Oecologia Montana 2010,
19, 42 - 46

Differential counts of white blood cells in the Alpine Accentor *Prunella collaris*

M. HAAS and V. KOSTKOVÁ-ZELINOVÁ

Institute of High Mountain Biology, Univerzity of Žilina, Tatranská Javorina 7, 059 56-SK, Slovak Republic, e.mail: haas.2m@gmail.com

Abstract: Differential count of white blood cells (WBC) provides information on the profile of their components. There has not been done any hematological research in the alpine species Alpine Accentor (*P. collaris*) yet. The research found out the dependence of changes in profile of WBC count in the relation to the sex, age and year period. The most changes in the number of WBC were found in the lymphocytes and heterophils. It has been proved, that those changes are not related to sex, but are related to the age of individual. The proportion of lymphocytes was in juveniles 64% and in adults 51.9%. The proportion of heterophils was in juve-niles 33.7% and in adults 38.6%. Seasonal changes in the WBC profile were also notified. The shifts of leucocyte values were recorded during spring, summer and autumn. In the spring period the number of hetrophils decreased (32.42%) in the comparison to the autumn levels (58.43%), while in case of lymphocytes the trend was inverted (in spring 45.24%, and in autumn 56.29%). The increase in eosinophil number is similar to that in case of lymphocytes, but this trend was not statistically significant. The number of monocytes and basophils has not been dependent on the season. Our results confirm higher number of lymphocytes compared to heterophils in the studied species.

Key words: differential count, white blood cells, *Prunella collaris*, age, sex, season

Introduction

Individual health condition is often assessed by blood count and especially its white components (Stejskalová 2001; Hauptmanová 2003, Hauptmanová *et al.* 2004). Differential count of leucocytes provides data for the relative numbers of different types of white blood cells (WBC), namely lymphocytes, heterophils, eosinophils, basophils and monocytes (Lucas and Jamroz 1961). During the stress load varies ratios of white blood cells significantly, since white blood cells participate in the immune response of the organism (Doubek *et al.* 2003). Changes in leucocytes are also dependent on season and time of sampling (Hauptmanová 2003). Differences in levels of leucocytes were also found in studies between juvenile and adult birds (Haptmanová 2003, Král and Suchý 2000, Paz Nava *et al.* 2001). There had been shown in numerous studies that psychical and physical stress, such as fasting frustration, water deprivation, cold, noise increases the heterophils/lymphocytes (H/L) ratio (e.g. Jones 1989, Wolford and Ringer 1962, Gross and Chickering 1987, McFarlane and Curtis 1989, Beuving *et al.* 1989, Cravener *et al.* 1992, Hacking *et al.* 1993). In the case of poultry the H/L ratio is often used as a reliable factor for determining the stress (Altan 2000). Maxwell (1993) lists the various forms of weight, e.g. nutritional, parental, climatic/environmental (physical or social) or psychological stressors, that all may affect this relationship. He states that despite the lower reliability of this indicator in comparison to the values of plasma corticosteroids, is adequate indicator for mild up to moderate stress. In sev-eral studies it was determined that the psychological and physiological stressors such as food frustration, water deprivation, increased the ratio to heterophils to lymphocytes (Jones 1989, Gross and Chickering 1987, Beuving *et al.* 1989, Cravener *et al.* 1992, Hacking *et al.* 1993). In the acute stress phase, increasing number of heterophiles and decreasing number of lymphocytes was revealed (Altan 2000).

Prunella collaris Scopoli, 1769 is an alpine bird species predominantly living in the alpine habitat (Glutz von Blotzheim and Bauer 1985, Cramp 1988). It lives in the high mountain regions from Western Europe through Central Asia to Japan, typically breeding in areas above the treeline at heights from 1,800-4,000m a.s.l. (Cramp 1988, Dyrcz and Janiga 1997). This species has diverse migration, when the juveniles and females are not fixed during the winter to breeding areas in higher altitudes, the males usually winter in the surrounding areas of breeding sites (Amat and Obeso 1989). The first breeding may occur in May, the second in June-July, the early breeding depends on weather conditions, in majority cases the adults rear the young only from one nest (Bub 1984). Molting occurs in case of the adults in August and September, in the high altitudes areas where the hatching period extends until August, molting may be delayed (Cramp 1988).

The aim of this study was to present the information on differential count of WBC in the Alpine Accentors and describe the changes in leucocyte numbers in dependence on age and sex of birds and on the different seasonal conditions.

Material and Methods

Birds were trapped in various locations of the breeding areas of *P. collaris* in alpine environment in the High Tatras and Malá Fatra (Slovakia), in the spring, summer and autumn in 2005 - 2007. Birds were captured in the ornithological folding traps or mist nets and after the blood sampling they were immediately released back into the wild.

