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BLOOD BIOCHEMICAL PROFILE OF FREE-LIVING EUROPEAN BROWN HARE (*LEPUS EUROPAEUS, PALLAS*) FROM BULGARIA

Iliyan Manev

University of Forestry, Faculty of Veterinary Medicine, Sofia, Bulgaria
E-mail: doc_man08@abv.bg

ABSTRACT

Estimation of blood biochemical profiles in free-living hares may serve to determine their health status and metabolic state as an element of conservation strategies. The objective of the current study was to determine blood biochemistry values for a population of free-living European brown hares. Blood samples from 34 adult hares shot during regular hunting season in South-Eastern Bulgaria, Europe (Kameno, Burgas region) were collected. The activities of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), glucose (GLUC), cholesterol (CHOL), triglycerides (TG), creatinin (CREAT), Urea (BUN), calcium (Ca), phosphorus (P) and magnesium (Mg) in serum were measured. All evaluated serum biochemistry values were within the physiological range with relatively low differences compared to references. Based on this we can conclude that there was an optimal health and nutritional status of the tested hare population.

Key words: Biochemical parameters, brown hare, Lepus europaeus.

Introduction

The European brown hare *(Lepus europaeus, Pallas 1778)* resides in open agriculturally landscapes of Europe (Sliwinski et al., 2019). The preferred habitat includes arable land, low grass and shrub vegetation with an altitude of up to about 900 m. Over recent decades, a widely observed population decline occurred, which was attributed to multiply factors including agricultural intensification, climate change, large-scale use of systemic insecticides, infectious diseases, increasing predator pressure (Edwards et al., 2000; Smith et al., 2005). The same decline trend is present in Bulgaria to the extent of a dramatic decrease in the population in some regions of the country. Zhelev et al. (2013) counted an average of 1.8 hares per 100 hectares, which indicates an extremely low population density of hares across large parts of Bulgaria, compared to census data 3 – 4 decades ago. Therefore, the determination of the parameters of the biochemical profile in free-living hares may serve to determine their health status and metabolic state as an element of conservation strategies (Peinado et al., 1999; Carey, 2005; Elarabany, 2018).

The aim of the current study was to determine some of the biochemical parameters including glucose, lipid profile, main serum proteins, liver function tests, kidney function tests and mineral profile reference values in population of European wild hare.

Materials and methods

Animals and sampling area

The blood samples were collected from 34 adult European brown hares in the south-eastern Bulgaria, Europe (Kameno, Burgas region). Animals were caught in December 2016. All hares were clinically healthy, based on the pathological exam.

The sampling area was chosen based on the research of Zhelev et al. (2013), which found a hare population density of 5–8/100 ha in the region. The area of Kameno is a flat-hilly area with

grassy and low shrubby vegetation and extensive vineyards. The altitude is about 15 m. above the sea level.

Sample collection and handling

Blood samples were obtained by heart tap immediately after shooting. Blood was left to clot, stored at 4–6 °C in thermal bag and transported to the laboratory. The blood serum was separated using centrifugation at 3000 rpm for 20 minutes, transferred in different tubes and stored at -20 °C until biochemistry analysis. The blood serum was used for the evaluation of selected parameters of liver activity (ALAT, ASAT, ALP), renal function (CREAT, UREA) nutritional and metabolic state (TP, ALB, GLUC, CHOL, TG) and mineral profile (Ca, P and Mg). Semi-automatic biochemistry analyzer BA-88A (Mindray, China) was used.

Statistical analysis was performed using SPSS 19.0.

Results and Discussion

The results of the estimated biochemical parameters are shown in Table 1**.**

Table 1: Serum biochemistry values of European brown hare from South-Eastern Bulgaria (n=34)

Parameter	Unit	Mean (X)	Standard deviation (SD)	Min	Max
ALAT	U/l	59.73	50.14	19	180
ASAT	U/l	164.35	163.84	45	492
ALP	U/l	81	31.02	39	104.4
TP	g/l	73.94	15.45	48.1	104.4
ALB	g/l	45.12	11.19	23.6	65.5
GLUC	mmol/l	8.78	4.80	3.62	12.66
CHOL	mmol/l	0.61	0.20	0.27	0.92
TG	mmol/l	1.62	0.64	1.05	3.41
CREAT	µg/l	92.56	15.07	69.1	117.2
BUN	mmol/l	10	1.91	7	12.8
Ca	mmol/	4.83	1.32	3.24	7.44
P	mol/l	4.02	1.57	2.36	5.71
Mg	mmol/l	1.25	0.07	1.2	1.39

Legend: ALAT – Alanine aminotransferase, ASAT- Aspartate aminotransferase, ALP – Alkaline phosphatase, TP – Total protein, ALB – Albumin, GLUC – Glucose, CHOL – Cholesterol, TG – Triglycerides, CREAT – Creatinin, BUN-blood urea nitrogen, Ca – Calcium, P – Phosphorus, Mg – Magnesium

