

Changes of haematological parameters in common pheasant throughout the year

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ABSTRACT: The haematological parameters of the common pheasant (*Phasianus colchicus*), the dynamics of changes in blood count parameters with regard to the season of the year and reproduction status, and the effects of sex and body condition on the haematological parameters of blood were studied. In total, 574 blood samples were collected. The samples were collected from pheasants from the age of 6 months, prior to the laying period, and after the laying period. Packed cell volume and haemoglobin concentration significantly increased in males during the period of growth and decreased during the period of reproductive activity. Significantly higher values were detected in males compared to females in erythrocyte count, packed cell volume, haemoglobin concentration and in mean corpuscular haemoglobin concentration. A statistically significant positive relationship was detected between weight and the values of most of the parameters related to red blood cells.

Keywords: haematology; *Phasianus colchicus*; erythrocytes; leucocytes

The common pheasant (*Phasianus colchicus*) is a gallinaceous bird not autochthonous in Europe, but at present commonly occurring and frequently hunted there. It originates from central and eastern Asia, from where it was introduced to Europe by the Romans during their years occupying Europe (Hudec and Cerny, 1977). In the Czech Republic, the common pheasant either lives in the wild or is kept in breeding facilities (pheasantries), from where it is released and subsequently hunted. At present, the pheasants are frequently kept in large flocks. Big pheasantries regularly order preventive health status examinations. The haematological examination is among the methods which may contribute to the detection of some changes in health status, which may not be apparent during physical examination but which affect the fitness of the birds (Kronfeld

and Medway, 1969; Bradley and Threlfall, 1974; Gavett and Wakeley, 1986).

The knowledge of haematological parameters in common pheasant still remains incomplete. The parameters of red blood cell count were presented by Torgowski and Kontecka (1998). Some parameters of red blood cell count and total leukocyte counts for phasianids were reported by Prinzinger and Misovic (1994). Selected parameters of white blood cell count in the common pheasant were specified by Lucas and Jamroz (1961), and Maxwell and Robertson (1995, 1998).

This work presents the analysis of the dynamics of changes in blood count parameters of the common pheasant with regard to the season of the year and reproduction status. The effects of sex and body condition (expressed as body weight) on the parameters of blood were studied as well.

Partially supported by the Ministry of Education, Youth and Sports of the Czech Republic (Grant No. MSM 6215712402) and partially by the Institute of Wildlife Ecology, Veterinary and Pharmaceutical University in Brno, Czech Republic.

MATERIAL AND METHODS

The pheasants used originated from a pheasantry in Jinacovice near Brno, Czech Republic, at an altitude of 320 m, close to the forest. The pheasants were kept in exterior aviaries, with the dimensions of $20 \times 4 \times 2.5$ m with a shelter provided by a closed structure. Prior to the laying period, the pheasants were placed into cages with the dimensions of $2 \times 0.8 \times 0.8$ m in groups consisting of 1 male and 9 females. After the laying period, the males and females were kept separately in the aviaries of $30 \times 5 \times 2.5$ m which were partially roofed. The number of birds per aviary was 100 to 300. The birds were fed with commercial feeding compounds BŽ 1, BŽ 2 and BŽ (ZZN, Czech Republic) supplemented with a commercial multivitamin compound (Aminofarm, Arco, Brno, Czech Republic).

Blood samples were collected from the birds of the same flock during three periods. The samples were taken for the first time in November 2000, then in March 2001 and finally in September 2001.

Birds were randomly sampled and caught with a net. The first sampling was during growth of young birds, the second prior to the laying period and the third were taken after the period of reproduction activity. Altogether 200 birds (101 males and 99 females) were sampled at the first occasion, 196 birds (101 males and 95 females) at the second sampling, and 178 birds (88 males and 90 females) at the third. Some birds were sampled only once, others twice or three times. Blood (1.0 ml) was collected from *vena ulnaris cutanea* into heparin. A blood smear was prepared from a blood droplet without heparin. Before blood sampling birds were weighed (with 0.02 kg precision).

