project had two objectives: establish baseline IgY levels in Steller's and spectacled eider egg yolk under controlled captive conditions and evaluate the effect of year, laying date, egg fertility, egg incubation duration, individual hen, hen age, hen mass, and laying order to determine which variables influenced IgY levels. The goal of this project was to characterize maternal transfer of IgY in Steller's and spectacled eiders.

2. Materials and methods

2.1. Study species

Captive adult Steller's and spectacled eiders were housed outdoors separately at the Alaska SeaLife Center (ASLC) in specially designed enclosures. The frame work of the habitat was aluminum and supported fiberglass pools that were 7′ × 6′ and 2′ deep. The decking was level with the edge of the pool and was perforated fiberglass, covered with 3M Nomad matting. Waste water drained through these substrates into an aluminum catchment drain and was piped to the ASLC waste water sump where it was sanitized. The eiders were kept in larger flocks during the non-breeding season with access to salt water pools. During the breeding season, breeding pairs were separated from the main flock and provided access to fresh water pools. Salt or fresh water was supplied to each of the pools, flowed constantly and skimmed off the surface. The habitat sides were enclosed with nylon mesh with holes of 1″ square and the roof panels were nylon gillnet with holes of 3″ square. Visual barriers were placed between flocks or breeding pairs. Birds were fed a diet of Mazuri (Purina, St. Louis, MO USA) sea duck pellets. These pellets were dispensed in automatic feeders which have a paddle that the birds push on when they want food. Their diet was supplemented with krill (*Euphasia pacifica*), mussels (*Mytilus trossulus*), silversides (*Menidia menidia*) and clams (*Spisula solidissima*), all of which comprised less than 5% of their total diet. Fresh drinking water was provided when the pools contained salt water. Pools were cleaned and disinfected every week and pen surfaces were disinfected every 2 weeks. Pen surfaces were hosed off as needed. The feeding areas were cleaned daily and the automatic feeders disinfected monthly. The eiders were weighed monthly except during the breeding season (June–July) to prevent disruption of nesting. Eggs were collected from the hens after laying, marked to note laying order, and incubated at least 5 days to assess fertility. Eggs were cut around the mid-section and the yolk and albumen were separated. The yolk was homogenized and stored at –20 °C until IgY extraction. Yolk samples were obtained from 180 Steller's and 223 spectacled eider eggs collected during the breeding seasons of 2007–2013.

2.2. IgY extraction from egg yolk

Proteins were extracted from the yolk by mixing 0.5 ml PBS with 0.5 ml yolk and adding 2.0 ml chloroform. The solution was vortexed for 10 minutes and incubated at room temperature for 1 hour. The mixture was centrifuged at 1400 rpm for 35 minutes and the supernatant removed and stored at –20 °C until analysis in the IgY assay.

2.3. IgY assay

Immunoglobulin Y levels were measured in the egg yolk extracts using an enzyme-linked immunosorbent assay. Flat-bottom 96-well plates were coated with 10 μg ml^{-1} rabbit anti-chicken IgY capture antibody (Sigma-Aldrich, St. Louis, MO, USA) in carbonate–bicarbonate coating buffer. The plates were washed 4 times with 0.25 ml of wash buffer (PBS with 0.05% Tween) using a plate washer and then 0.05 ml of yolk extract, standard or blank (PBS) was added to the plate. Dilutions of chicken IgY were used as the standard. The plates were incubated at 37 °C for 1 hour and then washed 4 times with wash buffer. Alkaline phosphatase labeled rabbit anti-chicken IgY (Sigma-Aldrich) was diluted 1:2000 and 0.05 ml added to each well. The plates were incubated at 37 °C for 1 hour then washed 4 times with wash buffer. Each well had 0.05 ml of p-nitrophenyl phosphate disodium salt (PNPP, Thermo Scientific, Rockford, IL, USA) substrate added to it and the plate was incubated for 30 minutes