

Calcium, phosphorus and magnesium levels and alkaline phosphatase activity in the blood of one-day-old ostriches

Miljenko Šimpraga^{1*}, Jelena Raukar², and Irena Lukač Novak³

¹Department of Physiology and Radiobiology, Faculty of Veterinary Medicine University of Zagreb, Croatia

²Postgraduate student, Faculty of Pharmacy and Biochemistry University of Zagreb, Croatia

³Poultry Centre, Croatian Veterinary Institute, Zagreb, Croatia

ŠIMPRAGA, M., J. RAUKAR, I. LUKAČ NOVAK: Calcium, phosphorus and magnesium levels and alkaline phosphatase activity in the blood of one-day-old ostriches. Vet. arhiv 74, 177-188, 2004.

ABSTRACT

Considering that climatic conditions, feeding and holding conditions as well as microflora and microfauna vary from one country to another, standard values of individual ostrich health status indicators should be established on a country to country basis. This particularly applies to ostriches at their earliest stage when they are extremely sensitive to stress induced by temperature fluctuations, initial feeding (starting), change of feeding regime, imbalanced ration composition, holding pen overpopulation, etc. Potential consequences include death or abnormal growth and development from the very beginning. It is widely known that problems associated with abnormalities in skeleton development, such as tibiotarsal rotation, reduce the young chick's resilience and increase the likelihood of its premature death and/or failure to meet production requirements. Calcium, phosphorus and magnesium metabolism disorder is one of the recognized causes of skeletal development problems. Since the available literature does not provide any information on calcium, phosphorus and magnesium levels and ALP activity in one-day-old ostriches, these elements were set as the objective of our research. The research was carried out on 10 healthy, one-day-old, unsexed ostriches of the variety *Struthio camelus domesticus*. The results have shown that calcium, phosphorus and magnesium levels were 2.33 mmol/L, 4.56 mmol/L, and 0.76 mmol/L, respectively, and ALP activity was 251.63 U/L. These results, although obtained on a relatively small number of birds, represent a valuable contribution to ostrich physiology because they may serve as reference values when investigating calcium, phosphorus and magnesium metabolism and/or abnormalities in skeletal development in these animals.

Key words: one-day-old ostrich, calcium, phosphorus, magnesium, alkaline phosphatase

* Contact address:

Prof. Dr. Miljenko Šimpraga, Faculty of Veterinary Medicine, University of Zagreb, 10000 Zagreb, Heinzelova 55, Croatia, Phone: +385 1 2390 170; Fax: +385 1 2441 390; E-mail: miljenko.simpraga@vef.hr

Introduction

Twenty years ago when hide and meat became the main objectives of ostrich breeding, ostrich farming began to expand intensively in Europe. The reasons for such expansion include their high adaptability to a variety of climatic conditions, relatively low infrastructural start-up costs and high price of the final product. Also, the utilization level in ostriches is far higher than in any other animal species (KREIBICH and SOMMER, 1995).

Considering that climatic conditions, ostrich feeding and holding conditions vary from one country to another and taking into account different microflora and microfauna, the values of individual indicators of relevance for the assessment of ostrich health status, should be standardized on a country to country basis.

For the same reason in Croatia, research work should be undertaken to determine the physiological values of biochemical indicators in peripheral circulation. This is particularly relevant to ostrich chicks at their earliest age, when they are sensitive to stress caused by temperature variations, starting, change in feeding regime, imbalanced ration composition, holding pen overpopulation, etc. Consequences may include death, or irregular growth and development from the very outset. It is widely known that problems associated with abnormalities in skeletal development, such as tibiotarsal rotation, reduce the young chick's resilience and increase the likelihood of its premature death and/or failure to meet production requirements (BEZUIDENHOUT et al., 1994; SQUIRE and MORE, 1998). Calcium, phosphorus and magnesium metabolism disorder is one of the recognized causes of skeletal development problems. However, in addition to the amounts of these minerals in serum, alkaline phosphatase activity is also an indicator of the metabolic activity of the minerals associated with skeletal ossification (HAYS and SWENSON, 1993; SIGLER, 1995; HOLLE and BENSON, 2001).

