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Variations in leucocyte profiles and plasma biochemistry are related to different aspects of parental investment in male and female Upland geese *Chloephaga picta leucoptera*

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ABSTRACT

The analysis of plasma biochemistry and haematology to monitor the condition of birds in the wild has been found a useful tool in ecological research. Despite biparental investment in most wild birds studied, some studies of condition indices found sex differences, and attributed these to the costs of egg formation or brooding in females or a higher contribution of males to chick rearing. We studied the natural variation of haematological and plasma biochemistry parameters (namely leucocyte, lymphocyte and heterophil counts, H/L ratio and plasma concentrations of proteins, triglycerides and carotenoids) in relation to the different measures of parental investment in males and females in the Upland goose (*Chloephaga picta leucoptera*), a socially monogamous species. We found no sex differences in haematological and most plasma biochemistry parameters, but a relation to different aspects of parental investment in breeding male and female Upland geese. H/L ratios were related to body condition and capture date in males while leucocyte counts, plasma protein and plasma carotenoid concentrations varied with clutch measures and hatching date in females. Higher H/L ratios of males in a low body condition and later in the year may reflect stress associated with the investment into the establishment and defence of the breeding territory. Females with higher clutch volumes had lower total leucocyte and lymphocyte numbers and higher levels of plasma protein. Earlier hatching dates were associated with lower numbers of all leucocyte types and higher values of plasma carotenoid concentrations. This indicates that differences in health state are reflected in reproductive performance in female Upland geese. We also found sexual differences in the repeatability of haematological and plasma biochemistry parameters between years and therefore suggest that their potential as a measure of individual quality differs between male and female Upland geese. Finally, numbers of leucocyte counts and plasma triglyceride concentrations of pair partners were significantly related. No study so far investigated these parameters in pair partners and we discuss possible reasons for our finding.

1. Introduction

Many studies assessing the relationship of condition indices and reproductive performance in wild birds have been carried out in species, where males and females share a great amount of the duties associated with chick rearing and territory defence (e.g. Kilgas et al., 2006b; Moreno et al., 1998; e.g. Moreno et al., 2002b; Shutler et al., 2004). Despite the biparental care some of the studies found sex differences in stress measures, and attributed this to the costs of egg formation (Jakubas et al., 2008; Kilgas et al., 2006a; Moreno et al., 2002b) or brooding (Hõrak et al., 1998a) in females or a higher contribution of males in the late phase of chick rearing (Jakubas et al., 2008).

Our study describes the natural variation of haematological and plasma biochemistry parameters (namely leucocyte, lymphocyte and heterophil counts, H/L ratio and plasma concentrations of proteins, triglycerides and carotenoids) in relation to the different measures of parental investment in males and females such as incubation and chick rearing in the Upland goose (*Chloephaga picta leucoptera*). Upland geese belong to the order of the sheldgeese (Tadornini), a group that resembles true geese and shows similar habits, but is more closely related to shelducks and ducks. The basic breeding biology and life-cycle of Upland geese has been studied in the Falklands from 1977 to 1980 (Summers, 1983). Upland geese are highly territorial and socially monogamous, usually returning to the same territory with the same mate every year. Take up of territories starts in August, egg laying commences in late September, and most clutches are finished by the end of October. Hatching mainly takes place between mid-October and mid-November and fledging starts when chicks are about 70 days old. Males and females differ in their specific parental roles, with males establishing and intensely defending the territory and females incubating and brooding. We predict that these differences in parental roles are reflected in haematological and blood chemistry parameters.

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The analysis of plasma biochemistry and haematology to monitor the condition of birds in the wild has been found a useful tool in ecological research, as it may give a more integrative picture of the state of an animal than condition indices based on body mass alone. Leucocyte profiles, i.e. the relative numbers of different leucocyte types in the peripheral blood, have been used to study the health and condition of birds in the wild (reviewed in Davis et al., 2008). Lymphocytes and heterophils make up the majority of white blood cells in birds. Lymphocytes are highly specific and involved in a variety of immunological functions like the modulation of the immune response and immunoglobulin production (Campbell, 1995; Hawkey and Dennet, 1989). They increase in numbers during parasitic infection (Bonier et al., 2007; Ots and Hõrak, 1998) and immunological challenges (Eeva et al., 2005). A decrease in lymphocyte numbers may either be linked to stress-induced immunosuppression (Hõrak et al., 1998b) or the absence of parasite infections. Heterophils are non-specific phagocytic cells that proliferate in response to infections, inflammation and stress (Campbell, 1995; Maxwell and Robertson, 1998) and increased numbers can be found during stress, trauma and chronic bacterial infections. The heterophil/lymphocyte ratio (H/L) is often used as stress indicator in birds (Gross and Siegel, 1983; Maxwell, 1993), that is known to increase in the presence of various stressors, such as infectious diseases or starvation.