The blood was taken by puncture from vena brachialis and a drop of blood was placed on the slide and made a thin smear. Other blood was absorbed on the paper tampon, air dried and left for later PCR analysis. The blood smears were left to dry, then fixed by 96% methanol and dried again. In the laboratory, the smears were stained with a combination of stains May-Grünwald and Giemsa-Romanowski, using the method based on Pappenheim (Lucas and Jamroz 1961). The stained smears were examined microscopically under 1000× magnification, 300 leucocytes were evaluated. Differential count of WBC has been set as percentage of different types of leucocytes from the total number of 300 cells. Sex was determined by PCR, protocols followed the study by Griffiths *et al.* (1998). Four juveniles - nestlings were sampled directly in the nest.

For statistical data evaluation Statistica 8 was used. One way ANOVA was used to compared the different types of cells between sexes. In total, 30 individuals of *P. collaris* were examined, thereout 14 males, 12 females and 4 juveniles. The sex of all birds was de-termined. The following white blood cells were differentiated: heterophils (He), lymphocytes (Ly), eosinophils (Eo), monocytes (Mo), basophils (Ba).

Results

Comparison of leucocytes count between the sexes

The differential count of WBC did not differ between sexes. (Fig. 1, Table 1).

Changes in white blood cells profile in relation to age

We did not statistically compare the ratio of WBC between adults and nestlings because of the small sample of nestling. But the ratio of WBC tend to be different between juveniles (n=4) and adults (n=26) (Fig. 2). While in the case of juveniles, the lymphocytes prevailed (64%), in adults the heterophils were prevalent (38.6%). Juveniles had a higher proportion of eosinophils (6.8%) compared to adults (3.7%). Basophils are more represented in adults (1.0%). The proportion of monocytes was approximately the same in both groups.

Adult individuals of *P. collaris* were captured during the spring (n=5), summer (n=10) and autumn (n=11), and there were visible seasonal trends in the numbers of different types of leucocytes values. Number of heterophils decreased in the spring (Fig. 3a), while lymphocytes increased (Fig. 3b) towards to the winter months. Number of eosinophils tend to increase from spring to autumn but this trend was not sstatistically significant (Fig. 3c). The numbers of monocytes (Fig. 3d) and basophils were not dependent on seasons. (Tab. 2, Fig 4.)

Comparison of the leucocyte profile to other passerine birds

The total WBC count *P. collaris* was determinated as arithmetic average of all adults individuals (n=26). For comparison to other passerine birds were used the results from the literature (Table 3; Zábojníková 1996, Hauptmanová 2003).

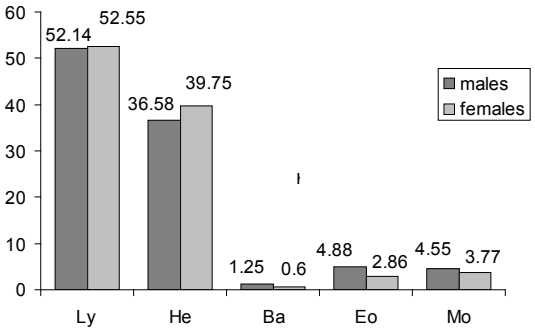

Fig. 1. The relative numbers of different types of WBC in relation to sex of *P. collaris*. **Ly** – lymfocytes, **He** – heterophils, **Ba** – basophils, **Eo** – eozinophils, **Mo** – monocytes.

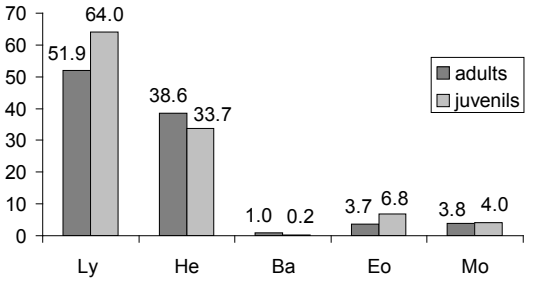

Fig. 2. The relative numbers of different types of WBC in juveniles and adults of *P. collaris*. **Ly** – lymfocytes, **He** – heterophils, **Ba** – basophils, **Eo** – eozinophils, **Mo** – monocytes.

	Males n=14		Females n=12		ANOVA		
Ly (mean)	52.14	(SD 13.96)	52.55	(SD 16.9)	F = 0.0045	p = 0.95	ns
He (mean)	36.58	(SD 14.82)	39.75	(SD 19.32)	F = 0.22	p = 0.64	ns
Eo (mean)	4.88	(SD 3.78)	2.86	(SD 2.58)	F = 2.45	p = 0,.13	ns
Mo (mean)	4.55	(SD 2.67)	3.77	(SD 1.49)	F = 0.79	p = 0.38	ns

Table 1. Comparison of the number of lymphocytes (**Ly**), heterophils (**He**), eosinophils (**Eo**) and monocytes (**Mo**) in relation to sex (n – number of individual).

