Italian Journal of Zoology

ISSN: 1125-0003 (Print) 1748-5851 (Online) Journal homepage: https://www.tandfonline.com/loi/tizo20

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To cite this article: E. Sengul, V. Gelen, A. Kara, N. Simsek, A. Karadeniz & H. Balkaya (2015) Enzyme histochemical, histometric and hematological features of peripheral blood cells in Sparrowhawk *Accipiter nisus* (Falconiformes: Accipitridae), Italian Journal of Zoology, 82:2, 151-156, DOI: 10.1080/11250003.2014.1001451

To link to this article: https://doi.org/10.1080/11250003.2014.1001451

Published online: 12 Jan 2015.

Italian Journal of Zoology, 2015, 151–156
Vol. 82, No. 2, http://dx.doi.org/10.1080/11250003.2014.1001451

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Enzyme histochemical, histometric and hematological features of peripheral blood cells in Sparrowhawk *Accipiter nisus* (Falconiformes: Accipitridae)

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(Received 24 December 2013; accepted 15 December 2014)

Abstract
The aim of this study was to determine the enzyme-histochemical, histometric and hematological features of peripheral blood cells in the Sparrowhawk (*Accipiter nisus*). Therefore, blood samples obtained from the wings (brachial vein) of nine Sparrowhawk were used. Red blood cell (RBC) and white blood cell (WBC) counts, enumeration of leukocyte types, hematocrit (Htc) value and hemoglobin concentration were determined by hemocytometric methods on blood samples. Also, the erythrocyte indices (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)) were calculated. Histometric analyses were estimated with an image analyzing system. Populations of positive and negative lymphocytes were counted by the alpha naphthyl acetate esterase (ANAE) staining method. When compared to other bird species, RBC counts were high, WBC counts and percentages of WBC except percentage of eosinophil, and percentage of ANAE-positive lymphocyte were similar, and diameters of RBC and nucleus were smaller. This preliminary study contributes by broadening the hematological research on wild bird species and provides a guideline for identifying blood cells in the Sparrowhawk.

Keywords: *Sparrowhawk, peripheral blood cells, hematological features, ANAE*

Introduction

Eurasian Sparrowhawk is the common name (Gustafson 2007) given to the bird species *Accipiter nisus* (Linnaeus, 1758) that belongs to the *Falconiformes* order, the *Accipitridae* family and the *Accipitrinae* subfamily (Kara et al. 2014). Some species of Sparrowhawk, having a size between hawks and kestrels and also known as goshawks, are used especially in the Black Sea Region of Turkey for hunting quail. The intimate relationship between these birds, which exist abundantly in nature, and people, especially with regard to hunting, indicates the importance of research on these animals.

Studies done to determine the hematological parameters of domestic and wild birds make important contributions in evaluating these animals' state of health or hematologic conditions, and are important sources in the literature (Campbell 1988; Gul et al. 2007; Campbell & Christine 2013). Since the morphologic features of blood cells are heterogenic in birds, the blood values that belong to each species need to be investigated (Lanzarot et al. 2005).

An enzyme-histochemical analysis of blood cells is one of the methods that can be used to distinguish mature cells from immature cells in living species, as well as to determine T- and B- lymphocyte rates. The alpha naphtyl acetate esterase (ANAE) staining method is one of these tests, along with the enzyme-histochemical analysis method, which provides different reactions to each of the blood cells (Mueller et al. 1975; Higgy et al. 1977; Basso et al. 1980; Çelik et al. 1994; Aştı et al. 1996). It was determined

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Published online 12 Jan 2015

that this lysosomal enzyme, which is a nonspecific esterase, is responsible for phagocytic and cytotoxic activities (Mueller et al. 1975; Catowsky 1991). The image that is evaluated as positive in the leukocytes stained with the ANAE method is a homogeneous reaction of brown-red granules under the cell membrane or in cytoplasm. This enzyme is generally stained with 1–5 granules in lymphocytes (Ozcan 2005; Karaca et al. 2006; Şimşek et al. 2009), with dense homogeneous granules in monocytes (Mueller et al. 1975; Higgy et al. 1977; Basso et al. 1980; Ramos et al. 1992), and with granulocytes, it is stained in different granular styles, which vary according to the positivity or negativity of the animal species. In this method, neutrophil granulocytes show a negative reaction in humans (Higgy et al. 1977), dogs, cats and horses (Aştı et al. 1996), and they show a positive reaction in rats, cattle (Aştı et al. 1996), sheep and goats (Osbaldiston & Sullivan 1978). Although eosinophil granulocytes show a negative reaction in rats, cats, sheep and goats (Osbaldiston & Sullivan 1978), Aştı et al. (1996) had a positive reaction in cattle, sheep and goats (Aştı et al. 1996).

