

HAEMATOLOGICAL STATUS OF ONE-DAY OLD OSTRICHES (*Struthio camelus domesticus*)

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*Measurement of haematologic and biochemical parameters is an important part of evaluating the health of ostrich chicks. Thus aimed to determine haematological and biochemical parameters in the blood of one-day old ostriches in intensive breeding. The blood count, iron and copper concentrations were determined in 30 clinically healthy, one-day old unsexed ostriches of the domesticated subspecies of ostrich *Struthio camelus domesticus*. Their importance in clinical evaluation and disease condition is discussed.*

In this study the following mean values were determined in 30 clinically healthy, one-day old unsexed ostriches: erythrocyte count = $1.48 \times 10^{12}/L$, MCV = 126.6 fl, MCH = 52.9 pg, MCHC = 418 g/L, haemoglobin concentration = 79 g/L, haematocrit = 18.87%, platelet count = $8.6 \times 10^9/L$, leucocyte counts $9.1 \times 10^9/L$, heterophils = 82%, eosinophils = 0.5%, basophils = 2.5%, lymphocytes = 11%, monocytes = 3.4%, iron = 4 mmol/L and copper = 2.6 $\mu\text{mol}/L$.

We hope that the data presented in this study will be an incentive to clinicians and scientists in the field, to use haematological and biochemical examinations as an aid to the diagnosis of diseases in ostriches.

Key words: one-day old ostriches, red blood cells, white blood cells, iron and copper concentrations

INTRODUCTION

Clinical haematology has been used for many years in avian medicine for evaluation of health in birds. Haematological and biochemical values can be helpful in assessing infection, organ function and many diseases. The fact that physiological and pathological factors may cause qualitative and quantitative changes in haematological values makes such studies an important aspect of the diagnostic panel and of the monitoring of sick birds (Levi *et al.*, 1989; Perelman, 1999). Qualitative and quantitative haematologic changes in ostriches depend on age, sex, different physiological and pathologic status, stress, nutrition and conditions in particular geographic areas. The results of haematologic parameters

in the blood of ostriches should be strictly interpreted because they are necessary together with good anamnesis and physical examination for reaching a proper diagnosis (Perelman, 1999; Raukar, 2004).

There are few published papers dealing with investigation of the haematological and biochemical status of ostriches in the first days of their life. Differential blood count has only been determined in blood samples of 6 one-day old ostriches according to published papers (Robertson and Maxwell, 1996). In previous studies we investigated calcium, phosphorus, magnesium levels and alkaline phosphatase activity in the blood of one-day old ostriches (Šimpraga *et al.*, 2004).

Measurement of haematologic and biochemical parameters is an important part of evaluating the health of ostrich chicks. However, very little is known about their physiological parameters in health and how these may affect its reproductive potential, in diseases or stress conditions. The clinical evaluation of blood indices and parameters is very important in this regard. In fact, it is often difficult to assess the correct health status of an animal without examining its blood. For the successful prevention of potential diseases it is essential to know the physiological values of blood indicators, because their variation may serve as a valuable indicator for early diagnosis of certain disorders and diseases (Perelman, 1999; Romdhane *et al.*, 2000). The fact that the available literature did not provide any data on complete blood count, iron and copper concentrations in clinically healthy one-day old ostriches and these parameters are baseline data for monitoring the health and productive indices in intensive breeding for ostriches, prompted us to investigate and discuss this issue in the present paper. Their importance in clinical evaluation and disease conditions is discussed.

MATERIAL AND METHODS

Animals

Research was carried out on 30 clinically healthy one-day old unsexed ostriches of the domesticated subspecies *Struthio camelus domesticus*. The birds were kept in an incubator for 24 hours after hatching in order to dry out, blood samples were taken the moment the birds were transferred from the incubator to the ostrich holding pen. They came from the Noster d.o.o. Farm, (Hodošan, Croatia).

Sampling and analyses

Blood samples were taken by jugular venipuncture (1 mL) and were stored the same day in sodium citrated tubes. Laboratory examination was carried out within four hours of sample collection. The total erythrocyte count (TRBC), haemoglobin (Hgb) concentration, haematocrit (PCV/HcT) and the mean corpuscular values (MCV, MCH, MCHC) were determined using the Abbott CellDyn 1700-haematology analyser. The total leucocyte count (TWBC) and platelet count were determined following the methods described for evaluation of avian blood (Campbell, 1995). The total leucocyte (TWBC) count was determined by a haemocytometer using the Natt and Herrick method. Blood was drawn to the

0.5 mark on a pipette and the Natt and Herrick solution was drawn to the 101 mark to dilute the blood. This gave a 1 : 200 dilution. After being mixed, the diluted blood was discharged into the haemocytometer counting chamber and allowed to settle for a few minutes before reading. The total leucocyte count was obtained by counting all leucocytes in the nine large squares in the ruled area of the haemocytometer chamber. Platelet counts were determined using haemocytometer (Campbell, 1995).

