BLOOD VALUES OF COMMON CRANES (*GRUS GRUS*) BY AGE AND SEASON

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Abstract—1. We studied the blood composition of common cranes (*Grus grus*) along the wintering period (October–March).

2. Plasma proteins decreased along the winter period whereas plasma urea increased. Both parameters were highly correlated.

3. Plasma levels of uric acid, triglycerides and cholesterol did not change during the winter. Young birds showed higher levels of plasma triglycerides.

4. Red blood cell number, hematocrit and blood hemoglobin content were similar in young and adult birds. Lower values of hematocrit and red cell number were recorded in early autumn.

5. Total white blood cell number did not change during the winter, young birds showing higher numbers than adults. Lymphocyte number decreased from November to March while heterophil counts increased.

INTRODUCTION

The annual cycle of many birds is characterized by two migratory periods, a spring migration that leads the birds to the breeding areas and an autumn migration that leads them to the most favourable environmental conditions of the winter quarters. It seems therefore apparent that functional status of a migratory bird may be changing throughout the year. However, there is an absence of literature about the exact nature of these changes. In a previous study we dealt with the blood composition of common cranes (*Grus grus*) (Puerta *et al.*, 1990). The present study analyses the changes in blood composition from November to March, i.e. during the wintering period of the common cranes.

MATERIALS AND METHODS

Cranes were captured in November 1988, January 1989, January 1990 and March 1990 at Gallocanta, northeastern Spain. In November and January birds were captured using oral tranquillizers, and in March 1990, with rocket-nets. Plumage differences were used to distinguish adult (older than 1 year) from young (first year) birds. Blood was collected from the radial vein with heparinized syringes (25 U/ml) and carried to the laboratory in cool containers.

Aliquots of blood were diluted (200 and 50 times for red and white cells, respectively) in hematological pipettes with Natt and Herrick's (1952) solution. Red and white blood cells were counted using 50–96 small squares for red cells and all the large squares for white cells of a cell counting Thoma chamber. Hematocrit was determined by centrifugation at 10,000 rpm/12 min. Hemoglobin was assayed according to the colorimetric method of Drabkin (1945).

Blood smears were fixed through 3 min immersion in methanol at the time of blood collection. They were stained with commercial Giemsa stain (Merck, Germany) diluted in phosphate buffer (1:4.5) pH 6.8 for 45 min. Leukocyte identification and counting were carried out using a light microscope with an oil immersion lens ($\times 100$). In each sample 180–400 white cells were counted.

Plasma was obtained by centrifugation and it was stored at -20°C until analysis. Plasma proteins were assayed after Lowry *et al.* (1951). Triglycerides, cholesterol, urea and uric acid were assayed with commercial kits (Cromatest, Knickerbocker Lab., Spain).

Statistical analysis was made by a two-way ANOVA (age and month), $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

During their first winter the common cranes start to moult their juvenile plumage. Accordingly, from their second year on, they are referred to as adults. However, they do not reach sexual maturity until about the age of 3–4 years (Glutz *et al.*, 1973). It should also be mentioned that the spring migration begins in late February, the mature birds migrating first (Alonso *et al.*, 1984). March captures took place in middle March, i.e. when most of the mature birds had left the study area. Accordingly, adults captured in March were probably “sexually immature birds”. Therefore, while the adult groups of November and January were made up of both sexually mature and immature adults, the March group of adults was fundamentally made up of sexually immature birds. These facts must be taken into account when analysing the present results.

The two-way ANOVA did not show differences in plasma levels of proteins, urea or uric acid between young and adult birds, although the levels of uric acid could be somewhat higher in young birds during early

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Fig. 1. Plasma levels of uric acid, proteins and urea of common cranes during the autumn–winter in Spain. Bars represent mean $\pm$ SE of the number of animals in parentheses. Open bars: young birds; dotted bars: adults.

winter, as shown in Fig. 1 and previous data (Puerta *et al.*, 1990). However, the same analysis showed a time-related variation in plasma proteins ($P < 0.001$) and in plasma urea ($P < 0.001$) whereas plasma uric acid remained unchanged during the studied period (Fig. 1). Indeed, plasma levels of proteins decreased along the study period ($r = -0.689$; $P < 0.001$) contrary to plasma urea, which was increasing ($r = 0.645$; $P < 0.001$). Considering the present data it seems difficult to state the reason for the decreased plasma proteins. However, if we take into account that March-sampled adults were sexually immature and that sexually mature birds had already left the winter quarters, a plausible explanation could be a competition for protein sources between both kinds of adults, those better fed migrating earlier. In fact, cranes reach their heaviest weight in January (Alonso *et al.*, unpublished results). Moreover, the high correlation between plasma levels of urea and proteins (Fig. 2) is remarkable whereas plasma uric acid was unaltered throughout the winter months (Fig. 1). Fasting or undernourishing are known to increase plasma urea in several species of raptors (Ferrer *et al.*, 1987; Garcia-Rodriguez *et al.*, 1987). Also, plasma urea has been related not only to plasma proteins but also to protein intake in underweight infants (Polberger *et al.*, 1990a,b). Therefore, on the whole, our data suggest that migratory trips could depend not only on lipid reserves (Marsh, 1983) but also on protein intake. Finally, although bird urine contains uric acid as the main nitrogenous end product, it seems that plasma levels of urea are a more sensitive parameter related to variations in protein metabolism, at least in common cranes.