Many researchers have stated that T and B lymphocytes can be distinguished from each other in people, cattle (Çelik et al. 1994), chickens, horses, goats, dogs (Aşti et al. 1997), turkeys (Ergun et al. 2004b), rats (Aştı et al. 1996; Karaca et al. 2006; Şimşek et al. 2009) and geese (Karadag Sarı et al. 2009) using the ANAE staining method. However, it is suggested that this reaction is not specific for T lymphocytes in rats (Fossum 1978) or in sheep (Dixon & Moriarty 1983). Because of this speculative situation, some researchers have tried to overcome this problem with the denotation of ANAE-positive and ANAE-negative lymphocytes instead of ANAE-positive T lymphocytes (Sur et al. 2004; Donmez & Sur 2007; Oznurlu et al. 2012).

Although there are a lot of studies about the hematologic features of wild winged birds (Karesh et al. 1997; Toro et al. 1997; Lanzarot et al. 2001, 2005), none has been conducted in the field of hematologic data belonging to the Sparrowhawks. This study, in which data were acquired by determining the histological and physical features of peripheral blood samples belonging to the Sparrowhawk, is aimed at contributing to the literature.

Materials and methods

In this study, nine Sparrowhawks (four males and five females) were used. Their wings were broken by a hunter or by accident and they were brought to the Atatürk University, Faculty of Veterinary Science, Clinic of Internal Diseases. Those Sparrowhawks that could not recover from their traumatic injuries, and did not have any infectious diseases, were selected for the study for a clinical examination by internal medicine experts. Blood samples of these predatory birds were appropriately collected from the wing vein (brachial vein) using a 12-gauge needle syringe into a sterile heparin tube into a sterilized heparin vacutainer tube, and then the birds were euthanized using ether anesthesia. In the samples, the number of erythrocytes, leukocytes and thrombocytes, the amount of hemoglobin and the hematocrit value (Htc), the leucocyte percentage and the rate of heterophil/lymphocyte in 1 mm^3 of blood were determined using the classic methods described by Konuk (1961). In addition, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formulas of Campbell and Christine (2013), which are given below:

$$\mathrm{MCV\,(L)} = \text{hematocrit} / \text{RBC count} \quad (1)$$

$$\mathrm{MCH\,(pg)} = \text{hemoglobin} / \text{RBC count} \quad (2)$$

$$\mathrm{MCHC\ (g/dL)} = \text{hemoglobin} / \text{haematocrit} \quad (3)$$

The heparin-containing blood samples were thinly smeared and used for histometric and enzyme-histochemical analyses. Some smears were stained with May–Grunwald–Giemsa staining to determine the red blood cell diameter and width. For the ANAE staining, some other smears were fixed in the glutardialdehyde–acetone solution (9 mL glutardialdehyde + 21 mL distilled water + 45 mL acetone) at −10°C for 3 minutes. After being washed with distilled water, they were incubated in the incubation solution [80 mL phosphate buffer + 4.8 mL hexazotize pararosaniline (2.4 mL of 4% sodium nitrite + 2.4 mL of 2% pararosaniline; Sigma Chemical Co., St. Louis, MO, Cat. No. T3750; –pH 6.4) + 0.8 mL alpha naphtyl acetate (20 mg alpha naphtyl acetate; Sigma Chemical Co., St. Louis, MO, Cat. No. N8505) dissolved in acetone] for 3 hours. Later, the smears were stained with 1% of methyl green (Merck Chem, Germany) for nucleus staining and then washed with distilled water 3 or 5 times for 20 minutes. In the ANAE-positivity analysis, the cells with a brown-red reaction on their cytoplasm were evaluated as ANAE-positive cells for the determination of ANAE-positive cell densities for each bird; the stained blood samples for each smear were counted in regard to an ANAE-positive or -negative reaction, and then the mean of positive and negative lymphocytes per bird was estimated. The ANAE-positive or ANAE-negative

lymphocyte counts per each bird were estimated. Histometric measurements were determined using an image analysis program (Kameram SLR, 1.4.1.0; Mikro Sistem Ltd., Istanbul, Turkey). The statistical average and standard deviation were calculated with the help of SPSS 17 program for Windows (Property of IBM Corp., Chicago, IL, USA).