We determined the values of total red blood cells (RBC), total white blood cells (WBC), packed cell volume (PCV), and haemoglobin content (Hb). We also determined the differential count of white blood cells of blood smears. On the basis of PCV, RBC and Hb, we calculated the values of MCV, MCH and MCHC (Campbell, 1995). The counts of red and white blood cells were determined in Bürker's

Table 1. Results of haematological examinations of male common pheasants (*Phasianus colchicus*) – values obtained in November 2000, March 2001 and September 2001

	November		March		September	
	<i>n</i>	mean \pm SD	<i>n</i>	mean \pm SD	<i>n</i>	mean \pm SD
RBC (T/l)	101	3.58 ± 0.78	96	4.09 ± 0.65	88	3.90 ± 0.73
PCV (l/l)	100	0.382 ± 0.041	96	0.442 ± 0.047	88	0.373 ± 0.033
Hb (g/l)	101	115.0 ± 20.5	89	141.1 ± 17.6	88	116.4 ± 14.7
MCH (pg)	101	33.0 ± 6.5	89	35.4 ± 6.0	88	30.8 ± 6.2
MCV (fl)	100	111.1 ± 20.4	96	109.6 ± 12.7	88	98.8 ± 19.1
MCHC (g/l)	100	301.7 ± 46.0	89	322.1 ± 28.3	88	312.3 ± 32.6
WBC (G/l)	101	24.53 ± 12.37	96	8.58 ± 3.54	88	12.58 ± 4.71
Lymphocytes (%)	82	68.8 ± 11.7	101	63.8 ± 10.0	81	66.1 ± 12.4
(G/l)	82	18.66 ± 9.82	96	5.57 ± 2.69	81	8.73 ± 4.14
Heterophils (%)	82	25.4 ± 11.2	101	29.1 ± 10.4	81	26.1 ± 12.3
(G/l)	82	6.38 ± 3.96	96	2.42 ± 1.25	81	3.06 ± 1.35
Eosinophils (%)	82	1.8 ± 1.5	101	1.0 ± 1.5	81	1.6 ± 1.4
(G/l)	82	0.42 ± 0.39	96	0.08 ± 0.12	81	0.20 ± 0.22
Basophils (%)	82	2.6 ± 3.5	101	4.6 ± 5.0	81	3.2 ± 2.5
(G/l)	82	0.62 ± 0.94	96	0.36 ± 0.36	81	0.40 ± 0.32
Monocytes (%)	82	1.1 ± 1.1	101	1.6 ± 1.9	81	3.0 ± 2.6
(G/l)	82	0.29 ± 0.32	96	0.15 ± 0.22	81	0.37 ± 0.36

T/l = 10^{12} /l; G/l = 10^9 /l

haemocytometer, after mixing with Natt-Herrick solution in the ratio of 1 to 200 (Natt and Herrick, 1952). PCV was determined after centrifugation in haematocrit capillaries. Haemoglobin content was determined by spectrophotometry (540 nm) after the blood was mixed with Drabkin solution in the ratio of 1 to 250 (Drabkin, 1945). Blood smears were stained with May-Grünwald and Giemsa-Romanowski stains (Lucas and Jamroz, 1961). One hundred white blood cells were evaluated for each smear. The type of blood cells was determined according to Lucas and Jamroz (1961).

For each variable of interest, we present the mean value and standard deviation (SD) in classification by gender and period.

For the purposes of analysis, all of the variables (excluding values in %) were supposed to follow the log-normal distribution. Linear mixed-effects model allowing for individual-specific intercepts was fitted by the maximum likelihood method in S+ software. Gender, period, and the interaction gender by period were fixed factors, with regression on weight added. This model solved a situation of the part of birds that were examined once and the other part

repeatedly. The significance level of 0.05 was used in all statistical tests to justify the claim of a statistically significant effect (Brown and Prescott, 1999).

RESULTS

We determined mean values of haematological parameters for male and female common pheasants (Tables 1 and 2). Males and females significantly differed in the following parameters: RBC, PCV, Hb, MCHC, WBC, heterophil count, and relative monocyte count (Table 3).

All parameters in both males and females significantly differed in various periods throughout the year (November, March, September) (Tables 1, 2 and 3). In most parameters concerning red blood cells the highest values in males and females were found in March prior to the laying period, and in September after the laying period, respectively. On contrary, the values of most of the parameters of white blood cell count in both males and females were the highest in November during growth of young birds.

