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# A comparative study of some haematological and biochemical parameters between two species from the Anatidae family within migration season

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## Abstract

**Background:** The study of haematological and biochemical parameters is important to evaluate the physiological status in wild birds. In order to carry out ecophysiological or conservation studies, it is important to know the baseline physiological parameters and how these vary with age, sex and life history events.

**Results:** Haematological parameters including red blood corpuscles (RBCs), white blood cells (WBCs), haematocrit, haemoglobin and related RBC indices and plasma and biochemical parameters including glucose, lipid profile and liver function tests, kidney function tests and electrolyte concentration reference values were determined in two long-distance migratory species from the family Anatidae, the northern shoveler (*Anas clypeata*) and Eurasian teal (*Anas crecca*), during migration season. Data revealed significant inter-species differences for most blood and biochemical parameters. These differences are likely due to different feeding habit, diet and species-specific migration behaviour rather than phylogenetic reasons or different environmental condition as they winter at the same place (Máñez et al., Conservation Monitoring in Freshwater Habitats, 2010).

**Conclusion:** To our knowledge, this is the first study that performs a comparative blood analysis on northern shoveler and Eurasian teal during their overwintering period in Manzala Lake, Egypt. Due to the lack of haematological studies on both species during migration, the current results represent a contribution to understanding the physiological adaptations that facilitate the use of different habitats in these species and for establishing reference physiological values and for comparison within the same species, or even with other species in different periods with regard to their use in future conservation efforts or other studies.

**Keywords:** Haematology, Plasma biochemistry, Anas spp., Migration, Overwintering site

## Background

Migratory northern shoveler (*Anas clypeata*) and Eurasian teal (*Anas crecca*) are two common and widespread freshwater ducks. They breed in northern areas of Europe and Asia (Clements, 2007) and wintering in southern Europe and Africa. Both species are of the species to which the Agreement on the Conservation of African-Eurasian Migratory Water birds (AEWA) applies.

The migratory water birds are classified as being "in danger of extinction" and its principle threats during migration include human disturbance including hunting, the loss and degradation of their overwintering habitat in wetlands and fishing (Amat & Green, 2010; Máñez et al., 2010). So far, no study has been conducted to assess haematological and biochemical conditions of those species during migration season, although the determination of haematological and biochemical parameters offers an efficient method to assess body condition in free-living birds. Both haematologic and biochemical parameters are good indicators of the metabolic state and are affected by different seasonal processes related to molting, breeding and migration as well as daily fluctuations due to circadian rhythm (Jenni-Eiermann et al., 2002). Moreover,

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this kind of studies may provide evidence that some physiological responses throughout the annual cycle may be species-specific (Cooke et al., 2013). The bird health status may help to detect possible pathologies (Cafarchia et al., 2006; Galkina, L'vov, Gromashevskiĭ, & Moskvina, 2004), monitor immunological status (L'vov et al., 2007) and complete parasitological surveys (Plutzer & Tomor, 2009).

During migration, birds are exposed to different stress situations such as high metabolic demands, physical activity, food quality and quantity or environmental contaminants which are able to induce changes in blood parameters (Sturkie & Griminger, 1986; Vleck & Vleck, 2002) that may lead to migratory and breeding constraints (Studds & Marra, 2005).

Although studies of the haematology and blood biochemistry of closely related species of birds are of a comparative physiological importance (Gee, Carpenter, & Hensler, 1981), those studies are scarce and focus mainly on birds from different taxa. Physiological studies on wildlife have proved to be relevant (Jabbour, Hayssen, & Bruford, 1997; Seiser et al., 2000). For this reason, it has been suggested that physiological ecology of wild animals should be incorporated into conservation policies and monitoring programmes of endangered populations (Carey, 2005; Seiser et al., 2000). However, a short-term plan aiming to evaluate and investigate the sudden environmental events, such as deaths of animals, rarely asks for immediate ecophysiological studies. They contribute to a greater understanding of the variation of blood characteristics in relation to such factors as phylogenetic position, ecological habitat, food selection and mode of life.