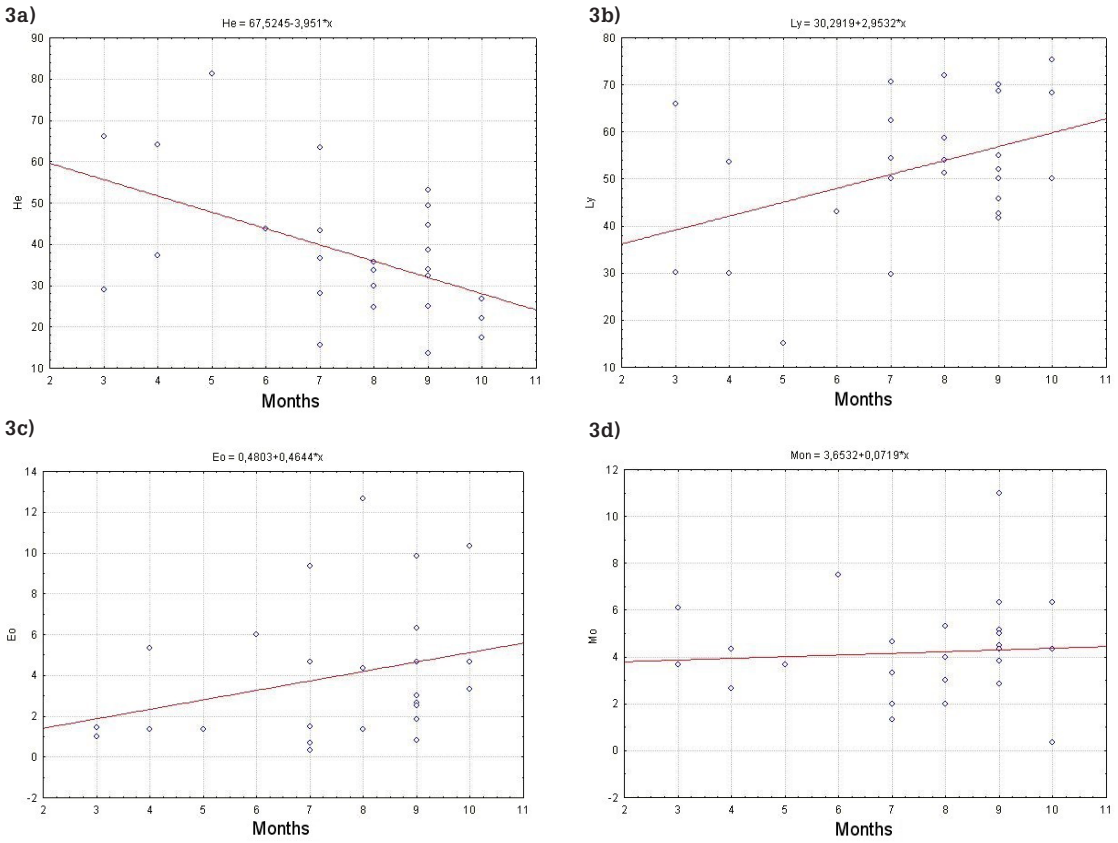

Fig. 3. Seasonal occurrence of heterophils (a), lymphocytes (b), eosinophils (c) and monocytes (d) in Alpine Accentor (March-October).

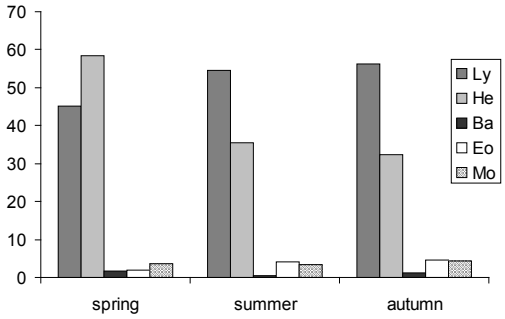

Fig. 4. Changes in the numbers of different types of WBC during the year. **Ly** – lymfocytes, **He** – heterophils, **Ba** – basophils, **Eo** – eozinophils, **Mo** – monocytes.

	Ly	He	Ba	Eo	Mo
Spring	45.24	58.43	1.76	2.02	3.62
Summer	54.60	35.47	0.38	4.22	3.45
Autumn	56.29	32.42	1.15	4.54	4.33

Tab. 2. The relative values of differential count of WBC in different seasons.