Results

In our study, the values according to the number of erythrocytes, leukocytes and thrombocytes belonging to nine Sparrowhawks, and morphologic data measured on these cells, are given in Table I. The number of erythrocytes is 3.40 ± 0.62 10^6/mm^3, the number of thrombocytes is 32.50 ± 1.40 10^3/mm^3, the amount of hemoglobin is 11.24 ± 4.25 g/dL, hematocrit is 40.56 ± 4.40%, MCV is 121.76 ± 5.60 fl, MCH is 32.98 ± 2.50 pg and MCHC is 27.39 ± 1.75 g/dL, and the total number of leukocytes is 9.16 ± 1.85 10^3/mm^3, heterophile number is 49.44 ± 2.75%, lymphocyte number is 40.33 ± 1.50%, monocyte number is 3.78 ± 0.80%, eosinophil number is 5.22 ± 0.50%, basophile number is 1.22 ± 0.5% and heterophile/lymphocyte number is 1.23 ± 0.70.

Table I. The blood values measured in Sparrowhawks (n = 9), the values expressed as mean (X) ± standard error mean (SEM).

Parameters	X ± SEM
Red blood cell (10^6/mm^3)	3.40 ± 0.62
Thrombocyte (10^3/mm^3)	32.50 ± 1.40
Hemoglobin (g/dL)	11.24 ± 4.25
Hematocrit (%)	40.56 ± 4.40
Mean corpuscular volume (fl)	121.76 ± 5.60
Mean corpuscular hemoglobin (pg)	32.98 ± 2.50
Mean corpuscular hemoglobin concentration (g/dL)	27.39 ± 1.75
White blood cell (10^3/mm^3)	9.16 ± 1.85
Heterophil (%)	49.44 ± 2.75
Lymphocyte (%)	40.33 ± 1.50
Monocyte (%)	3.78 ± 0.80
Eosinophil (%)	5.22 ± 0.50
Basophil (%)	1.22 ± 0.50
Heterophil/lymphocyte	1.23 ± 0.70

In addition, in the histometric evaluation of blood cells belonging to the Sparrowhawks, the diameter and width of some blood cells were measured (erythrocytes 8.84 ± 0.78 × 5.34 ± 0.70; reticulocytes 9.08 ± 1.00 × 7.08 ± 0.84; lymphocytes 6.16 ± 1.08). The histometric results are shown in Figure 1.

It was determined that lymphocytes, heterophile and eosinophil, which are some of the blood cells examined under light microscopy, had a reaction and showed spots, and monocytes had a homogeneous brown-red reaction (Figure 2). Some of the lymphocytes among these cells had an ANAE-positive reaction and showed 1–5 pieces with spots, and some of the lymphocytes did not have a reaction (Figure 2). As a result of the examinations, it was determined that the number of ANAE-negative cells was greater than the number of positive ones. The results of ANAE analysis are presented in Table II.

Discussion

The blood cells' number, diameter, amount of hematocrit and hemoglobin, along with enzyme-histochemical features, the health condition and the immunologic system of the organism are

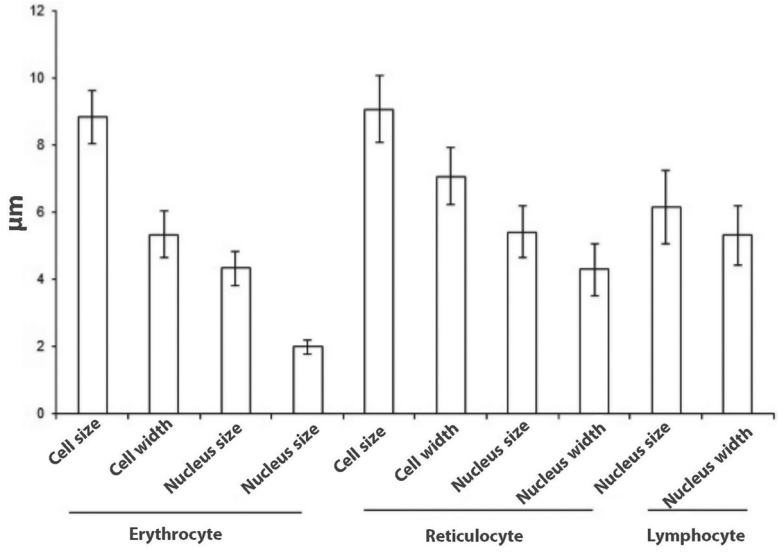

Figure 1. Histometric analysis for measurement of cell and/or nucleus diameter and width belonging to some blood cells of Sparrowhawks.