This study is a comparative analysis of haematologic and plasma biochemical parameters in migratory northern shoveler and Eurasian teal overwintering in northern Egypt. To our knowledge, this is the first study dealing with haematological and biochemical parameters in both species during the overwintering stage. The aim of this study was to quantify and compare some key parameters of haematology and blood biochemistry and to provide reference values for normal physiologic parameters for both species with respect to migration.

## Methods

### Study area

This study was conducted at Manzala Lake where the northern shoveler and Eurasian teal used to overwinter along the edges of the lake in Shata area, northern Egypt (at the borderline between Damietta Governorate and Port Said Governorate; 31° 16′ 2″ N, 32° 2′ 5″ E) (Elarabany, Abdallah, & Said, 2012). Birds were caught using mist nets or baited "Potter" traps during overwintering season (November–March 2014). A total of 30 adult birds were chosen for the study (shoveler = 14 and Eurasian teal = 15). Birds considered as unhealthy by physical examination were not included in this study. Licences for capturing and blood sampling of birds were obtained from the Egyptian Environmental Affairs Agency (EEAA).

### Blood sampling

Birds were weighed, and blood samples were collected from the brachial vein representing about 1% of the body weight (Lumeij, 1997) using a heparinized syringe. To minimize variation in the blood parameters due to the circadian rhythm, all samples were collected between 11:00 AM and 12:00 PM (García-Rodríguez, Ferrer, Recio, & Castroviejo, 1987). The time elapsing from capture to blood withdrawal was less than 3 min to avoid any change in the physiologic parameters due to handling associated stress. Each blood sample was divided into three aliquots, the first aliquot was used for blood cells count, the second aliquot was used for haematocrit and haemoglobin determination and the third aliquot was left for 1 h and then centrifuged at 3000 rpm for 10 min to isolate plasma; the harvested plasma was stored at − 20 °C until assayed later in the same day.

### Blood cells (RBCs, WBCs and thrombocytes)

Erythrocytes (RBCs) were counted in a haemocytometer (improved Neubauer chamber) after diluting the blood sample in saline (Ferrer, 1929). The raw data of RBCs was multiplied by 10,000 to obtain the final values (Campbell, 1995; Pierson, 2000). WBC total number was determined by counting all leucocytes in the chamber and multiplying the raw data by 220 to obtain the final values (Campbell, 1995; Pierson, 2000). A drop of blood was smeared on a microscope slide, air-dried, and stained with Wright-Giemsa stain for both total thrombocyte count and leucocytes' differential count (Tavares-Dias & de Moraes, 2006; Tavares-Dias & Moraes, 2007). Absolute differential count of WBCs was calculated from the relative of examining a total of 100 leucocytes under oil immersion. Differential leucocyte counts were obtained by multiplying their proportions with WBCs.

### Haematocrit, haemoglobin and RBC indices

In order to evaluate haematocrit value or the packed cell volume (PCV), blood samples were placed in microcapillary tubes up to 75% of the tube length then sealing the tube bottom by using wax. Sealed tubes were centrifuged at 14000 rpm for 5 min (Morris & Davey, 2001), and PCV results were determined by using a microhaematocrit reader.

Haemoglobin (Hb) was determined photometrically by using cyanohemoglobin method (Drabkin & Austin, 1935; Hawk & Oser, 1965), where 10 μl of the whole blood was added to 5 ml of Drabkin's reagent. After

10 min, this mix was centrifuged at 3000 rpm for 5 min to avoid the interference of lysed RBC nuclei in optical density. Optical density was measured in a spectrophotometer at wavelength 540 nm.

The haematocrit (%), haemoglobin (g/dl) and RBCs values were used to calculate RBC indices including the mean corpuscle volume (MCV), the mean corpuscle haemoglobin (MCH) and the mean corpuscle haemoglobin concentration (MCHC) (Campbell, 1995; Seiverd, 1964).

### Biochemical parameters

Plasma chemistry for the following 15 parameters were determined photometrically using GENWAY spectrophotometer: albumin (ALB), total protein (TPP), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), cholesterol (CHOL), triglycerides (TRI), creatinine (CREA), blood urea nitrogen (BUN), uric acid (UR AC), glucose (GLU), calcium (CA), sodium (SOD), potassium (POT) and inorganic phosphorus (PHOS). Methods used for their assay are listed in Table 1. Absorbance values of samples and standards were measured with a microplate spectrophotometer, all samples were measured in duplicates.

