Journal of Microbiology, Biotechnology and Food Sciences

Trusinová et al. 2012/13 : 2 (3) 1058-1067

REGULAR ARTICLE

EVALUATION OF HEMATOLOGY AND BIOCHEMICAL PARAMETERS OF FREE-LIVING EUROPEAN BROWN HARE

Miriam Trusinová[*1], Anton Kováčik*[*2], Jozef Gašparík[1], Jaroslav Slamečka[3,4], Rastislav Jurčík[3], Peter Čupka[2], Eva Tušimová[2], Norbert Lukáč[2]*

Address: Ing. Miriam Trusinová, PhD.

[1]Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Department of Poultry Science and Small Animal Husbandry, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic

[2]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic

[3]Animal Production Research Centre Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic

[4]Czech University of Life Sciences Prague, Kamýcká 129, CZ-165 21 Praha 6 – Suchdol, Czech Republic

*Corresponding authors: xtrusinova@is.uniag.sk ; anton.kovacik@yahoo.com

ABSTRACT

The objective of our study was to determine blood hematological and serum chemistry values for a population of free-living European brown hares and to investigate differences related to age. The European brown hare (*Lepus europaeus*) is a species of hare naturally occurring in northern, central and western parts of Europe and southwestern Asia. The blood samples were collected from 25 hares (15 adults and 10 juveniles) shot during regular hunting seasons in 4 hunting grounds located in Danube lowland. Following parameters were analyzed: WBC, LYM, GRA, RBC, HGB, HCT, MCV, MCH, MCHC on blood samples, and glucose, total proteins, urea, bilirubin, cholesterol, Ca, P, Na, K, Cl on serum. WBC count was higher in adults ($2.86.10^9.l^{-1}$) than in young hares ($2.71.10^9.l^{-1}$). RBC count and HGB concentration were higher in young hares (RBC=$8.94.10^{12}.l^{-1}$, HGB=184.43 $g.l^{-1}$) than in adults (RBC=$8.38.10^{12}.l^{-1}$, HGB=177.59 $g.l^{-1}$). No significant differences were

found in other hematology parameters. Biochemical indicators of age were evaluated and an increased content of glucose in adult individuals was found (a = 9.39 mmol.l^{-1}, j = 8.48 mmol.l^{-1}). The increased level of urea in blood serum was observed as almost significant in juvenile individuals (a = 6.11 mmol.l^{-1}, j = 6.94 mmol.l^{-1}), but also reduction of bilirubin was observed (a = 5.19 mmol.l^{-1}, j = 4.23 mmol.l^{-1}). Other monitored biochemical parameters were fairly balanced. All evaluated hematology and serum chemistry parameters of brown hares were within the physiological range with relatively low differences.

Keywords: European brown hare, *Lepus europaeus*, hematology, serum chemistry

INTRODUCTION

The European brown hare (*Lepus europaeus*) is a species of hare naturally occurring in northern, central and western parts of Europe and southwestern Asia. It is a mammal adapted to temperate, open country and their likes warm and dry parts of plains and uplands (**Slamečka *et al.*, 1997**). The brown hares are very important not only for their economic and hunting aspects, but they are also a part of natural biodiversity in our country. Populations of European brown hares (*Lepus europaeus*) have declined dramatically over the past 50 years (**Wincentz Jensen, 2009**), although all the causes have not yet been identified, disease have been considered as a main factor in the decline of local hare populations and in some cases also predation by the red fox and developments in agricultural land use (**Schmidt *et al.*, 2004**). The management of brown hare population in Slovakia was analyzed by **Miškeje *et al.* (2011)**.

Hematological, biochemical and mineral values can be useful for evaluating the health of free-living European brown hares, but information about these parameters is fragmented and incomplete (**Marco *et al.*, 2003; Massányi *et al.*, 2009**). The blood system and its components are closely related to respiration, metabolism, immunity against infection, homeostatic maintenance, and with nervous system, they regulate interaction between parts of the whole organism (**Slamečka *et al.*, 1997**).

There are published hematology and serum chemistry values for the European brown hare (**Bukovjan *et al.*, 1991; Šebová *et al.*, 1993; Slamečka *et al.*, 1997; Feldman *et al.*, 2000; Marco *et al.*, 2003; Paci *et al.*, 2007; Fik *et al.*, 2008; Massányi *et al.*, 2009**). The

objective of our study was to determine blood hematological and biochemical values for a population of free-living European brown hares and to investigate differences related to age.