Table 2. Results of haematological examinations of female common pheasants (*Phasianus colchicus*) – values obtained in November 2000, March 2001 and September 2001

	November		March		September	
	<i>n</i>	mean ± SD	<i>n</i>	mean ± SD	<i>n</i>	mean ± SD
RBC (T/l)	98	3.47 ± 0.77	94	3.30 ± 0.47	88	3.98 ± 0.63
PCV (l/l)	94	0.378 ± 0.034	94	0.375 ± 0.036	87	0.385 ± 0.038
Hb (g/l)	96	104.0 ± 21.5	93	117.6 ± 15.0	87	123.9 ± 15.6
MCH (pg)	95	30.7 ± 7.2	93	36.0 ± 4.8	87	31.8 ± 5.9
MCV (fl)	93	112.1 ± 21.7	94	115.0 ± 12.8	87	98.6 ± 14.0
MCHC (g/l)	94	277.9 ± 47.8	93	313.5 ± 24.9	86	322.3 ± 29.7
WBC (G/l)	102	27.41 ± 12.02	94	9.34 ± 4.23	88	13.31 ± 4.56
Lymphocytes (%)	77	68.8 ± 12.8	88	65.7 ± 12.8	90	63.9 ± 11.7
(G/l)	77	19.89 ± 7.73	87	6.46 ± 3.70	88	8.70 ± 3.80
Heterophils (%)	77	25.3 ± 12.6	88	27.0 ± 12.6	90	31.1 ± 12.4
(G/l)	77	7.31 ± 4.78	87	2.30 ± 1.18	88	3.94 ± 1.66
Eosinophils (%)	77	2.4 ± 2.1	88	1.2 ± 1.5	90	1.1 ± 1.4
(G/l)	77	0.63 ± 0.53	87	0.11 ± 0.16	88	0.15 ± 0.22
Basophils (%)	77	2.3 ± 2.7	88	4.5 ± 4.1	90	2.4 ± 2.7
(G/l)	77	0.65 ± 0.78	87	0.40 ± 0.38	88	0.32 ± 0.36
Monocytes (%)	77	1.3 ± 1.7	88	1.6 ± 1.6	90	1.4 ± 1.2
(G/l)	77	0.34 ± 0.43	87	0.15 ± 0.18	88	0.19 ± 0.19

T/l = 10¹²/l; G/l = 10⁹/l

Table 3. Statistical evaluation of differences of values of haematological parameters in male and female pheasants (Gender), in pheasants tested in November, March and September (Period) and in pheasants according to weight (Weight); *P*-values

	Gender	Period	Weight
RBC	0.0001	<0.0001	0.001
PCV	<0.0001	<0.0001	<0.0001
Hb	<0.0001	<0.0001	<0.0001
MCH	0.5975	<0.0001	0.1111
MCV	0.3275	<0.0001	0.8870
MCHC	0.0106	<0.0001	0.0119
WBC	0.0306	<0.0001	0.0105
Lymphocytes (%)	0.6841	0.0018	0.0012
(G/l)	0.1620	<0.0001	0.0017
Heterophils (%)	0.2140	0.0470	0.0014
(G/l)	0.0356	<0.0001	0.6692
Eosinophils (%)	0.5519	<0.0001	0.2157
(G/l)	0.1338	<0.0001	0.7568
Basophils (%)	0.1824	<0.0001	0.2943
(G/l)	0.7843	0.0004	0.2296
Monocytes (%)	0.0053	<0.0001	0.1901
(G/l)	0.0760	<0.0001	0.8830

G/l = 10⁹/l

A statistically significant positive relationship was detected between weight (data of the weight of pheasants examined are not shown) and the values of RBC, PCV, Hb, MCHC, WBC, lymphocyte count, and relative lymphocyte and heterophil counts (Table 3).

DISCUSSION

To our knowledge there are only few reports on the parameters of blood count in pheasants. Torgowski and Kontecka (1998) reported haematological parameters in the group of 50 common pheasants (45 females, 5 males) during the laying period (day 0, 5 and 10). In the present study we found markedly higher erythrocyte and leukocyte counts, similar PCV values and lower haemoglobin concentrations than Torgowski and Kontecka (1998). There was no reference on the staining method for the determination of RBC and WBC counts in that paper. Another staining method might have caused the

differences in blood element counts (Lukacova and Fried, 1962). The results of our work produced a characterisation of blood count parameters which surpassed the descriptions published so far.

The parameters of white blood cell count in pheasants were referred to in other works. Maxwell and Robertson (1995, 1998) reported the following values in pheasants with no regards to their sex: 29.0% of heterophils and 1.9% of basophils. These are similar values as in our study. In contrary to our observations, Lucas and Jamroz (1961) found in adult males of common pheasant produced a lower lymphocyte count and higher heterophil, basophil and monocyte counts. The same authors reported similar eosinophil count in comparison to our results. The results might have been due to the small number of samples collected, as in wild birds a high variability of haematological parameters is rather typical, particularly in regards to leukocytes (Lucas and Jamroz, 1961).