**Table 1** Methods of analysis

| Parameter | Unit | Method (test kit/reagent) |
|---|---|---|
| Albumin (ALB) | g/dl | Bromocresol green (biodiagnostics) |
| Total protein (TP) | g/dl | Biuret reagent (biodiagnostics) |
| Aspartate aminotransferase (AST) | U/l | Kinetic method (BIOADWIC) |
| Alkaline phosphatase (ALP) | U/l | Kinetic method (BioMED) |
| γ-Glutamyl transpeptidase (GGT) | U/l | Activity Colorimetric (Sigma-aldrich) |
| Cholesterol (CHOL) | mg/dl | Enzymatic colorimetric method (bio-diagnostic) |
| Triglycerides (TRI) | mg/dl | Glycerol phosphate oxidase-peroxidase enzymatic method (BioMED) |
| Creatinine (CREA) | mg/dl | Colorimetric method, endpoint (bio-diagnostic) |
| Blood urea (UREA) | mg/dl | Enzymatic colorimetric method (BioMED) |
| Uric acid (UR AC) | mg/dl | Enzymatic colorimetric method (BioMED) |
| Glucose (GLU) | mg/dl | Enzymatic glucose oxidase method (BioMED) |
| Calcium (CA) | mg/dl | Color. E. point method (bio-diagnostic) |
| Sodium (SOD) | mmol/l | Color. E. point method (bio-diagnostic) |
| Potassium (POT) | mmol/l | Turbidimetric method (bio-diagnostic) |
| Inorganic phosphorus (PHOS) | mg/dl | Color. E. point method (bio-diagnostic) |

### Statistical analysis

Values are presented as mean ± SD for each species, normal distribution of all variables was tested, student *t* test was used to compare haematological and biochemical parameters between the two species, t value and df, are also provided. Statistical analyses were carried out using the SPSS software package version 19.0. A probability value (*P*) of less than 0.05 was considered to be significant.

## Results

### Haematocrit, haemoglobin and RBCs indices

Mean values, standard errors and ranges are summarized in Table 2. Only haemoglobin value was significantly higher in northern shoveler than Eurasian teal ($P = 0.007$, $t_{df} = 1.94$). However, no significant differences were found between the two species for PCV, MCV, MCH, MCHC.

### Blood cell count

Comparative haematological results are provided in Table 2. Statistical analysis revealed that RBC number was significantly higher in northern shoveler than Eurasian teal ($P = 0.025$, $t_{df} = 2.13$). On the other hand, thrombocyte number and lymphocyte percentage were significantly lower in northern shoveler than Eurasian teal ($P = 0.0005$, $t_{df} = 1.89$ and $P = 0.027$, $t_{df} = 1.94$, respectively). No significant differences were found in reticulocytes, WBCs, eosinophil, basophils or monocytes between the two species. Northern shoveler had higher reticulocytes, neutrophils and eosinophils, and lower WBCs, lymphocytes and monocytes count than Eurasian teal. The heterophils/lymphocytes (H/L) ratio was significantly higher in the Northern shoveler than in European teal (2.33 and 1.92, respectively; $P = 0.03$, $t_{df} = 1.98$).

### Plasma biochemical analysis

Means and ranges of whole blood biochemical parameters are listed in Table 3. Results showed significant differences in ALT activity, glucose, cholesterol, uric acid potassium and sodium levels between the two species. In particular, northern shoveler showed higher glucose, cholesterol and uric acid levels (GLU, $P = 0.0007$, $t_{df} = 2.01$; CHO, $P = 0.01$, $t_{df} = 2.13$; and UR AC, $P = 0.03$, $t_{df} = 2.77$, respectively) and lower ALT activity ($P = 0.0005$, $t_{df} = 2.57$), potassium ($P = 0.01$, $t_{df} = 2.13$) and sodium ($P = 0.04$, $t_{df} = 2.77$) than Eurasian teal. The other biochemical parameters did not show any significant differences. The highest of albumin, total proteins, creatinine, BUN levels, ALP activity and GGT activity were found in Eurasian teal, whereas the highest levels of triglycerides, calcium, uric acid and