MATERIAL AND METHODS

Collection and sample preparation

The blood samples were collected from 25 hares (*Lepus europaeus*) that were caught in the West Slovak Lowland (Čataj, Veľká Mača, Piešťany, Lehnice). In our experiments we used 15 adult and 10 juvenile hares. Animals were divided into two age groups: juveniles and subadults (born in this year) and adults (with the age more than 1 year) according to the eye lens weight (**Slamečka *et al.*, 1997**) with the 280 mg threshold. Animals were caught in December 2011. Blood samples were obtained by heart tap. In order to restraint blood coagulation chelatone was added to the tubes for hematological analysis and transport to laboratory immediately after sampling. Blood samples for serum chemistry were left to clot at room temperature (21°C). The blood serum was separated using centrifugation at 3000 rpm for 20 minutes and kept at 4°C until arrival at the laboratory, where it was stored at -20°C until serum chemistry analysis.

Hematological analysis

In blood samples selected hematological parameters (WBC – total white blood cell count, LYM – lymphocytes, GRA – granulocytes, RBC – red blood cell count, HGB – hemoglobin, HCT – hematocrit, MCV - mean corpuscular volume of erythrocytes, MCH - Mean Corpuscular Hemoglobin, MCHC - Mean Corpuscular Hemoglobin Concentration) were measured using hematology analyzer Abacus junior VET (Diatron, Austria).

Biochemical analysis

The serum was used for the evaluation of selected parameters of mineral profile (calcium, phosphorus, sodium, potassium, chloride) and other biochemical parameters (urea, total proteins, glucose, cholesterol, and bilirubin). Sodium, potassium and chloride were measured by the automated analyzer EasyLyte (Medica, Bedford, USA), other parameters

were measured by the semi-automated clinical chemistry analyzer Microlab 300 (Vilat Scientific, Dieren, The Netherlands) (**Massányi *et al.*, 1995; Massányi *et al.*, 2009**).

Statistical analysis

Statistical analysis was performed with statistical software SAS. To compare results, the analysis of variance Student´s t-test was used. Descriptive statistical characteristics (mean, minimum, maximum, standard deviation and coefficient of variation) were evaluated. The level of significance was set at *** ($P<0.001$); ** ($P<0.01$); * ($P<0.05$).

RESULTS AND DISCUSSION

In this study, we evaluated hematology and serum chemistry values in a single group of animals, with the same methodologies and at the same time.

Hematology

The results of hematological parameters from four observed sites are shown in Table 1. Results of analyzes of selected hematological parameters of brown hare were compared with reference values which presented by **Feldman *et al.* (2000)**. Based on the analysis of hematological parameters, we found out that our measured average values were relatively balanced in accordance with the reference values by **Feldman *et al.* (2000)**.

The investigated population of hares was statistically compared according to age (adult and juvenile). WBC count was higher in adults than in young hares. RBC count and HGB concentration were higher in young hares then in adults, where juveniles were slightly above the reference value according to **Feldman *et al.* (2000)**. No significant differences were found in other hematology parameters. Evaluation of hematological parameters of hares by age is shown in Table 2.

Table 1 Hematology values of the European brown hare (*Lepus europaeus*)

Measurement (n=25)	(x)	(s)	Min	Max
WBC ($10^9.l^{-1}$)	2.79	1.34	0.66	5.93
LYM ($10^9.l^{-1}$)	2.39	1.48	0.14	5.69
GRA ($10^9.l^{-1}$)	0.30	0.57	0.01	2.29
RBC ($10^{12}.l^{-1}$)	8.60	0.79	7.31	10.12
HGB ($g.l^{-1}$)	180.31	14.87	155.18	216.25
HCT (%)	49.78	3.86	43.41	58.81
MCV (fl)	57.95	2.92	53.00	63.10
MCH (pg)	20.98	0.91	19.00	22.60
MCHC ($g.dl^{-1}$)	36.23	12.74	33.50	38.90

Legend: WBC – total white blood cell count, LYM – lymphocytes, GRA – granulocytes, RBC – red blood cell count, HGB – hemoglobin, HCT – hematocrit, MCV - mean corpuscular volume of erythrocytes, MCH - Mean Corpuscular Hemoglobin, MCHC - Mean Corpuscular Hemoglobin Concentration