Thin blood smears of peripheral blood were prepared from a small drop of fresh blood directly from the needle and were made for each sample for the determination of differential blood count and examination of leucocyte cell morphology. Differential leucocyte counts were made on monolayer blood films and fixed in absolute methanol for 2 to 5 min. They were subsequently stained with May – Grunwald – Giemsa. Percent eosinophils, percent heterophils, percent lymphocytes, percent monocytes and percent basophils were examined and were determined under a light microscope (Levi *et al.*, 1989; Romdhane *et al.*, 2000). Cells were classified as described by Campbell (1995).

Blood samples for biochemical tests with lithium – heparin as an anticoagulant were collected at the same time as blood samples for haematological tests. Iron concentration was determined by the colorimetric method (Olympus AU800). Copper concentration was determined by atomic absorption spectrophotometer (Perkin Elmer AA800).

Statistical analysis

Results were statistically analysed and presented in tabular form. Statistical processing was carried out using a computer program, the SAS system for Windows, release 6.12, SAS Institute Inc. Cary, NC 27513, USA.

RESULTS

The results of total blood counts determined in the blood of 30 examined one-day old ostriches are shown in Tables 1, 2 and 3.

Table 1 shows that the mean value of erythrocyte count was $1.48 \times 10^{12}/L$, the mean value of haemoglobin concentration was 79 g/L, the mean value of haematocrit was 18.87%, the mean value of the mean corpuscular volume (MCV) was 126.6 fL, the mean value of the mean corpuscular haemoglobin (MCH) was 52.9 pg, the mean value of the mean corpuscular haemoglobin concentration (MCHC) was 418 g/L and the mean value of the platelet count was $8.60 \times 10^9 /L$. Results of differential blood count in the blood of one – day old ostriches are shown in Table 2.

Table 2 shows that the mean value of the leucocyte count was $9.10 \times 10^9/L$, the mean value of percent heterophils was 82%, the mean value of percent eosinophils was 0.5%, the mean value of percent basophils was 2.5%, the mean value of percent lymphocytes was 11% and the mean value of percent of monocytes was 3.4%. Results of copper and iron concentrations in the blood of one-day old ostriches are shown in Table 3.

Table 1. Erythrocyte count, haemoglobin concentration, haematocrit, MCV, MCH, MCHC and platelet count in the blood of 30 clinically healthy one-day old ostriches (n = 30)

Parameters	minimum	maximum	x	S _x	S ²	SD	CV (%)
Erythrocytes [x 10 ¹² /L]	1.28	1.60	1.48	0.018	0.009	0.099	6.672
Haemoglobin [g/L]	65	88	79	1.105	36.64	6.053	7.672
Haematocrit [%]	15.8	21.5	18.87	0.283	2.404	1.550	8.216
MCV [fL]	116.8	134.3	126.6	0.802	19.33	4.396	3.470
MCH [pg]	49.6	55.2	52.9	0.276	2.285	1.511	2.855
MCHC [g/L]	400	430	418	1.592	76.06	8.721	2.086
Platelets [x 10 ⁹ /L]	5.90	12.5	8.60	0.3	2.706	1.644	19.157

x = Mean; S_x = Standard Error Mean; S² = Variance; SD = Standard Deviation; CV (%) = Coefficient of variation; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

Table 2. Total leucocyte count, percent heterophils, percent eosinophils, percent basophils, percent lymphocytes and percent monocytes in the blood of clinically healthy one-day old ostriches (n = 30)

Parameters	minimum	maximum	x	S _x	S ²	SD	CV (%)
Leucocytes [x 10 ⁹ /L]	6.30	13.30	9.10	0.312	2.924	1.710	18.875
Heterophils [%]	74	88	82	0.625	11.747	3.427	4.162
Eosinophils [%]	0	2	0.5	0.124	0.465	0.682	136.45
Basophils [%]	0	5	2.5	0.247	1.843	1.357	53.59
Lymphocytes [%]	6	17	11	0.539	8.736	2.955	26.31
Monocytes [%]	2	5	3.4	0.156	0.731	0.855	25.14

x = Mean; S_x = Standard Error Mean; S² = Variance; SD = Standard Deviation; CV (%) = Coefficient of variation.

Table 3. Copper and iron concentrations in the blood of clinically, healthy one-day old ostriches (n = 30)

Parameters	minimum	maximum	x	S _x	S ²	SD	CV (%)
Copper [µmol/L]	1.7	3.9	2.6	0.112	0.377	0.614	22.87
Iron [mmol/L]	3	6	4	0.161	0.786	0.886	21.11

x = Mean; S_x = Standard Error Mean; S² = Variance; SD = Standard Deviation;
CV (%) = Coefficient of variation.