Increased or decreased physiological values of these biochemical indicators suggest certain changes and problems in ostriches. For example, hypercalcemia is an indication of increased parathyroid hormone activity, low Ca in diet or lowered Ca:P ratio in diet and hens in lay or coming into lay. Hypocalcemia is indicative of: decreased absorption of calcium; decreased vitamin D₃ intake; decreased parathyroid hormone activity, with

increased phosphorus and hypoproteinemia. Hyperphosphoremia indicates: possible renal disease, imbalance in diet and low parathyroid hormone. Hypophosphoremia indicates: low dietary calcium; increased parathyroid hormone, with increased excretion by kidney; deficient diet or anorexia; increased vitamin D₃ intake and increased tissue catabolism. Hypermagnesemia is indicative of high levels in diet and ingestion of Epsom Salt (MgSO₄). Hypomagnesemia indicates: anorexia or decreased dietary intake; deficiencies in diet; inhibited absorption by another mineral component of an imbalanced diet, and over-hydration. Increased alkaline phosphatase activity is an indication of bone repair or remodelling, and biliary or liver disease (HOPKINS, 1995). Increased ALP activity in ostrich blood serum may also indicate problems in the gastrointestinal tract (HOPKINS, 1995; YUKSEK et al., 2002).

Furthermore, for the successful prevention of potential diseases it is essential to know the physiological values of certain blood indicators, because their variations may serve as a valuable indicator for early diagnosis of certain disorders and diseases (PERELMAN, 1999).

The fact that the available literature did not provide any data on calcium, phosphorus and magnesium levels or on ALP activity in one-day-old ostriches, prompted us to investigate and discuss this issue in the present paper.

Materials and methods

Animals. The investigation was carried out on 10 healthy, one-day-old, unsexed ostriches of the variety *Struthio camelus domesticus*. Since the birds were kept in an incubator for 24 hours after hatching in order to dry well, blood samples were taken at the moment when the birds were transferred from the incubator to the ostrich chick-holding pen. The animals originated from the Noster d.o.o. Farm, (Hodošan, Croatia).

Biochemical analyses. Blood samples for biochemical analyses (1 ml each) were taken by venipuncture from *v. jugularis* into gel-containing test tubes. Levels of calcium (Ca), phosphorus (P) and magnesium (Mg) and alkaline phosphatase (ALP) activity were then determined in the serum.

Total calcium, phosphorus and magnesium level in serum was determined by the colorimetric method using reagent kits supplied by Herbos dijagnostika, Sisak (Croatia). The stained complex intensity was read out on spectrophotometer HELIOS Thermo Spectronic (UK) at a wavelength of 575 nm for calcium, 680 nm for phosphorus, and 540 nm for magnesium.

ALP activity in serum was determined by the colorimetric (IFCC) method for the measurement of catalytic ALP activity level, using reagent kits supplied by Herbos dijagnostika, Sisak (Croatia). The stained complex intensity was read out on spectrophotometer HELIOS Thermo Spectronic (UK) at a wavelength of 405 nm.

Statistical data processing. Results were statistically analyzed and presented graphically. Statistical processing was carried out using computer program, the SAS system for Windows, release 6.12, SAS Institute Inc. Cary, NC 27513, USA.