Discussion

Blood sampling was not possible in larger volumes because the samples were carried out directly in the high altitude and the long transport to the laboratory would destroy the blood samples. Therefore it was not performed to the total number of leucocytes in Bürker counting chamber.

Our results do not demonstrate effect of sex on differential count of WBC. This is in agreement to the results by Hauptmanová (2003) who also did not find difference in WBC conuts between sexes of several passerine bird species .

The age is an important factor influencing the ratio of leucocytes in birds. Hauptmanová (2003) states that in case of juveniles European Robin *Erithacus rubecula* values of lymphocytes are higher young birds than in adults, while adults posses higher levels of heterophiles, basophils and eosinophils than young birds, the number of monocytes remained constant. Comparative representation of lymphocytes and heterophils for juveniles was also found in the Great Tit *Parus major*. Eosinophils prevailed injuveniles, while the basophils and monocytes in adults (Hauptmanová 2003). Lower number of lymphocytes and a higher number of eosinophils and heterophils were also found in adult Dark-rumped Petrel *Pterodroma phaeopygia* when compared to juvenile birds (Work 1996). Kráľ and Suchý (2000) observed the greatest changes in the decrease of lymphocytes in cocks (*Gallus gallus domesticus*) during sexual maturation. Our findings support the general knowledge in birds that the juveniles have a higher number of lymphocytes than adults, while matures have higher levels of heterophils. Those two types of WBC are probably the most affected by the age of the individual. Other white cells (eosinophils, basophils and monocytes) probably depend on different factors (species specific conditions, infection etc.).

Each year period is characterized by the specific conditions that influence any bird individual - its breeding, feeding young, moulting, migration. If we consider these periods of a bird life as potential stressors, then we can understand to changes in the

	Prunella collaris	*Muscicapa striata* [1]	*Parus major*[1]	*Parus major*[2]	*Cyanistes caeruleus*[1]	*Cyanistes caeruleus*[2]	*Sitta europaea*[1]	*Erithacus rubecula*[2]	*Turdus merula*[2]	*Serinus serinus*[2]	*Chloris chloris*[2]	*Cardueli spinus*[2]	*Coccothraustes coccothraustes*[2]
Ly	51.9	74.7	76.4	69.0	57.6	49.0	79.5	69.0	68.0	46.0	49.0	47.0	56.0
He	38.6	20.6	16.4	20.0	30.7	24.0	12.7	28.0	27.0	37.0	33.0	32.0	40.0
Ba	1.0	1.4	0.9	5.0	0.0	17.0	3.8	2.0	4.0	14.0	9.0	14.0	2.0
Eo	3.7	0.9	3.4	5.0	7.1	5.0	1.4	1.0	1.0	4.0	8.0	6.0	1.0
Mo	3.8	2.2	2.4	1.0	4.6	1.0	2.6	0.0	1.0	0.0	1.0	2.0	1.0

[1] Zábojníková 1996; [2] Hauptmanova 2003

Table 3. Comparison of WBC counts in Alpine Accentor to other passerine birds. **Ly** – lymfocytes, **He** – heterophils, **Ba** – basophils, **Eo** – eozinophils, **Mo** – monocytes.

H/L ratio in different seasons of a year. From our results it is clear, that the highest values of heterophils and the lower values of lymphocytes are in the spring. In the autumn, the H/L ratio is lowest, since the number of heterophils decreases and the number of lymphocytes increases. The spring period is characterized by limited availability of food sources and the overall composition of food (Janiga and Novotná 2006). We can also assume that migration from the wintering areas to breeding areas is a considerable pressure on this species. According Hauptmanová (2003), a higher number of lymphocytes and lower number of heterophils in winter may reflect an adaptability *P. major* to unfavourable weather conditions and conversely a higher number of heterophils and lower number of lymphocytes in the spring is due to beginning of reproductive period. The same conclusions were confirmed in the reasearch on Common Pheasants *Phasianus colchicus* (Hauptmanová *et al.* 2006).

Our results confirm that the number of lymphocytes in *Prunella collaris* is higher than the number heterophils. The same was found by Lucas and Jamroz (1961), Zábojníková (1996) and Hauptmanová (2003) in other passerine bird species. Hauptmanová (2003) discussed some differences in comparison to results by Dufva and Allander (1995) and Ost *et al.* (1998) in *P. major*, nad explained them in terms of different season and sampling periods.

Acknowledgements

This study was partly funded by grants VEGA 1/6064/99, VEGA 1/4369/07, APVT-20-026102, AV4/0020/05 and EEA No. SK0061 Grant.

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Received 23 April 2010; accepted 8 August 2010.