**Table 2** Haematological parameters of migratory Northern shoveler (*Anas clypeata*, $n = 14$) and Eurasian teal (*Anas crecca*, $n = 15$) during overwintering period in Manzala Lake, Northern Egypt

| Parameter | *Anas clypeata* | | *Anas crecca* | |
|---|---|---|---|---|
| | Mean ± SD | Min–Max | Mean ± SD | Min–Max |
| Erythrocytes ($10^6$/μl) | 5.75 ± 0.92* | 4.8–6.9 | 4.2 ± 0.36 | 3.6–4.6 |
| PCV (%) | 46.25 ± 8.14 | 34–55 | 38.2 ± 4.21 | 30–42 |
| Hb (g/dl) | 15.125 ± 1.13* | 14.2–17 | 12.36 ± 0.96 | 11–13.9 |
| MCV (fl) | 88.075 ± 13.71 | 70.8–106.1 | 91.52 ± 13.30 | 69–108.3 |
| MCH (PG) | 28.975 ± 2.58 | 24.6–31.1 | 29.38 ± 1.12 | 27.8–30.9 |
| MCHC (g/dl) | 33.5 ± 4.97 | 41.8–28.8 | 32.66 ± 4.43 | 27.7–40.7 |
| Reticulocytes (%) | 0.35 ± 0.05 | 0.3–0.4 | 0.32 ± 0.04 | 0.3–0.4 |
| Thrombocytes ($10^3$/μl) | 237.5 ± 32.6 | 210–290 | 388 ± 41.66* | 320–450 |
| Leucocytes ($10^3$/μl) | 6.05 ± 1.8 | 3.2–8.5 | 6.68 ± 1.3 | 4.5–8.2 |
| Neutrophils ($10^3$/μl) | 3.9 ± 0.05 | 1.9–5.6 | 4.05 ± 0.05 | 2.5–5.4 |
| Neutrophils (%) | 64.5 ± 2.60 | 60–66 | 60.6 ± 3.6 | 55–66 |
| Eosinophil ($10^3$/μl) | 0.23 ± 0.02 | 0.10–.50 | 0.2 ± 0.00001 | 0.13–0.25 |
| Eosinophil (%) | 3.75 ± 1.30 | 3–6 | 3 ± 0.001 | 2.9–3 |
| Lymphocyte ($10^3$/μl) | 1.68 ± 0.02 | 0.86–2.6 | 2.1 ± 0.04 | 1.2–3 |
| Lymphocyte (%) | 27.75 ± 1.30 | 27–30 | 32 ± 3.2* | 27–37 |
| Monocytes ($10^3$/μl) | 0.24 ± 0.00001 | 0.12–0.34 | 0.29 ± 0.01 | 0.18–0.41 |
| Monocytes (%) | 4.00 ± 0.001 | 3.9–4 | 4.4 ± 0.5 | 4.0–5 |
| Heterophil/lymphocyte (H/L) | 2.33 ± 0.19* | 2.00–2.44 | 1.92 ± 0.31 | 1.49–2.44 |

*$p < 0.05$

**Table 3** Plasma biochemical values for migratory northern shoveler (*Anas clypeata*, $n = 14$) and Eurasian teal (*Anas crecca*, $n = 15$) during overwintering period in Manzala Lake, Northern Egypt

