

Seasonal variations in the blood biochemistry of brown hare^{*)}

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Summary

The aim of this study was to analyze the annual concentration of some biochemical parameters in the blood plasma of brown hares (*Lepus europaeus*). During the course of a one-year period 36 male and 38 female adult hares were analyzed. The highest concentration of calcium in blood plasma was recorded in the summer with significant differences ($p < 0.05$) between the summer and autumn and between the winter and spring periods. The concentration of phosphorus in blood plasma manifested seasonal oscillations, the highest concentration being recorded in the summer. The Ca : P ratio changed slightly during the year. Differences in the concentration of plasma magnesium were found depending on the season; significantly higher concentrations of magnesium were found in the autumn (1.63 mmol.l^{-1}) than in winter (1.37 mmol.l^{-1}). The concentration of sodium ranged from $142\text{--}158.2 \text{ mmol.l}^{-1}$. There was also a significant difference in the content of this parameter between the summer and autumn. The lowest sodium concentration was detected in the autumn and the highest in summer. In the winter the highest concentration of potassium (9.80 mmol.l^{-1}) in the blood plasma of hares was measured. The Na : K ratio decreased from spring to winter. The average concentration of glucose in the blood plasma of hares ranged during the whole year from 5.72 to 8.99 mmol.l^{-1} . The highest content was found in June and the lowest in September. The annual average year concentration of urea was 5.00 mmol.l^{-1} , with the highest amount in the spring. A significantly higher cholesterol concentration was measured in the spring than in summer. The concentration of total proteins in blood plasma achieved the average value of $56.49 \text{ mmol.l}^{-1}$ and a significant difference was recorded in spring and summer. In total lipids, a significant difference was calculated between the spring (1.46 g.l^{-1}) and summer (0.91 g.l^{-1}). The highest amount of bilirubin was found in the spring ($4.25 \text{ }\mu\text{mol.l}^{-1}$) and the lowest in autumn ($3.70 \text{ }\mu\text{mol.l}^{-1}$), with an average concentration of $3.97 \text{ }\mu\text{mol.l}^{-1}$. Depending on the season the highest ALT and AST concentration was in spring and the lowest in summer. Annual differences in blood biochemical parameters of brown hares found in this study reflect the actual nutritional and health status as well as the ability of physiological adaptation to the appropriate seasonal period in relation to food intake, condition, health status and stress effect.

Keywords: *Lepus europaeus*, biochemical parameters, blood, seasons, metabolism

The hare came to Europe from North America and Asia. In Slovakia there is only one member: the brown hare (*Lepus europaeus*). The brown hare is common throughout Europe except in the northernmost part where the mountain hare (*Lepus timidus*) occurs. The habitat of brown hares is shared with that of mountain hares (2). The most favorable place for brown hares is dry and warm parts of plains and uplands (32).

The brown hare is an important game animal. It is important both from economic and hunting aspects. The economic importance depends both on its population

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density, venison production and export entity (27, 34, 37). Hare is endangered by various infections and diseases. The main reasons of deaths in the years 1986-1994 were the following: liver necrosis syndrome (18.70%), pseudo tuberculosis (14.6%), trauma (13.6%), pasteurellosis (8.3%), coccidiosis (7.3%) and tularaemia (3.3%) (8, 15).

In the last decades, a significant decrease in the hare population has been observed (7, 14). Although all the reasons have not yet been identified, diseases have been suggested to play a role in the decline of local hare populations and in some cases also predation by the red fox and developments in agricultural land-use during

the last 45 years have affected brown hare numbers negatively (30). Among the free-living, this species acts as a very sensitive bioindicator reacting to all adverse environmental influences (34).

Serum biochemistry values can be useful for evaluating the health of populations of free-living European brown hares. However, current information is fragmented and incomplete (16). The establishment of reference intervals is problematic since parameter values determined from apparently normal animals are assumed to follow a certain distribution and deviations from these distributions are used to identify disease processes.

Therefore, the establishment of accurate reference intervals by each laboratory is essential for both hematologic and serum biochemical parameters (4, 23).