Results

Total calcium levels determined in serum of examined animals are shown in Fig. 1. Results show that the median value was 2.33 mmol/L,

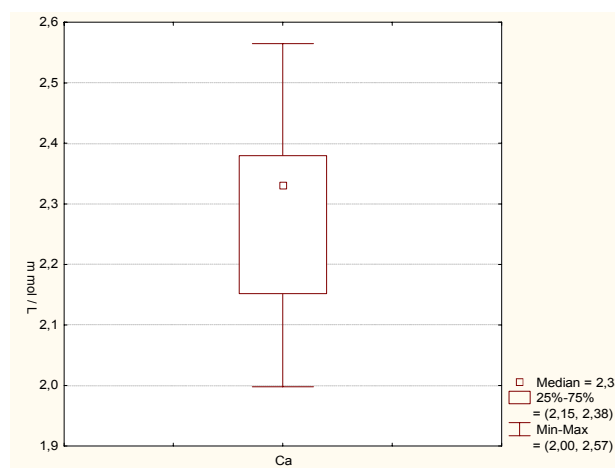


Fig. 1. Calcium level in serum of one-day-old ostriches

and that 50% of all values around the median value ranged from 2.15 to 2.38 mmol/L. It is also visible that minimum and maximum values were 2.0 and 2.57 mmol/L, respectively.

Total phosphorus levels determined in serum of tested animals are shown in Fig. 2. Results show that the median value was 4.56 mmol/L, and that 50% of all values around the median ranged from 4.27 to 5.28 mmol/L. Minimum and maximum recorded values were 3.77 mmol/L and 6.18 mmol/L, respectively.

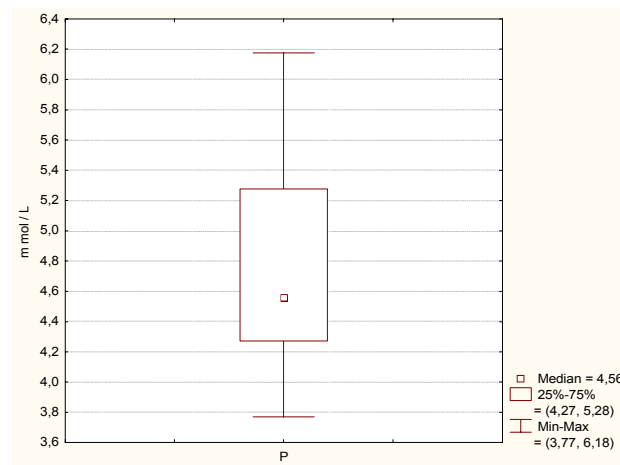


Fig. 2. Phosphorus level in serum of one-day-old ostriches

Total magnesium levels determined in serum of tested animals are shown in Fig. 3. Results show that the median value was 0.76 mmol/L, and that 50% of all values around the median ranged from 0.71 to 0.88. Minimum and maximum values were 0.63 mmol/L and 1.02 mmol/L, respectively.

ALP activity determined in serum of examined animals is shown in Fig. 4. Results show that the median value of the ALP activity was 251.63 U/L and that 50% of all values around the median ranged from 238.74 to 295.72 U/L. Fig. 4. also shows that minimum and maximum activities were 204.83 and 467.99 U/L, respectively.

M. Šimpraga et al.: Calcium, phosphorus and magnesium levels and alkaline phosphatase activity in the blood of one-day-old ostriches