| Parameter | *Anas clypeata* | | *Anas crecca* | |
|---|---|---|---|---|
| | Mean ± SD | Min–Max | Mean ± SD | Min–Max |
| Albumin (g/dl) | 1.325 ± 0.08 | 1.2–1.4 | 1.35 ± 0.11 | 1.2–1.5 |
| Total protein (g/dl) | 6.975 ± 0.42 | 6.5–7.6 | 7.1 ± 0.29 | 6.9–7.6 |
| AST (U/l) | 16 ± 2.55 | 12–19 | 34 ± 3.94* | 30–40 |
| ALP (U/l) | 131.5 ± 11.19 | 119–145 | 145.25 ± 9.34 | 132–155 |
| GGT (U/l) | 5.75 ± 0.43 | 5–6 | 6.0 ± 1.41 | 4.0–8.0 |
| Cholesterol (mg/dl) | 391.67 ± 23.61* | 359–414 | 321.25 ± 22.73 | 290–352 |
| Triglycerides (mg/dl) | 144.67 ± 12.68 | 132–162 | 126.5 ± 5.02 | 121–132 |
| Creatinine (mg/dl) | 0.475 ± 0.11 | 0.32–0.63 | 0.53 ± 0.11 | 0.36–0.62 |
| Blood urea (mg/dl) | 20.5 ± 3.84 | 15–25 | 21.5 ± 1.12 | 20–23 |
| Uric acid (mg/dl) | 6.4 ± 0.90* | 5.1–7.5 | 4.65 ± 0.45 | 4.5–40 |
| Glucose (gm/dl) | 188.25 ± 7.26* | 177–196 | 136.5 ± 12.46 | 122–155 |
| Calcium (gm/dl) | 6.65 ± 0.34 | 6.2–7.1 | 6.35 ± 0.75 | 5.6–7.6 |
| Sodium (mmol/l) | 109.25 ± 15.88 | 82–121 | 131 ± 5.74* | 122–136 |
| Potassium (mmol/l) | 3.1 ± 0.07 | 3–3.2 | 3.425 ± 0.15* | 3.2–3.6 |
| Inorganic phosphorus (mg/dl) | 3.925 ± 0.41 | 3.4–4.5 | 3.475 ± 0.33 | 2.9–3.7 |

*$p < 0.05$

inorganic phosphorus were found in northern shoveler. No differences were found within the same species.

## Discussion

Both haematological and biochemical parameters have been frequently used as a useful and non-invasive indicator of general condition for many species of vertebrates (Jenni & Schwilch, 2001; Sánchez-Guzmán et al., 2004). However, these advantages are limited by some factors such as lack of reference values and sensitivity of the haematological and biochemical variables, to uncontrolled environmental conditions and the state of birds (Newman, Piatt, & White, 1997; Nyholm, 1998). Migration is a very critical event which promotes many physiological and behavioural adaptations (Schwilch, Grattarola, Spina, & Jenni, 2002; Scott, Mitchell, & Evans, 1994).

### Haematocrit, haemoglobin and RBC indices

Haematocrit value has often been used as an indicator of health status in both wild and captive or domestic birds (Fair, Whitaker, & Pearson, 2007). Approximately, 15% of the adult avian species showed a PCV value of 35–40%. Mean value is 44% (Hawkey, Bennett, Gascoyne, Hart, & Kirkwood, 1991). Northern shoveler showed a higher haematocrit values than European teal (46.25% and 38.2%, respectively), although these values lie within those estimated for another bird species with values of 29% for hens and 58.5% for pigeons (Sturkie & Griminger, 1986), but still lower than those values for diving duck, Lesser scaup (*Aythya affinis*), which shows a PCV value higher than 50% (Glomski & Pica, 2011) and higher than Pilgrim goose (*Anser anser*). This is in agreement with other studies showing that migration in temperate climates causes haematocrit to be higher in winter probably due to dehydration, oxygen demand during migration, molt and preparation for reproduction (Fair et al., 2007).

Concerning RBCs and haemoglobin, Northern shoveler had higher haemoglobin concentration than European teal (Table 2). It is likely that birds with high levels of haematocrit are accompanied by their high Hb levels (Kostelecka-Myrcha, Zukowski, & Oksiejczuk, 1997; Palomeque, Palacios, & Planas, 1980). The difference in haemoglobin concentration is related to the changes in blood volume per unite of body weight, generally birds with bigger body mass have higher PCV and Hb content (Nirmalan & Robinson, 1971).

RBC indices are values that indicate the size, haemoglobin content, and concentration of the average erythrocyte circulating in a subject's blood (Glomski & Pica, 2011) and were calculated indirectly with reference to RBCs, Hb, and PCV values; therefore, their changes are directly related to these blood measurements. MCV, MCH and MCHC values were the range described in other bird species; all birds showed the phylogenetic modification of the efficacy of the circulating red cells, e.g. reduction in the erythrocyte size which is reflected in MCV less than 100 fl (Hauptmanova, Literak, & Bartova, 2002; Kostelecka-Myrcha & Jaroszewicz, 1993). This is in accordance with our results where both Northern shoveler and European teal showed MCV lower than 100 fl (88 fl and 91.52 fl, respectively).