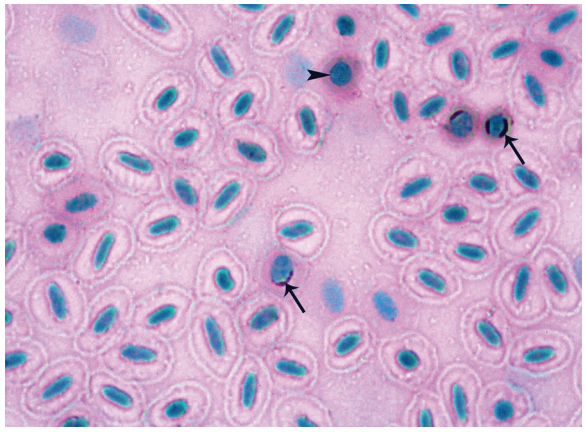

Figure 2. Blood smear of Sparrowhawks stained with the enzyme-histochemical alpha naphthyl acetate esterase (ANAE) staining method; arrows: ANAE positive lymphocytes; arrowhead: ANAE negative lymphocyte, ANAE staining, 40× objective.

Table II. Semi-quantitative analysis of lymphocytes belonging to Sparrowhawks (n = 9), alpha-naphthyl acetate esterase (ANAE).

Number/sex	ANAE (positive) lymphocyte (%) ± SEM	ANAE (negative) lymphocyte (%) ± SEM
1. ♀	40.00 ± 2.40	60.00 ± 3.50
2. ♀	38.00 ± 3.70	62.00 ± 1.40
3. ♀	43.00 ± 1.50	57.00 ± 1.90
4. ♀	48.00 ± 4.20	52.00 ± 2.10
5. ♂	45.00 ± 1.70	55.00 ± 3.50
6. ♂	37.00 ± 3.40	63.00 ± 1.60
7. ♂	50.00 ± 1.90	50.00 ± 1.80
8. ♂	42.00 ± 2.20	58.00 ± 2.40
9. ♂	47.00 ± 3.50	53.00 ± 2.60
X ± S.E.M	43.30 ± 2.70	56.60 ± 2.30

evaluated as important types of data in the analysis of some diseases.

The health conditions of predicator animals are highly difficult to determine and follow, especially in regards to hematologic parameters, data acquired through these studies (Weber et al. 2002). The hematologic values, and histometric and ANAE enzyme-histochemical features, of Sparrowhawks' blood cells were determined for the first time in this study.

Hematologic values are important in evaluating clinic pathologies such as traumatic injuries, parasitic diseases, organ damage, bacterial septicemia and nutritional deficiencies. In addition, the fact that the number of erythrocytes is low or normal can be defined as MCV anemia. The MCV, MCH and MCHC levels of hemoglobin and hematocrit that were detected in this study are found to be similar to the values declared in previous studies (Smith & Bush 1978; Lanzarot et al. 2001). While the erythrocyte reference range is usually 1.90–5.00 $10^6/mm^3$ in birds, this range is 2.08–2.90 $10^6/mm^3$ in hawks (Rehder et al. 1982). The reference range is 2.30–2.40 $10^6/mm^3$ in chickens (Aslan et al. 2005), 2.97–3.15 $10^6/mm^3$ in Japanese quails (Nazifi & Asasi 2001), 2.60–3.30 $10^6/mm^3$ in geese (Karadag Sarı et al. 2009), 2.05–2.33 $10^6/mm^3$ in partridges (Keskin et al. 2002), 1.94–2.94 $10^6/mm^3$ in eagles, 2.18–2.90 $10^6/mm^3$ in hawks, 2.70–3.23 $10^6/mm^3$ in owls, 2.10–3.70 $10^6/mm^3$ in secretary birds (Smith & Bush 1978), 2.19–2.87 $10^6/mm^3$ in vultures (Polo et al. 1992) and 1.52–2.92 $10^6/mm^3$ in kestrels (Shen et al. 2008), as determined by various researchers. Also, the diameter of the erythrocyte is 12.60 × 9.80 μm and the diameter of the nucleus is 12.60 × 9.80 μm in eagles; the diameter of the erythrocyte is 16.50 × 6.50 μm and the diameter of the nucleus is 7.50 × 2.00 μm in vultures (Polo et al. 1992); and the diameter of the erythrocyte is 27.30 × 13.00 μm and the diameter of the nucleus is 7.20 × 3.30 μm in kestrels (Shen et al. 2008). As for this study, it was determined that the Sparrowhawks' average number of erythrocytes is 3.40 × $10^6/mm^3$, their erythrocyte diameter is 8.80 × 5.30 μm and their nucleus diameter is 4.30 × 2.00 μm. It was also determined that the number of erythrocytes is higher than the reference range for predatory birds. Rehder et al. (1982) stated that their nucleus diameter is also smaller than the nucleus diameter of eagles, vultures and kestrels.