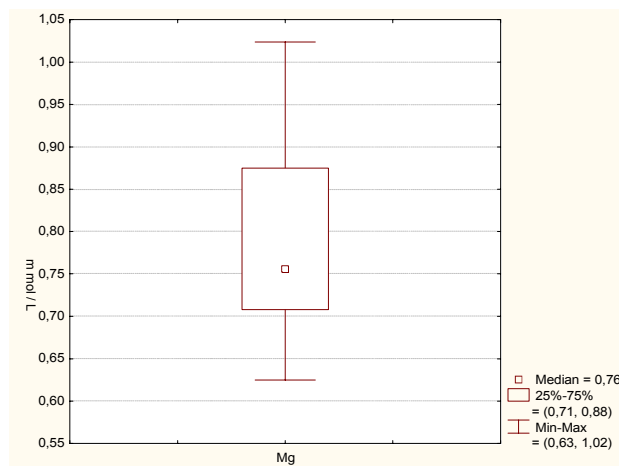


Fig. 3. Magnesium level in serum of one-day-old ostriches

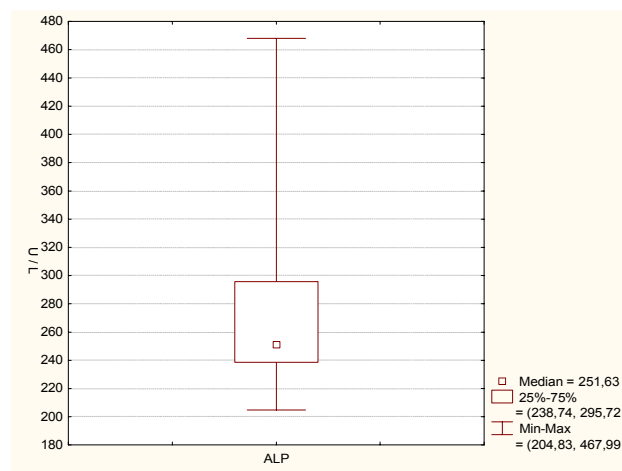


Fig. 4. ALP activity level in serum of one-day-old ostriches

Discussion

Results of the investigation of calcium, phosphorus and magnesium levels and of ALP activity in the serum of one-day-old ostriches have shown that the calcium level was 2.33 mmol/L, phosphorus level 4.56 mmol/L, magnesium level 0.766 mmol/L; ALP activity level was 251.63 U/L.

Considering the fact that no information as to the values of these biochemical indicators in the serum of one-day-old ostriches was provided in the available literature, the achieved results will be compared to those reported for ostriches at an age of one month and over.

With regard to age, for comparison purposes our research was closest to that carried out by LEVY et al. (1989), who reported achieved values of these indicators in 1-3 month-old ostriches. In ostriches of that age, mean calcium, phosphorus and magnesium levels were 2.2 mmol/L, 2.0 mmol/L, and 1.2 mmol/L, respectively; ALP activity was 531 U/L. The same paper reports the values of these indicators in ostriches of age groups 4-5, 6-9 and 12-72 months, and in adult ostriches - for female and male birds separately. Results achieved in these age groups have shown that 4-5 month-old ostriches had calcium, phosphorus and magnesium levels of 2.4 mmol/L, 1.6 mmol/L, and 0.9 mmol/L, respectively; ALP activity was 730 U/L. At the age of 6-9 months established calcium, phosphorus and magnesium levels were 2.0 mmol/L, 1.3 mmol/L and 1.0 mmol/L, respectively; ALP activity was 531 U/L. In the 12-72-month age group established calcium, phosphorus and magnesium levels were 2.5 mmol/L, 1.4 mmol/L, and 1.4 mmol/L; ALP activity was 330 U/L. In adult ostriches established calcium level was 2.4 mmol/L for male birds and 2.1 mmol/L for hens. Phosphorus level was 1.5 mmol/L for both male and female birds. Magnesium level was also equal in males and females, amounting to 1.1 mmol/L. With regard to ALP, its activity was 626 U/L in males and 532 U/L in hens.

QUINTAVALLA et al. (2001) carried out similar research involving ostriches of 4 different age groups, but not one-day-old ostriches. In the first group, including 3-12 month-old ostriches, mean calcium levels for male and female birds were 3.12 mmol/L and 2.86 mmol/L, respectively. Phosphorus level was measured only in males and amounted to 1.76 mmol/L. ALP in males was 300.9 U/L and in females 258.81 U/L. In the 12-24-month age

group, calcium levels in male and female birds were 2.46 mg/dl and 2.90 mg/dl, respectively. Phosphorus level in male birds was 0.62 mmol/L; ALP activity in male birds was 195 U/L and in hens 176.83 U/L. In the 24-36-month age group, calcium level in male birds was 2.46 mmol/L, although in female birds it was not measured. Phosphorus levels in male and female birds were 0.75 mmol/L and 0.15 mmol/L, respectively. ALP activity was measured only in male birds and amounted to 162.33 U/L. At the age of 36-48 months calcium levels in male and female birds were 3.26 mmol/L and 2.98 mmol/L, respectively. The phosphorus level established in male birds was 1.1 mmol/L. ALP activity in male birds was 120.61 and in female birds 113.90 U/L.