### Blood cells count

White blood cells are the defence cells of the body; their levels have a great participation in the immune responses and the ability of animals to fight infection (Schalm, Jain, & Carroll, 1975). Birds with higher WBC count will be able to resist infection more than those with lower WBCs level. Our finding showed that Northern shoveler had lower mean WBCs level than European teal.

Only mean lymphocyte number was significantly different between the two species, where Northern shoveler showed lower lymphocytes percentages (Table 2). On the other hand, no significant differences were found between other leucocyte types. This could be due to many factors such as age, nutritional status and ambient temperature. (Sturkie, 2012). Moreover, these WBCs types usually show low range of variation in most species (0 to 5% in basophils and 0 to 10% in monocytes), which might be an additional explanation for the absence of any significant differences between species. Briefly, the leucocyte number of both study species is the same as that of the domestic hen (Jain, 1993).

Heterophils/lymphocytes (H/L) ratio was significantly higher in the Northern shoveler (2.33) than in European teal (1.92); this ratio usually provides important information for immune system function, following the prolonged effect of stress factors such as starvation or fasting due to the lack of food resources in overwintering site (Scope, Filip, Gabler, & Resch, 2002; Totzke, Fenske, Hüppop, Raabe, & Schach, 1999).

Avian thrombocytes have the capability to be involved in the immune response by producing and releasing a vast array of bioactive proteins (Semple, Italiano, & Freedman, 2011; Weyrich & Zimmerman, 2004). In the present study, thrombocyte count (TC) followed the same trend of WBCs, where Northern shoveler showed lower TC than European teal (Table 3). Several studies suggested that bird's thrombocytes have phagocytic ability and represent a link between innate and adaptive immunity (Kogut et al., 2005; Kogut, Rothwell, & Kaiser, 2002).

### Plasma biochemical analysis

Changes in plasma biochemical parameters have been used frequently as indicators of nutritional and health status in birds (Artacho, Soto-Gamboa, Verdugo, & Nespolo, 2007; Rodríguez, Tortosa, & Villafuerte, 2005). Measurement of

plasma metabolites offers a rapid and reproducible method to study the metabolism and physiological state of free-living birds (Jenni & Jenni-Eiermann, 1998). Multiple blood metabolites (lipids, proteins and glucose) are regulated by dietary intake and metabolism and serve as an indicator of the nutritional status of wild marine birds, which is strongly related to the health status (Artacho et al., 2007; Rodríguez et al., 2005).

Total plasma protein (TPP) is an important parameter for evaluating the nutritional status in birds and, thus, can be useful in determining some infectious diseases (Harr, 2002). Northern shoveler showed lower mean plasma protein concentration (Table 3); TPP concentration in both species was higher than those reported in other *Anas* sp., 3.5–5.5 g/dl (Lumeij, 1997). This is likely related to the feeding habit in migratory birds which have a high food absorption rate and higher liver ability to synthesize proteins (Dawson & Bortolotti, 1997). Moreover, some proteins are catabolized during flight in order to obtain endogenous protein (Jenni & Jenni-Eiermann, 1998; Klaassen, Kvist, & Lindstrom, 2000). Protein could also be mobilized to provide water during dehydration caused by endurance flight (Klaassen, 1996), or as an adaptive phenotypic plasticity of flight muscle size as result of decreasing body mass during migration (Jenni & Jenni-Eiermann, 1998).

Aspartate aminotransferase (AST) is best described as a very sensitive indicator of liver disease and muscle damage (Harr, 2002). The values of AST in both study species were similar to those described for other species (Garcia, Hermosa, & Aguirre, 2010; Tlak, Milinković--Tur, Stojević, & Piršljin, 2008) and in the same range for many Anatidae, 12–73 U/l (Lumeij, 1997). In addition, the significant difference observed in AST between Northern shoveler and European teal (Table 3) could be related to nutritional rate, bird physiology or inflammatory processes that might occur in migratory birds (Calabuig, Ferrer, & Muriel, 2010).