Some simple measures of blood chemistry in relation to health and condition of individuals include plasma protein concentrations, which are considered to be linked to nutritional status in birds, with rising concentrations when dietary protein intake increases or depressed levels indicating nutritional inadequacies (Jenni-Eiermann and Jenni, 1996; Jenni-Eiermann and Jenni, 1997, 1998; Ots et al., 1998; Rodríguez et al., 2005). High values of protein may also be caused by hemoconcentration, e.g. due to dehydration. Plasma triglycerides have also been linked to health state and fat reserves (Lloyd and Gibson, 2006; Masello and Quillfeldt, 2004; Quillfeldt et al., 2004), as they reflect the deposition of lipids into adipose tissues and thereby recent nutritional changes. Fasting individuals in a post-resorptive state, where triglycerides are hydrolysed from adipose tissues generally exhibit lower values of triglycerides (e.g. Jenni-Eiermann and Jenni, 1997; Jenni-Eiermann and Jenni, 1998). A third indicator of health state is the plasma concentration of carotenoids. Carotenoids are natural pigments that cannot be synthesized by vertebrates and hence must be obtained via the diet (Brush, 1981; Fox, 1979; McGraw, 2005). They have a range of health-related functions and are known to work as antioxidants and immune-enhancers (Lozano, 1994; Olson and Owens, 1998) and both direct (plasma concentration) and indirect (plasma hue) measures of carotenoids could be linked to body condition (Mougeot et al., 2009), immunocompetence (Mougeot et al., 2007; Perez-Rodriguez et al., 2008b; Quillfeldt et al., 2004) and ornamentation (Masello and Quillfeldt, 2004; Mougeot et al., 2009).

The specific aims of the current study were

(1) to determine possible sex differences in haematological and plasma biochemistry parameters in Upland geese,
(2) to determine whether haematological and plasma biochemistry parameters are linked to sex-specific parental investment,
(3) to estimate repeatability within individuals in consecutive years and to determine possible influences on variability, and
(4) to compare haematological and plasma biochemistry parameters between pair members of Upland geese.

2. Materials and methods

2.1. Study site

The study was carried out in the New Island Nature Reserve, Falkland Islands (51°43′S, 61°17′W) from October to December 2007 and 2008. The island has been established as a nature reserve in 1970 when all livestock was removed from the island. This led to an increase in the density of Upland geese, which is now one of the highest in the Falkland Islands (Quillfeldt et al., 2005).

2.2. Field measurements and sampling

At the start of each field season we mapped nests using GPS. For each nest, we determined clutch size, measured length (L, expressed in cm) and breadth (B, expressed in cm) of each egg to the nearest 0.1 mm using callipers and weighed each egg to the nearest 0.1 g using a digital balance. Egg volume (V; in cm^3) was calculated as $V=(L\times B^2\times 0.507)$ following Furness and Furness (1981). We defined total clutch volume as the sum of the volumes of each egg in the clutch. Avian eggs decrease in density with increasing length of incubation mainly due to water loss. We estimated density loss during a preliminary study in 2005 by weighing 41 eggs (from 6 clutches) twice within a period of 3–10 days. Eggs lost an average of 0.0063 ± 0.0005 SE g/cm^3 per day. We determined expected hatching date as follows: (1) we calculated egg density (D) from egg mass M (expressed in g) and V as $D=M/V$. (2) Our 2005 data showed that hatching occurred at a mean egg density of $0.89\ g/cm^3\pm0.01\ g/cm^3$ SE ($n=14$ eggs). (3) Thus, the number of days to hatching T was estimated as $T=(D-0.89\ g/cm^3)/0.0063\ g/cm^3$. We visited nests at least once a day, starting at the estimated hatching date; all eggs hatched within 0–2 days from the estimated hatch date.

We caught adults during the period when they attended their brood (chick ages 0 to 45 days, mean 11 ± 1.4 days) using a 3×5 m whoosh net. One person herded the family of geese slowly to the catching area, and when they arrived directly in front of the furled net, the other researcher pulled the trigger. Adults were marked with individual metal rings and weighed to the nearest 10 g using a digital spring balance. Head length, culmen length, and tarsus length were measured to the nearest 0.1 mm using callipers; wing length (maximum flattened chord) was measured to the nearest 1 mm using a foot rule. A blood sample (approximately 300 μL) was collected from the brachial vein. Blood samples were kept cold (4 °C) and centrifuged within 4 h. The separated plasma was stored at −20 °C until plasma protein, triglyceride and carotenoid levels were determined (see below).

Chicks were marked individually using web-tags and weighed to the nearest 1 g using a spring balance (<300 g) or to the nearest 10 g using a digital spring balance (>300 g). We measured head length, culmen length, wing length and tarsus length (±0.1 mm) using callipers. Chick ages were determined from a growth curve for head and tarsus established from chicks of known age in 2005. For each clutch, the mean chick age and thereby mean hatching date were calculated. As hatching dates could not be determined from egg density for all pairs, we used this estimated hatching date for further analyses. Estimated hatching dates from egg measures and chick measures were highly correlated ($r=0.973$, $P<0.001$, $N=41$).

The body condition of adults was determined accounting for structural size based on a regression of body mass on the first principal component (PC1) of measurements of wing, head, bill and tarsus. Body condition was then calculated as the ratio of the observed body mass to the derived expected body mass.

2.3. Leucocyte counts

The differential leucocyte count was determined as described by Ruiz et al. (2002) by examining whole blood air-dry smears. Immediately after returning from the field (no later than 4 h after sampling), the blood sample was well shaken and a drop of blood was smeared on a glass slide, using the standard two slide wedge procedure. All samples were fixed with absolute methanol. In the laboratory, smears were stained using Giemsa stain at a 1:10 dilution, for 20 min. Differential leucocyte counts were carried out with a light microscope (1000×, magnification with oil immersion), crossing the