Ritchie et al. (1994) presented the ranges of values of RBC, PCV, Hb and MCV in common pheasants.

Our mean RBC value is slightly higher than the upper limit of the range given by these authors. Our PCV value is slightly below the upper limit of their range (0.28–0.42 l/l). The values of Hb and of erythrocyte size expressed as MCV are similar to our results.

We are aware of the fact that it is difficult to determine standard values of haematological examination, because the results of the analyses may be influenced by numerous factors. These factors include the general health status of the birds. Another potentially important factor is the stress caused by catching, handling and blood sampling (Bounous et al., 2000). Largest variability was found in total leukocyte count, and differential leukocyte count (Lucas and Jamroz, 1961; Campbell, 1994). The birds must be handled very carefully and blood must be collected as soon as possible after catching (Fudge, 2000). In our work, we tried to follow this principle as much as possible. At the same time it is well known that haematological parameters in birds depend on age and sex and that they may also vary due to season or based on time of sampling and, according to some authors, even due to feed (Prinzinger and Misovic, 1994; Maxwell and Robertson, 1998; Fudge, 2000).

We found most of the differences between males and females of common pheasants in the parameters related to red blood cells. Males showed significantly higher values than females in most parameters, which conforms to similar findings in geese (Lazar et al., 1991), Japanese quail (Mihailov et al., 1999) and budgerigars (Itoh, 1992). In all species mentioned above, higher erythrocyte counts were found in males. This was also reflected in PCV and Hb. In addition, we also found higher MCHC values in males. We assumed that the reason for the difference is a higher level of oestrogens in blood of female birds, which reduces the values of red blood cell count. At the same time an opposite effect is caused by testosterone in males (Itoh, 1992).

The time of sampling significantly influenced all haematological parameters in pheasants. So, it seems that the age, sexual maturation, and phase of reproduction cycles influence these parameters in both males and females. For values related to red blood cells, it was already mentioned in Japanese quail and domestic chicken (Strakova et al. 1994; Suchy et al. 1997; Kral and Suchy, 2000).

Significant differences were found in certain values of absolute numbers of various leukocyte types. In almost all parameters the lowest values

were found in March both in males and in females. The leukocyte count is reduced again the period of maturation of the birds (Prinzinger and Misovic, 1994), and this finding may be also explain why their numbers decreased during the period from November to March. Leukocyte count was found to be very variable in pheasants. The same finding was described by Campbell (1994). The variability is due to many factors, such as the season, individual properties of birds, stress, immune status, as well as the time of blood sampling during the day (Maxwell and Robertson, 1998; Fudge 2000).

Changes throughout the year were different in males and females. In males the values related to red blood cells were increasing from November to March. This finding confirmed the results of Suchy et al. (1997) and Kral and Suchy (2000) in domestic chickens. The authors reported that the erythrocyte count, PCV and haemoglobin concentrations increased during the process of maturation of the birds in connection with ongoing spermiogenesis. During the period of reproductive activity of pheasants, some values related to RBC were reduced. In the situations with increased energy output, the parameters related to red blood cells are increased to a certain extent (Prinzinger and Misovic, 1994). We suggest that the reduction was due to significant load on the organism during the period of reproductive activity (Fudge, 2000). The demands on males were high and the ratio was not adjusted accordingly. The male birds also showed a marked reduction in weight. Similarly to our results, Torgowski and Kontecka (1998) also recorded reduction in RBC, PCV and Hb in the flock of common pheasant monitored in their work.

In females during the period of growth, the erythrocyte count was slightly decreasing. Due to this trend the concentration of haemoglobin increased (increase in MCH and MCHC), which compensated the decrease in red blood cells. During the laying period in hens the erythrocyte count increased, while MCH and MCV decreased. This suggests that the size of erythrocytes was reduced, as we observed in males. Since their number increased at the same time, the concentration of haemoglobin and PCV remained the same, and therefore the capacity of blood to transfer oxygen remained basically unchanged.

The body weight changed response to nutrition, age and load of the bird. The pheasants showed best body conditions and the highest values of most of the parameters related to red blood cells prior

to the start of reproductive activity, which always requires an increased output of energy.

Acknowledgments

We wish to thank Ing. Frantisek Vitula, Jana Smidova and Anna Pleskacova for their help.

The experiments comply with the current laws of the Czech Republic where the experiments were performed.

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Received: 05–03–30

Accepted after corrections: 06–01–21

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