There were no monthly differences in cholesterol or in triglycerides, which agrees with Kocan and

Pitts (1976). On the other hand, plasma cholesterol was similar in young and adult common cranes while young birds showed higher levels ($P < 0.05$) of plasma triglycerides than adults (Fig. 3).

The two-way ANOVA did not reveal any difference in red blood cell (RBC) number, hematocrit and blood hemoglobin content between young and adult birds (Fig. 4), which is in accordance with a previous study (Puerta *et al.*, 1990). However, the same statistical analysis indicates that the RBC number and hematocrit do change along the winter period ($P < 0.05$). Available data from other species show that only during summer is there an appreciable drop in RBC number, hematocrit and blood hemoglobin content, together with a tendency for autumn values to be lower than in spring and winter (deGraw *et al.*, 1979; Ronald and George, 1988). Figure 4 shows that the values of hematocrit and RBC number were lower in November, which agrees with previous data (Kocan and Pitts, 1976; deGraw *et al.*, 1979; Ronald and George, 1988). Since blood hemoglobin content was however unchanged, it seems that blood hemoglobin content is a more precisely regulated blood parameter.

Total white blood cell (WBC) number seems influenced by age (Puerta *et al.*, 1990; Alonso *et al.*, 1991; Puerta *et al.*, 1992) or by captivity conditions (Puerta *et al.*, 1992). In fact, we have again found that young common cranes have a greater number of WBC than adults ($P < 0.05$) (Fig. 5). We found no changes in total WBC number during the winter but, unexpectedly, there was a significant seasonal variation in the relative proportions of heterophils ($P < 0.001$) and lymphocytes ($P < 0.001$), the other types (namely, eosinophils, basophils and monocytes) remaining

Fig. 2. Urea versus total proteins plasma levels of common cranes during the autumn–winter in Spain.

Fig. 3. Plasma levels of triglycerides and cholesterol of common cranes during the autumn–winter in Spain. Bars represent mean $\pm$ SE of the number of animals in parentheses. Open bars: young birds; dotted bars: adults.

Fig. 4. Red blood cell number, hematocrit and blood hemoglobin content of common cranes during the autumn-winter in Spain. Bars represent mean $\pm$ SE of the number of animals in parentheses. Open bars: young birds; dotted bars: adults.

unchanged. As can be seen in Fig. 5, lymphocytes show a tendency to decrease during the winter, this trend being more apparent in young birds, where they are more abundant than in adults. On the contrary, during March heterophils are the most abundant WBC type. The reason for this change in the relative proportion of lymphocytes and heterophils cannot be deduced from the present data. However, it could be that the animals sampled in November had just reached the winter quarters and were probably exposed to new infectious or immunotoxic agents which led to an increase in lymphocytes. The even higher lymphocyte proportion in young birds (which were in the winter quarters for the first time) compared with that of adults (which have visited the same geographic area at least once) gives some support to this hypothesis.

It is well known that differential leukocyte counts differ among different avian species. Thus, while some species show lymphocytes as the main WBC type (Fairbrother and O'Loughlin, 1990; Puerta *et al.*, 1992) others show mainly heterophils (Conetta *et al.*, 1974; Hawkey *et al.*, 1983; Alonso *et al.*, 1991) and even others show both types in similar proportions (Leonard, 1982; Puerta *et al.*, 1990; Puerta *et al.*, 1991). Age (Puerta *et al.*, 1989; Fairbrother and O'Loughlin, 1990; Alonso *et al.*, 1991), reproductive state (Nirmalan and Robinson, 1971) and moult (Driver, 1981) are known to influence differential counts. This study indicates that the annual cycle should be considered as another factor, at least in migratory birds.

Fig. 5. Total and differential white blood cell counts of common cranes along the autumn-winter in Spain. Bars represent mean $\pm$ SE of the number of animals in parentheses. Y: Young birds; A: adults.

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