The mean value of copper concentration was 2.6 µmol/L and the mean value of iron concentration was 4 mmol/L (Table 3).

DISCUSSION

Tables 1, 2 and 3 summarize the haematological and biochemical values of one-day old ostriches. Considering the fact that no information as to the values of haematological and biochemical indicators in the blood of one-day old ostriches was provided in the available literature, the results achieved will be compared to those reported for ostriches at an age of one month and over. We were able to compare only the differential blood count with just one published paper (Robertson and Maxwell, 1996).

Our study has shown that there are variations in the haematological and biochemical parameters among one-day old ostriches, younger age groups and adult ostriches.

Red blood cell parameters may be important indicators of the functioning of the haematopoietic system in ostriches. Erythrocyte parameters (erythrocyte count, haemoglobin concentration, haematocrit, MCV, MCH and MCHC) of one-day old ostriches in our research were lower in relation to values reported in the blood of younger groups (1 - 20 months) and in adult birds (Levi *et al.*, 1989; Palomeque *et al.*, 1991; Hopkins, 1995; Brown and Jones, 1996; Romdhane *et al.*, 2000; Yksek *et al.*, 2002; Agaoglu *et al.*, 2003).

In determining red blood cell parameters it is important to notice anaemia in ostriches. Anaemia occurs in ostriches with lower haemoglobin, haematocrit and erythrocyte values. These lower values are accompanied by microcytosis and hypochromia (Levi *et al.*, 1989; Brown and Jones, 1996). MCV may be an important indicator of the haematopoietic activity (Perelman, 1999). Mean corpuscular values (MCV, MCH and MCHC) are important indicators in determining the morphological characteristics of anaemia. The types of anaemias can be classified according to mean corpuscular values, as well (Campbell, 1995).

Lower erythrocyte, haemoglobin and haematocrit values in one-day old ostriches in relation to older age groups have shown that during growth they

suffer more from conditions that result in anaemia. Due to this, anaemia is most often present in ostrich chicks. According to investigations carried out by Levi *et al.* (1989) low values accompanied by microcytosis and hypochromia gradually increase with age. This condition may be due to effective haematopoietic capacity of the ostrich's bone marrow. Therefore the haematopoietic capacity of the ostrich's bone marrow should be monitored from the first day for early diagnosis of anaemia. Besides mean corpuscular values, erythrocyte, haemoglobin and haematocrit values are also important indicators for monitoring haematopoietic capacity of the bone marrow and for proper diagnosis of anaemia in ostrich chicks. We would point out that better production results may be reached by knowing the physiological values of ostrich chicks' blood.

Because of the lack of research referring to platelet count in ostriches we were able to compare our results with only one published paper reported by Romdhane *et al.* (2000). The values of the platelet count achieved were lower in relation to the investigated platelet count in ostriches up to 20 months of age but they were higher in relation to the investigated platelet count in ostriches over 20 months of age (Romdhane *et al.*, 2000). A lower platelet count may be the expression of an immature system. Platelets have an important role in haemostasis, they have a phagocytic function and they help in removing foreign material from the blood (Roland and Birrenkott, 1998).

Iron and copper are the most essential metals in ostriches. They participate in haemoglobin synthesis. Copper metabolism disorder is also associated with abnormalities in the development of the skeleton. Because of the lack of research referring to iron concentration in younger age groups of ostriches we were only able to compare our results with adult ostriches.

Values of iron concentration were lower than previously measured levels in the blood of adult ostriches (Huchzermeyer, 1994; Lien and Lu, 1994). Iron deficiency leads to anaemia and to a shortened erythrocyte life (Brown and Jones, 1996; Raukar, 2003).

Values of copper concentration were lower in relation to reported results of copper concentration measurements in the blood of ostriches at the age of 1 to 3 months (Bezuidenhout *et al.*, 1994; Yüsek *et al.*, 2002) and in adults (Yüsek *et al.*, 2002.; Agaoglu *et al.*, 2003). Copper is essential for feather pigmentation, bone formation and egg production. Signs of copper deficiency include lameness, swelling of joints and other leg disorders, anaemia and nervous system disorders (Brown and Jones, 1996; Perelman *et al.*, 2001; Raukar, 2003).

Differences in values between iron and copper concentrations in one-day old ostriches and in older groups can be associated with dietary, environmental, seasonal and disease factors. This shows that when changes are observed in mineral levels in the blood of ostriches any of the above stated factors, especially those associated with diet and disease should be properly investigated and followed by appropriate prophylactic or curative measures.

Comparing the results of this study with the results in the published papers we can conclude that the age of an ostrich has a strong impact on total erythrocyte count, haemoglobin concentration, haematocrit, MCV, MCH, MCHC, platelets, iron and copper concentrations. Therefore the mean values of

erythrocytes, haemoglobin concentration, haematocrit, MCV, MCH, MCHC, iron and copper concentrations in the specimens of 30 clinically healthy one-day old ostriches were lower in relation to the older age groups.