Table 2 Hematology values of the European brown hare by age

Measurement	Adult Hares (n = 15)		Juvenile Hares (n = 10)	
	mean ± SD	CV	mean ± SD	CV
WBC ($10^9.l^{-1}$)	2.86 ± 1.17	41.00	2.71 ± 1.64	60.54
LYM ($10^9.l^{-1}$)	2.37 ± 1.38	58.22	2.44 ± 1.71	69.94
GRA ($10^9.l^{-1}$)	0.38 ± 0.64	168.00	0.18 ± 0.47	26.19
RBC ($10^{12}.l^{-1}$)	8.38 ± 0.62	7.35	8.94 ± 0.95	10.64
HGB ($g.l^{-1}$)	177.59 ± 12.32	6.94	184.43 ± 17.97	9.74
HCT (%)	49.15 ± 3.32	6.75	50.75 ± 4.60	9.06
MCV (fl)	58.65 ± 2.77	4.72	56.91 ± 2.98	5.23
MCH (pg)	21.20 ± 0.88	4.16	20.67 ± 0.92	4.46
MCHC ($g.l^{-1}$)	361.59 ± 12.44	3.44	363.46 ± 13.79	3.79

Legend: WBC – total white blood cell count, LYM – lymphocytes, GRA – granulocytes, RBC – red blood cell count, HGB – hemoglobin, HCT – hematocrit, MCV - mean corpuscular volume of erythrocytes, MCH - Mean Corpuscular Hemoglobin, MCHC - Mean Corpuscular Hemoglobin Concentration

Characteristics of individual hematological parameters of the blood elements in hare were studied by **Marco *et al.* (2003)** in the group of 43 individuals. They reported $3.49.10^9.l^{-1}$ the level of white blood cells in juvenile hares and $3.19.10^9.l^{-1}$ in adult hares, which is compared with our results slightly higher. Total count of lymphocytes was recorded at $1.92.10^9.l^{-1}$ respectively $2.11.10^9.l^{-1}$, which corresponds with our results. **Slamečka *et al.* (1997)** subjected 17 blood tests of hares from West-Slovak lowland. Their results showed WBC count in juveniles $3.56 . 10^9.l^{-1}$ and in adults $3.815 . 10^9.l^{-1}$, which means higher number in adults, as well as in our findings. **Bukovjan *et al.* (1991)** indicates relatively wide range of WBC ($1.23 - 6.34.10^9.l^{-1}$) of juvenile hares living in the cage breeding.

Marco *et al.* (2003) found the statistically significant higher count of RBC in adult hares ($10.00.10^{12}.l^{-1}$) comparison in juvenile ($9.26.10^{12}.l^{-1}$), similar to **Slamečka *et al.* (1997)**, which found count of RBC in adult hares $9.25.10^{12}.l^{-1}$ and in juvenile $9.15.10^{12}.l^{-1}$, which is inconsistent with our results.

Marco *et al.* (2003) reported statistically significant higher concentration of HGB in adult hares (208.1 $g.l^{-1}$) comparison in juvenile (187.4 $g.l^{-1}$), which was not confirmed in our study group. MCV, MCH, MCHC parameters were consistent with our findings.

Serum Chemistry

The results of biochemical indicators from 4 monitored areas in 2011 are shown in Table 3. Results of analyzes of selected biochemical indicators of brown hare were compared with reference values which presented by **Marco *et al.* (2003)** and **Fik *et al.* (2008)**. Based on the analysis of biochemical parameters, we found out that our measured average values were relatively balanced in accordance with the reference values.

Biochemical indicators of age were evaluated and an increased content of glucose in adult individuals was found. The increased level of urea in blood serum to the evidential threshold was observed in juvenile individuals, but also reduction of bilirubin was observed. Other monitored biochemical parameters were fairly balanced. Evaluation of biochemical indicators of age are shown in Table 4.

Table 3 Serum chemistry values of the European brown hare (*Lepus europaeus*)

Measurement (n=25)	(x)	(s)	Min	Max
Gluc (mmol.l^{-1})	9.02	2.56	4.20	13.60
TP (g.l^{-1})	64.78	10.30	47.16	83.80
Urea (mmol.l^{-1})	6.44	1.54	3.70	9.90
Bil (mmol.l^{-1})	4.80	1.83	2.02	8.59
Chol (mmol.l^{-1})	1.49	0.89	0.29	3.55
Ca (mmol.l^{-1})	3.26	0.62	2.04	4.64
P (mmol.l^{-1})	2.45	0.77	1.22	3.88
Na (mmol.l^{-1})	134.24	9.74	101.20	154.80
K (mmol.l^{-1})	10.80	2.57	5.33	15.24
Cl (mmol.l^{-1})	107.63	5.26	98.60	118.70

Legend: Gluc – Glucose, TP – Total proteins, Bil – Bilirubin, Chol – Cholesterol, Ca – Calcium, P – Phosphorus, Na – Sodium, K – potassium, Cl - Chloride