To the best of our knowledge no studies on the biochemical profile of European brown hare from Bulgaria are available. The comparison of the current results should be estimated with appropriate reference range. However there are no accepted standardized reference biochemistry blood values for adult free-living brown hare. The comparison with available data from different regions of Europe should take into account the influence of geographical and climatic features, as well as differences in study design. Based on the survey of Marco et al. (2003) for north-eastern Spain, Paci et al. (2007) for central Italy, Nicpoń et al. (2010) for south-eastern Poland and Trusinová et al. (2012/13) for Slovak Republic (Table 2) and on the current results for south-eastern Bulgaria several trends can be highlighted. The activity of aspartate aminotransferase compared to the activity of alanine aminotransferase is higher in all the studies mentioned. An acceptable explanation for this physiological feature in hare can be the intense muscle activity level (Brancaccio et al., 2007; Kanda et al., 2014). Another feature of the rabbit blood biochemistry is the physiological hyperglycemia, probably associated with intense metabolism rate (Simon and Wittmann, 2019) or can be stress-

induced (Marik and Bellomo, 2013). The blood urea level varies in the reference studies (from 6.44 to 20.2 mmol/l) which may be due to distinct levels of protein metabolism in relation to the season and nutritional features (Carabaño et al., 2009). While most of the blood biochemical parameters in the current study are in line with the reference ones, some discrapancy can be noted in the levels of calcium and phosphorus with mean values of 4.83 and 4.02 mmol/l respectively in the current study compared to 2.7-3.0 mmol/l and 1.15-2.45 mmol/l respectively in the reference studies.

Table 2: Blood biochemistry parameters (mean values) of adult European brown hare from different regions of Europe

Parameter	Reference			
	Marco et al.	**Paci et al.**	**Nicpoń et al.**	**Trusinová et al.**
ALAT (U/l)	20.47	60	135.87	-
ASAT (U/l)	85.91	160	280.8	-
ALP (U/l)	203.32	-	50.07	-
TP (g/l)	40.6	-	48.41	64.78
ALB (g/l)	25.7	-	35.45	-
GLUC (mmol/l)	11.1	12.1	18.21	9.02
CHOL (mmol/l)	0.58	0.39	0.6	1.49
TG (mmol/l)	-	-	0.9	-
CREAT (µg/l)	95.5	-	101.21	-
UREA (mmol/l)	11.47	20.2	7.17	6.44
Ca (mmol/l)	-	3.0	2.7	3.26
P (mmol/l)	-	1.2	1.15	2.45
Mg (mmol/l)	-	1.3	0.97	-

Annual differences in blood biochemical parameters of brown hare reflect the actual nutritional and health status as well as the seasonal variations related to food intake. Some seasonal variations in the blood biochemistry of brown hare were detected. Massányi et al. (2009) reported that the highest concentration of calcium in blood plasma was recorded in the summer (3.39 mmol/l), significantly higher concentrations of magnesium were found in the autumn (1.63 mmol/l) than in winter (1.37 mmol/l). The highest amount of urea was found in spring (6.07 mmol/l). A significantly higher cholesterol concentration was measured in the spring than in summer (1.93 vs. 1.22 mmol/l) but glucose was highest in summer (10.64 mmol/l). On the other side the highest value of total proteins was in autumn (61.67 mmol/l). Depending on the season the highest ALT and AST concentration was in spring and the lowest in summer.

The influence of blood collection method and the time of sampling after death can also affect severely the blood tests values and their proper interpretation (Wesson et al., 1979; Bateson and Bradshaw, 2000).

Blood biochemical profiles can support the assessment of health and nutritional status of hare populations. Important topic in understanding hare density decline is the relationship between the habitat characteristics and physiological and nutritional condition of the animals. Paci et al. (2007) reported no winter nutritional deficiency base on the biochemical values. Their results showed that the highest density and best body condition can be found in highlands, open fields with low tree presence and wooded borders, medium mixture soils, scarce predator presence and limited anthropogenic presence. The European brown hare decline trend in Denmark for the period 1955–2000 can be attributed to predation by red fox and intensive agricultural activity. Additionally, though mild winters were beneficial to European brown hares, the increasing frequency of mild winters was

insufficient to reverse the negative population dynamics (Schmidt et al., 2004). The lack of perennials, large vegetable areas and especially diverse crops in combination with the high number of predators are the main reasons for the dramatic decline of the brown hare in Bulgaria (Zhelev, 2015). From this point of view, interdisciplinary studies and comprehensive measures must be implemented in order to maintain and restore the hare population in the country.

Conclusion

All evaluated serum biochemistry values were within the physiological range with relatively low differences compared to references. Based on this we can conclude that there was an optimal health and nutritional status of the tested hare population. Due to the lack of data from this part of Europe the current results represent an effort to understand the health status of a hare population in selected area and contributes to the establishment of reference value data for this wild species with regard to their use in future conservation programs or other studies.

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