BROWN and JONES (1996) investigated the impact of age on certain biochemical parameters, including calcium, phosphorus and magnesium levels. Blood samples were taken at two-week intervals from ostriches ranging from 8 to 26 weeks of age. Mean calcium, phosphorus and magnesium levels were 2.35 mmol/L, 5.04 mmol/L and 0.90 mmol/L, respectively. A statistically significant increase ($P < 0.001$) was noted only in the calcium level between the 8th and 10th week, and then again after 18 weeks of age.

PALOMEQUE et al. (1991) determined the values of haematological and biochemical indicators in the blood of Masai ostriches in the Barcelona ZOO, at the age of 5 and 17 months. At the age of 5 months, calcium, phosphorus and magnesium levels were 2.65 mmol/L, 3.86 mmol/L and 0.72 mmol/L, respectively; ALP activity was 339.1 U/L. At the age of 17 months, calcium, phosphorus and magnesium levels were 4.51 mmol/L, 4.43 mmol/L and 9.92 mmol/L; ALP activity was 171.5 U/L.

ROMDHANE et al. (2000) investigated the influence of age and sex on the values of biochemical and haematological parameters in 10-60-month-old ostriches. Results have shown that mean calcium levels in male and female birds were 2.4 mmol/L and 2.6 mmol/L, respectively. Phosphorus levels in male and female birds were 1.3 mmol/L and 1.2 mmol/L, respectively. Sex-based differences were not statistically significant. However, significant differences were noted in respect of age, especially in phosphorus levels. In particular, phosphorus levels were significantly higher

($P < 0.05$) in ostriches belonging to the age group of over 20 months (1.4 mmol/L) as compared to the age group below 20 months (1.2 mmol/L).

VERSTAPPEN et al. (2002) also determined the values of biochemical indicators in blood plasma of adult ostriches ranging from 24 to 36 months of age. Mean calcium and phosphorus levels were 3.0 mmol/L and 1.7 mmol/L, respectively; ALP activity was 126 U/L.

The above results show that calcium levels determined in our research based on one-day-old ostriches correspond to the results reported by a majority of other authors, which range from 2.1 mmol/L to 3.0 mmol/L for ostriches ranging from 1 month old to adult age. Levels above 3.0 mmol/L have been reported only by two authors (PALOMEQUE et al., 1991; QUINTAVALLA et al., 2001)

With regard to phosphorus levels, the values established in our experiments are somewhat higher than those reported by other authors. The reason for such differences cannot be identified with any certainty, based as they are on a single research project involving one-day-old ostriches and with a limited number of other research works. This may be due to the fact that during the embryonal development stage and in the first few days after hatching, the energetic metabolism is very active, and phosphorus, as a component of energy-rich compounds, in particular creatine phosphate and adenosin triphosphate, plays a very important role. One of the possible reasons is also reduced activity of the parathyroid hormone. Although it was not observed in our research, a slight sample haemolysis should also be taken into account as a possible cause of increased phosphorus levels in serum, as explained by BROWN and JONES (1996). It is thought that future research on one-day-old ostriches will solve this dilemma.

Magnesium levels established in our research correspond to magnesium levels in the blood plasma of 5-month-old ostriches, reported by PALOMEQUE et al. (1991). Other authors, including PALOMEQUE et al. (1991) who investigated 17-month-old ostriches, report somewhat higher values, ranging from 0.9 mmol/L to 1.4 mmol/L in ostriches of different age groups (BEZUIDENHOUT et al., 1994; BROWN and JONES, 1996; PERELMAN, 1999). Lower values on the first day after hatching are most probably due to incomplete

activation of physiological processes involving magnesium, the activity of which rapidly intensifies immediately after transition to autonomous feeding.