Table 4 Serum chemistry values of the European brown hare by age

Measurement	Adult Hares (n = 15)		Juvenile Hares (n = 10)	
	mean ± SD	CV	mean ± SD	CV
Gluc (mmol.l^{-1})	9.39 ± 2.60	27.74	8.48 ± 2.53	29.83
TP (g.l^{-1})	63.43 ± 9.31	14.68	66.81 ± 11.86	17.76
Urea (mmol.l^{-1})	6.11 ± 1.46	23.88	6.94 ± 1.60	23.05
Bil (mmol.l^{-1})	5.19 ± 1.91	36.87	4.23 ± 1.64	38.80
Chol (mmol.l^{-1})	1.55 ± 1.02	66.05	1.42 ± 0.71	49.80
Ca (mmol.l^{-1})	3.22 ± 0.56	17.22	3.32 ± 0.75	22.57
P (mmol.l^{-1})	2.46 ± 0.78	31.48	2.43 ± 0.82	33.77
Na (mmol.l^{-1})	133.17 ± 11.38	8.55	135.85± 6.82	5.02
K (mmol.l^{-1})	11.00 ± 2.33	21.16	10.51 ± 3.02	28.74
Cl (mmol.l^{-1})	107.48 ± 5.66	5.27	107.86 ± 4.89	4.53

Legend: Gluc – Glucose, TP – Total proteins, Bil – Bilirubin, Chol – Cholesterol, Ca – Calcium, P – Phosphorus, Na – Sodium, K – potassium, Cl - Chloride

Basic biochemical parameters are needed to assess the health status of wild hare. Relatively high level of glucose was noticed (9.02 mmol.l^{-1}), compared to **Massányi *et al.* (2009)**, who observed 74 adult subjects throughout the year. Average annual rate of glucose was 6.56 mmol.l^{-1}, but in winter 5.72 mmol.l^{-1}. **Marco *et al.* (2003)** reported higher glucose values in adult subjects (12.9 mmol.l^{-1}) compared with juveniles (11.1 mmol.l^{-1}) which was confirmed in our work.

Marco *et al.* (2003) reports significantly higher levels of total protein in adult subjects (52.5 g.l^{-1}). **Slamečka *et al.* (1997)** states average level of total protein in the winter of 55.77 g.l^{-1} and annual average of 56.53 g.l^{-1}, similarly **Massányi *et al.* (2009)**, who found an average value of 56.49 g.l^{-1} which was lower compared with our findings. Blood serum urea, cholesterol and bilirubin levels observed in our work correspond with authors **Slamečka *et al.* (1997)** and **Massányi *et al.* (2009)**. **Marco *et al.* (2003)** confirmed the significantly higher levels of bilirubin in juvenile individuals (1.71 mmol.l^{-1}) compared with adult hares (1.03 mmol.l^{-1}).

Paci *et al.* (2007) examined 172 blood serum of brown hare from 33 areas around Florence (Italy) during two years focused on metabolic and mineral profile based on age. Their results indicate a high uniformity of results between juvenile and adult individuals (Na: a - 136.8 mmol.l^{-1}, j - 136.2 mmol.l^{-1}, Ca: a - 3.0 mmol.l^{-1}; j - 2.9 mmol.l^{-1}, P: a - 1.2 mmol.l^{-1}, j - 1.3 mmol.l^{-1}, Cl: a - 104.0 mmol.l^{-1}, j - 105.2 mmol.l^{-1}) except potassium, there were confirmed statistically higher levels in adult subjects (K: a - 4.7 mmol.l^{-1}, j - 4.4 mmol.l^{-1}).

Massányi *et al.* (2009) noticed the concentration of minerals in the blood serum of brown hares in winter: Ca 3.22 mmol.l^{-1}, P 2.02 mmol.l^{-1}, Na 142 mmol.l^{-1} and K 9.80 mmol.l^{-1}, which is consistent with our findings.

CONCLUSION

We can conclude that our findings are comparable with the results of other authors. All evaluated hematology and serum chemistry parameters of brown hares were within the physiological range with relatively low differences. These results may be partly considered as a representative for free living brown hare in the West-Slovak Lowland. The overall conclusion of our experiments states that the observed population had no significant pathologic characteristics. It would be appropriate to verify observed results on more free-living hares of various areas, as well as on subjects bred in captivity.

Acknowledgments: This work was supported by projects APVV 0368-10, VEGA No. 1/0532/11, VEGA No. 1/1101/11 and KEGA 035 Spu-4/2012 and KEGA Cultural and Educational Grant Agency no. 013SPU-4/2012.

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