Alkaline phosphatase (ALP) is another enzyme that is usually related to the metabolic rate in many Anatidae (Boettcher, 2004; Calabuig, Ferrer, Muriel, & Tilgar, 2010; Olsen, Rininger, Ets, & Sladen, 2002). The higher values of alkaline phosphatase (AP) found European teal could be explained by the higher metabolic rate than Northern shoveler (Table 3).

Cholesterol, triglycerides and glucose levels are related to the quantitative and qualitative composition of the diet (Calabuig, Ferrer, & Muriel, 2010). Mean cholesterol values (Table 3) differed very significantly between the two study species. Mean values of plasma cholesterol exceeded those found for other non-migratory Anatidae, 104–244 mg/dl (Lumeij, 1997), and those found for Northern shoveler during preparation spring migration, 106 mg/dl (Elarabany, 2014), but very close to those concentrations which were found previously in Northern shoveler during mid-winter in Egypt, 454.4 mg/dl (Elarabany, 2014) and to those in starving birds, 391.8 mg/dl (Jeffrey, Peakall, Miller, & Herzberg, 1985). Starvation has been related to an increase in blood cholesterol levels due to fat reserve mobilization (Black, 1981; Galvin, 1980).

Triglyceride levels (Table 3) obtained in the present study were similar to those reported in the same species previously at arrival to overwintering site and during mid-winter time, 86.44–200 mg/dl (Elarabany, 2014) and to those reported for gruiform birds, 102–190 mg/dl (Gee et al., 1981; Livezey, 1998). The higher values found in Northern shoveler (144.6 mg/dl) and European teal (126 mg/dl) compared to non-migratory birds, e.g. Black-necked swan (*Cygnus melanocoryphus*), 90 mg/dl (Artacho et al., 2007), may be due to elevation of triglyceride levels in migratory birds since they constitute the important source of energy mobilized from the fat deposits in order to permit them to migrate (Jenni-Eiermann et al., 2002; Jenni-Eiermann & Jenni, 1994).

Creatinine is widely used as an indicator of renal integrity and its concentration is related to diet (Woerpel & Rosskopf, 1984). Creatinine level in both Northern shoveler and European teal (Table 3) lie within the range for many birds, 0.30–1.90 mg/dl (Lumeij, 1997).

No significant differences in BUN concentration were found between the two species; their BUN concentration was higher compared to that found in other bird species which ranged from 7 mg/dl in the common buzzard (*Buteo buteo*) to 14.9 mg/dl in the vulture (Balasch, Musquera, Palacios, Jimenez, & Palomeque, 1976). Additionally, our BUN values were very similar to those found previously in Northern shoveler, 18.0 mg/dl, in Egypt during mid-winter (Elarabany, 2014). High levels of BUN were reported before in common buzzard where it reached 50 mg/dl when going through a period of poor or unavailable food supply (Rodríguez et al., 2005). Since birds were sampled during the winter during migration season, a season characterized by changing in food habit and aggregation of different dabbling ducks (Balasch et al., 1976), high BUN values, as well as cholesterol levels, might suggest a deficient feeding of both ducks, the same as occurred in the common buzzard.

Mean uric acid values obtained in this study for both species of ducks (Table 3) were within the range reported for arriving Northern shoveler and during mid-winter period, 2.8–8.6 mg/dl (Elarabany, 2014), and many anatids, 2–12 mg/dl (Dombrowski, Bourgeois, Couture, & Linard, 2003; Levengood et al., 2000), for species of the Gruiformes order (coots, cranes and rails), 0.9–12.4 mg/dl, and for red-legged partridge (*Alectoris rufa*), which ranged between 6.8 and 10.6 mg/dl (Rodríguez et al., 2005).

The highly significant inter-species difference for uric acid concentration could be due to fasting, similar results were found in common buzzard in a fasting period, 3 mg/dl (García-Rodríguez, Ferrer, Carrillo, & Castroviejo, 1987). Moreover, uric acid levels are highly associated with diet (Polo, 1995). This may explain the relatively low uric acid in European teal as they usually change their feeding habit from feeding on small invertebrates to be granivorous during migration. In captive and wild Mallards (*Anas platyrhynchos*), the levels of uric acid during, and following, molt were higher than levels before the molt. In wild *B. canadensis*, uric acid levels varied between annual cycle phase (Mori & George, 1978). Apparently, there are specific physiologic reasons (e.g. higher demand for uric acid) during specific periods; birds have decreased levels of uric acid because the nutrients available during molting, when the birds are flightless, differ from other times of the year.