The total leucocyte number and leucocyte percentages show a considerably wide distribution among bird species. For instance, the leucocyte number is 2.64 ± 0.30 $10^3/mm^3$ in Japanese quails (Nazifi & Asasi 2001), 20.80 ± 0.58 $10^3/mm^3$ in rock partridges (Keskin et al. 2002), 19.00 ± 8.30 $10^3/mm^3$ in eagles, 15.60 ± 9.50 $10^3/mm^3$ in owls, 17.00 ± 9.00 $10^3/mm^3$ in hawks, 13.80 ± 7.50 $10^3/mm^3$ in secretary birds (Smith & Bush 1978), 6.02 ± 1.42 $10^3/mm^3$ in kestrels (Shen et al. 2008) and 13.19 ± 7.32 $10^3/mm^3$ in vultures (Polo et al. 1992). Among the possible reasons for this are interspecific differences, the methods of being caught and captured, the taking of blood from these birds and the different anti-coagulant items used (Padilla et al. 2003). The percentage of leukocytes in the peripheral blood is an important parameter for evaluating the healthy status of humans and animals. Leukocytosis is defined as an increase in the number of WBC in the blood. Infection, trauma, poisoning, bleeding in the body cavity, fast-growing neoplasm and leukemia are the common causes of leukocytosis (Aengwanich et al. 2002). In our study, it was determined that the average leucocyte number and percentage detected in the Sparrowhawks are proximate with the averages (9.16 × $10^3/mm^3$) that were stated by previous studies on Sparrowhawks, except for the eosinophil percentage. It was found that the eosinophil

percentage was high in most of the animals that were examined. It is thought that the possible reason for this is that Sparrowhawks have protozoal or parasitary disease in their blood or gastrointestinal system. Also, many researchers (Kirkpatrick & Lauer 1985; Barnard & Bair 1986; Peirce et al. 1990; Lanzarot et al. 2001) frequently state that there might be hematozoon or gastrointestinal parasites in the blood of wild birds like Sparrowhawks. On the other hand, our parasitological examination did not find any types of blood parasite infection or gastrointestinal parasites.

The lymphocyte percentage is 27.8% in swans (Milani et al. 2012), 30.79% in eagles, 35% in hawks (Smith & Bush 1978), 43.8% in kestrels (Shen et al. 2008), 45.2% in painted stork (Salakij et al. 2003), 46% in 12-week-old rock partridges (Donmez & Sur 2008), 53.5% in 14-week-old rock partridges (Keskin et al. 2002), 48.6% in male pigeons and 49.3% in female pigeons (Oznurlu et al. 2012), 52.9% in young and adult pheasants (Sur et al. 2004) and 67% in adult ducks (Donmez & Sur 2007). In this study, it was determined that the Sparrowhawks' peripheral blood lymphocyte rate (40.33%) is lower than those of the winged species stated above. Many viral or bacterial diseases like fowl plague can increase or decrease the enzyme activity and hematologic blood values in chickens (Jeurissen et al. 1989). Studies conducted in the last 20 years state that the ANAE-positive lymphocyte rate is 32.65% in adult ducks (Donmez & Sur 2008), 57.90% in mallard ducks, 54.80% in Muscovy ducks, 55.10% in Peking ducks (Karaca et al. 2006), 33.73% in young and adult pheasants (Sur et al. 2004), 47.80–48.80% in pigeons (Oznurlu et al. 2012), 51.80% in turkeys (Ergun et al. 2004b), 53.60% in geese (Karadag Sarı et al. 2009), 56.00% in chickens (Maiti et al. 1990; Aştı et al. 1999) and 59.30% in ostriches (Ergun et al. 2004a). Conversely, in the study relating to rock partridges by Donmez and Sur (2008), it was reported that the ANAE-positive lymphocyte rate differs from 37.00% in 1-day-old birds to 29.83% in 5-week-old birds and 47.83% in 12-week-old birds, depending on age. In this study, it was determined that the ANAE-positive lymphocyte rate is 43.30% in the peripheral blood of Sparrowhawks.

Finally, it was determined that the data acquired by this study will be a reference for future studies and will be used to follow and control the health condition of these animals in the wild. Also, the results of this study revealed the differences among bird species in regards to hematologic parameters.

Funding

This work was supported by the Atatürk University Research.

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