Our study showed that heterophil and eosinophil counts were higher, basophil, lymphocyte and monocyte counts were lower compared to the reported investigation in one-day old ostriches (Robertson and Maxwell, 1996).

The leucocyte count and the levels of percent heterophils, basophils and monocytes were higher in relation to reported results in ostriches at the age of 1 to 20 months (Levi *et al.*, 1989; Romdhane *et al.*, 2000). The leucocyte count and the levels of percent heterophils and monocytes were higher than in adults (Levi *et al.*, 1989; Robertson and Maxwell, 1996; Spinu *et al.*, 1999; Romdhane *et al.*, 2000; Polat *et al.*, 2001). The results of percent lymphocytes, eosinophils and basophils were lower compared to adults (Levi *et al.*, 1989; Robertson and Maxwell, 1996; Spinu *et al.*, 1999; Romdhane *et al.*, 2000; Polat *et al.*, 2001).

The values of percent eosinophils were lower in relation to results of investigations in ostriches at the age of 1 to 3 months (Levi *et al.*, 1989).

In our study, one-day old ostriches had more leucocytes than adults. It is possible that a higher risk of infection in the young ostriches is reflected in a higher number of leucocytes.

Newly hatched chicks have been shown to have peripheral blood lymphopenia and heterophilia. Whether this is a normal leucocyte profile occurring during this physiological stage, a simple stress response, or both is not clearly understood at this time (Wells *et al.*, 1998). Therefore, the cause of heterophilia and lymphopenia cannot be clarified in our one-day old ostriches.

The age of the ostrich and the impact of stress on the total number of leucocytes affects the differential blood count. Compared to ostrich chicks hatched in a natural habitat ostrich chicks in intensive breeding from immediately after hatching are subjected to a large number of high – stress circumstances: transport from the hatchery to the ostrich farm, temperature variations, holding pen overpopulation, etc.

Heterophils protect ostriches from bacterial infections. Eosinophils protect them from infections caused by parasites (worms and protozoa). Basophils in ostriches function as a mediator in early inflammatory response and in parasitic diseases (Hopkins, 1995; Spinu *et al.*, 1999). Lowered eosinophils and basophils values in the one-day old ostriches examined in relation to adults have shown that one-day ostriches are more sensitive to infections induced by parasites. Granulocyte function is closely linked with lymphocyte and monocyte functions i. e. with cells that are part of the defence mechanism from infection. Owing to this the determination of the total leucocyte count in peripheral blood and determining of the differential blood count and of morphological cellular changes within leucocytes and within the differential blood count may be significant indicators of the stage of a inflammatory process.

CONCLUSION

Ostriches, as a newly domesticated species, have not been fully studied up to now and each new information is a step forward towards a better understanding of this particular animal species. Even more so when one-day old ostriches are concerned as for these not even the most elementary data is available from the existing literature. Also, in view of the fact that ostriches are good breeding animals, it is essential to know the physiological values of the haematological and biochemical parameters, because any variations in these values could be an early diagnostical indicator of certain problems which may reduce the animal's production capacity and/or pose a risk to its health.

The results of our research should therefore be considered as a valuable contribution to the knowledge of ostrich physiology, and may provide a basis for monitoring the health of ostrich chicks in intensive breeding and serve also as a basis for better reproduction.

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HEMATOLOŠKI STATUS JEDNODNEVNIH NOJEVA (*Struthio camelus domesticus*)

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SADRŽAJ

Merenje hematoloških i biohemijskih parametara je značajno za procenu zdravstvenog statusa nojevih pilića. U ovom radu su prikazani rezultati određivanja hematoloških i biohemijskih parametara u krvi jednodnevnihi nojeva u farmakim uslovima. Kompletna krvna slika, koncentracije gvožđa i bakra određene su u 30 klinički zdravihi jednodnevnihi nojeva domaće podvrste nojeva *Struthio camelus domesticus*. U ovom radu se raspravlja o kliničkom značenju ovih parametara.

U našem istraživanju utvrđene su sledeće srednje vrednosti u 30 klinički zdravihi jednodnevnihi nojeva: broj eritrocita $E = 1.48 \times 10^{12}/L$, MCV = 126.6 fl, MCH = 52.9 pg, MCHC = 418 g/L, koncentracija hemoglobina = 79 g/L, hematokrit = 18.87%, broj trombocita = $8.6 \times 10^9/L$, broj leukocita = $9.1 \times 10^9/L$, heterofili = 82%, eozinofili = 0.5%, bazofili = 2.5%, limfociti = 11%, monociti = 3.4%, gvožđe = 4 mmol/L i bakar = 2.6 $\mu\text{mol}/L$.