Glucose is the main metabolite of the carbohydrate metabolism; the main source of glucose is the diet or, in the fasting state, glycogen stores or from gluconeogenesis. Its plasma concentration is kept within very narrow limits, regulated by hormonal control, even in fasting stat, because glucose is the main source of energy for the central nervous system (Rodríguez et al., 2005; Totzke et al., 1999). Glucose values in this study were significantly higher in Northern shoveler than in European teal (Table 3), suggesting a greater removal of plasma glucose in European teal that can be used as an energy source during feeding and migration (Scanes & Braun, 2013). Glucose concentration in both species was extremely lower than those estimated for other Anatidae, 232–369 mg/dl, indicating lower intake of food and probably minor glycogen reserves that could happen after long migration flight.

All plasma electrolytes measured in this study were within the normal range for other birds (Table 3). Although sodium levels in plasma were significantly different between the two species but still similar to the values reported in other birds, 130–160 mmol/l (Balasch et al., 1973; Balasch, Palomeque, Palacios, Musquera, & Jimenez, 1974). The potassium values for Northern shoveler were statistically lower than those of European teal and lie within the range of most birds, 2–4 mmol/l (Campbell, 2004).

On the other hand, no significant inter-species differences were found for calcium and phosphorus (Table 3). Phosphorus values were in the same range of other species of birds, 1–5 mg/dl (Campbell, 2004; Ghebremeskel, Williams, Keymer, Horsley, & Gardner, 1989). Calcium concentration was similar to those reported for 13 different non-breeding species, less than 8 mg/dl (Campbell, 2004). They were very low than those concentrations that have been found in egg-laying hens, 11 mg/dl (Campbell, 2004; Taylor, Simkiss, & Stringer, 1971).

## Conclusions

To our knowledge, this is the first study that performs a comparative blood analysis on northern shoveler and Eurasian teal during their overwintering period in Manzala Lake, Egypt. Due to the lack of haematological studies on both species during migration, the current results represent a contribution to understand the physiological adaptations that facilitate the use of different habitats in these species and for establishing reference physiological values and for comparison within the same species, or even with other species in different periods with regard to their use in future conservation efforts or other studies.

The present results showed similar values of haematological parameters for the two studied species along with some differences in RBC counts and total and differential WBC counts, where European teal was more stressed than Northern shoveler. Future sex-specific and long-term studies are necessary to establish seasonal baseline physiological values for a better understanding of the mechanisms underlying variation in body condition that affects reproductive fitness and subsequent life events.

#### Abbreviations

AEWA: Agreement on the Conservation of African-Eurasian Migratory Water birds; ALB: Albumin; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; BUN: Blood urea nitrogen; CA: Calcium; CHOL: Cholesterol; CREA: Creatinine; EEAA: Egyptian Environmental Affairs Agency; GGT: Gamma-glutamyl transpeptidase; GLU: Glucose; H/L ratio: Heterophils/lymphocytes ratio; Hb: Haemoglobin; MCH: Mean corpuscle haemoglobin; MCHC: The mean corpuscle haemoglobin concentration; MCV: Mean corpuscle volume; PCV: Packed cell volume; PHOS: Inorganic phosphorus; POT: Potassium; RBCs: Red blood corpuscles; SOD: Sodium; TC: Thrombocyte count; TPP: Total protein; TRI: Triglycerides; UR AC: Uric acid; WBCs: White blood cells

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#### Availability of data and materials

No raw data are deposited.

#### Authors' contributions

Single author planned the work and did all the field and lab work, tabulating the data and all analysis, and writing of the manuscript. The author read and approved the final manuscript.

#### Ethics approval

Licences for capture, handling and blood sampling of birds were obtained from the Egyptian Environmental Affairs Agency (EEAA). All experiments were done according to research protocols approved by the animal care committee of the National Research Center, Egypt.

#### Consent for publication

No human subjects are included.

#### Competing interests

The author declares that she has no competing interests.

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