With regard to ALP activity values determined in our research work, we can state that this activity corresponds to the intensity of the ossification process and skeletal development during the embryonal stage of ostrich chick development. The increase in ALP activity, as reported by other authors, is proportional to age, i.e. skeletal development and growth of the bird. Once this growth has been completed, these values fall once more and reach the lowest value in adult birds in the interval between two breeding cycles. Naturally, due to eggshell mineralization during the reproduction stage, ALP activity in ostrich hen serum increases due to intensive calcium metabolism, which is also the case in other birds (JOHNSON, 1995).

Finally, we can conclude that ostriches, as a new domesticated species, have not been fully researched to date and each new item of information is a step forward towards a better understanding of this particular animal species. Even more so where one-day-old ostriches are concerned, for which not even the most elementary data are available from the existing literature. Also, in view of the fact that ostrich is a producing animal, it is essential that we know the physiological values of its biochemical parameters, because any variation 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.

Therefore, the results of our research, regardless of the relatively small number of birds involved in the research, should be considered a valuable contribution to the knowledge of ostrich physiology, as they may serve as reference values when investigating calcium, phosphorus and magnesium metabolism and/or abnormalities in skeletal development in these animals.

Acknowledgements

We are grateful to Zdravko Pandur, owner of the Noster d.o.o. Ostrich Farm, and to Jasna Sačer, who provided valuable material and technical assistance in the realization of this research.

References

- BEZUIDENHOUT, A. J., W. P. BURGER, F. REYERS, J. T. SOLEY (1994): Serum and bone - mineral status of ostriches with tibiotarsal rotation. *Onderstepoort J. Vet. Res.* 61, 203-206.
- BROWN, C. R., G. E. JONES (1996): Some blood chemical, electrolyte and mineral values from young ostriches. *S. Afr. Vet. Ass.* 67, 111-114.
- HAYS, V. W., M. J. SWENSON (1993): Minerals. In: *Dukes' Physiology of Domestic animals*, 11th ed., (Swenson, M. J., W. O. Reece, Eds.). Cornell University Press. Ithaca and London. pp. 517-535.
- HOLLE, D., F. BENSON (2001): Understanding Production. *Ostrich Nutrition*. 2001 Blue Mountain Feeds, Inc. South Africa. pp. 8-13.
- HOPKINS, B. A. (1995): Blood count and serum chemistry analysis. In: *Ratite Encyclopedia, Ostrich - Emu - Rhea*, 1st ed., (Drenowatz, C., Ed). Charley Elrod & Helen Wilborn. pp. 409-412.
- JOHNSON, A. L. (1995): Reproduction in the female. In: *Sturkie's Avian Physiology*, 5th ed., (Whittow, G. C., Ed). Academic Press, San Diego, USA. 569-596.
- KREIBICH, A., M. SOMMER (Eds.) (1995): *Ostrich farm management*. Landwirtschaftsverlag GmbH, Münster-Hiltrup, Germany. pp. 36-49.
- LEVY, A., B. PERELMAN, T. WANER, M. Van GREVENBROEK, C. Van CREVELD, R. YAGIL (1989): Reference blood chemical values in ostriches (*Struthio camelus*). *Am. J. Vet. Res.* 50, 1548-1550.
- PALOMEQUE, J., D. PINTO, G. VISCOR (1991): Hematological and blood chemistry values of the Masai ostrich (*Struthio camelus*). *J. Wildl. Dis.* 27, 34-40.
- PERELMAN, B. (1999): Health management and veterinary procedures. In: *The Ostrich. Biology, Production and Health*. (Deeming, D. C., Ed.) CABI Publishing. CAB International. Wallingford. UK. pp. 321-346.
- QUINTAVALLA, F., E. BIGLIARDI, P. BERTONI (2001): Blood biochemical baseline values in the ostrich (*Struthio camelus*). *Annali de la Facolte di Medicina Veterinaria Parma* 21, 61-71.
- ROMDHANE, S. B., M. N. ROMDANE, S. MHIRI, M. A. BEN MILED, M. KORTAS (2000): Les parametres biochimiques et hematologiques chez l'autruche (*Struthio camelus*) dans un elevage tunisien. *Revue Med. Vet.* 151, 231-238.
- SIGLER, D. H. (1995): Basic nutrition for ratites. In: *Ratite Encyclopedia, Ostrich - Emu - Rhea*, 1st ed., (Drenowatz, C., Ed.), Charley Elrod & Helen Wilborn, Ratite Records, San Antonio, TX, USA. pp. 287-294.
- SQUIRE, B. T., S. J. MORE (1998): Factor on farms in eastern Australia associated with the development of tibiotarsal rotation in ostrich chick. *Aust. vet. J.* 76, 110-117.
- VERSTAPPEN, F. A. L. M., J. T. LUMEIJ, R. G. G. BRONNEBERG (2002): Plasma chemistry reference values in ostriches. *J. Wildl. Dis.* 38, 154-159.

M. Šimpraga et al.: Calcium, phosphorus and magnesium levels and alkaline phosphatase activity in the blood of one-day-old ostriches

YUKSEK, N., Z. AGAOGLU, A. KAYA, L. ASLAN, H. M. ERDOGAN, Y. AKGUL (2002): Stomach impaction in ostriches (*Struthio camelus*): blood chemistry, hematology and treatment. Avian Dis. 46, 757-760.

Received: 19 December 2003

Accepted: 3 June 2004

ŠIMPRAGA, M., J. RAUKAR, I. LUKAČ NOVAK: Koncentracija kalcija, fosfora i magnezija te aktivnost alkalne fosfataze u krvi jednodnevnih nojeva. Vet. arhiv 74, 177-188, 2004.

SAŽETAK

Zbog različitih klimatskih uvjeta, različitih uvjeta hranidbe i držanja u pojedinoj zemlji, te zbog različite mikroflore i mikrofaune, vrijednosti pojedinih pokazatelja procjene zdravlja nojeva moraju se standardizirati za svaku pojedinu zemlju. To naročito vrijedi za nojeve u najranijoj dobi, osjetljive na stres kao što su promjena temperature okoliša, početak hranidbe, izmjena režima hranidbe, neuravnotežen sastav obroka, prenapučenost i tome slično. Posljedice toga mogu biti uginuća, ili nepravilan rast i razvoj od najranije dobi. Poznati su problemi s nepravilnostima u razvoju koštanog sustava, kao što je npr. tibiotarzalna rotacija, koji imaju za posljedicu smanjenje vitalnosti mlade jedinke s realnim izgledima da ne doživi duži vijek i/ili ne udovolji proizvodnim potrebama. Jedan od uzroka problema u razvoju skeleta je i poremećaj u metabolizmu kalcija, fosfora i magnezija. Budući da u literaturi nema podataka o koncentracijama kalcija, fosfora i magnezija te aktivnosti alkalne fosfataze u jednodnevnih nojeva, cilj nam je bio upravo to istražiti. Istraživanje je provedeno na 10 zdravih jednodnevnih nojeva, neodređenog spola, podvrste *Struthio camelus domesticus*. Rezultati istraživanja pokazali su da je koncentracija kalcija iznosila 2,33 mmol/L, koncentracija fosfora 4,56 mmol/L, koncentracija magnezija 0,76 mmol/L, a aktivnost alkalne fosfataze 251,63 U/L. Rezultati istraživanja, iako dobiveni na relativno malom broju životinja, predstavljaju vrijedan doprinos fiziologiji nojeva jer mogu poslužiti kao referentne vrijednosti u praćenju metabolizma kalcija, fosfora i magnezija, odnosno poremećaja u razvoju skeleta u tih životinja.

Ključne riječi: jednodnevni noj, kalcij, fosfor, magnezij, alkalna fosfataza