Calcium (Ca^{2+}) is the two-ionic-charge ion that is used most widely in animals and plants; it is used not only to generate membrane voltage but also to control many signal transduction systems once it has entered the cell (1). Phosphorus directly controls parathyroid hormone (PTH) synthesis and secretion (19). Potassium (K) and magnesium (Mg) are essential macro-nutrients (36). All living cells have an absolute requirement for K^{+} , which must be taken up from an external medium (29). Magnesium is one of essential minerals for humans and animals. It plays an important role in energy metabolism, protein synthesis, bone metabolism and muscle contraction. Magnesium deficiency causes tetany, muscular incoordination, growth retardation and death. Mg is necessary for normal growth; however the mechanism is unclear (25). As production is closely related to the health and nutrition status of the animals, plasma biochemical evaluation is considered to be a basic aid in their determination (28). The establishment of reference values for a variety of enzymes and other biochemical parameters are an important prerequisite for the recognition and diagnosis of illness and health status of the wild and domestic animals as well as. Therefore the determination of their blood composition becomes quite vital (35).

The aim of this study was to monitor and find possible differences in serum biochemical parameters of hares in accordance with the season. The results could provide useful information and will help in monitoring the health status of hares.

Material and methods

The samples were collected from hares (*Lepus europaeus*) that were caught in the surroundings of the town Trnava in West Slovakia. This is a hunting area with optimum soil and climate conditions for game and crop plant production.

In a one-year period 36 male and 38 female adult hares were analyzed. The animals were typed into the season according to the date of the hunt. The spring included March, April and May;

the summer, June, July and August; the autumn consisted of September, October and November; while the winter period included December, January and February.

Immediately after killing the animals, samples of blood were obtained by heart tap. From blood samples the plasma was separated using centrifugation at 3000 rpm for 30 minutes. The blood plasma was used for the evaluation of selected parameters of mineral profile (calcium, phosphorus, magnesium, sodium, potassium) and other parameters of energy and enzymatic profile (glucose, total proteins, urea, total lipids, bilirubine, AST and ALT). These parameters were measured by the semi-automated clinical chemistry analyzer Microlab 300 (Vilat Scientific, Dieren, The Netherlands) (10, 17).

To compare results the analysis of variance as well as Student's t-test and Scheffe's test were used.

Results and discussion

The highest calcium concentration in blood plasma was recorded in the summer (3.39 mmol.l^{-1}). The lowest content of calcium was measured in the spring (2.98 mmol.l^{-1}). Significant differences ($p < 0.05$) between summer (3.39 mmol.l^{-1}) and autumn (3.03 mmol.l^{-1}) and also between winter (3.22 mmol.l^{-1}) and spring (2.98 mmol.l^{-1}) were determined (fig. 1). The concentration of phosphorus in blood plasma manifested seasonal oscillations, probably because of different concentrations of nutrients in the obtained food. The highest concentration was recorded in the summer (2.34 mmol.l^{-1}) in comparison with other seasons (fig. 1). The lowest concentration was in the spring (2.02 mmol.l^{-1}), probably due to the lack of food. The Ca : P ratio changed during the year slightly. In the spring it was 1.4 : 1, in the summer 1.31 : 1, in the autumn 1.36 : 1 and in the winter 1.50 : 1. Differences were found in the concentration of plasma magnesium depending on the season. The highest concentration of magnesium was significantly ($p < 0.05$) found in the autumn (1.63 mmol.l^{-1}) in comparison with winter (1.37 mmol.l^{-1}). Marked variations in the content of follow-up elements were also among individual months (fig. 1).

In the winter the highest concentration of potassium (9.80 mmol.l^{-1}) in the blood plasma of hare was measured.

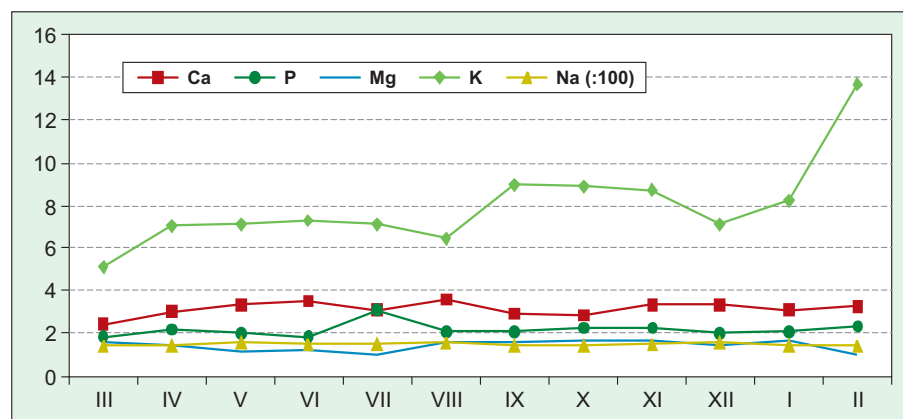


Fig. 1. Dynamics of the concentration of calcium, phosphorus, magnesium and potassium in blood plasma of brown hare during the year

red. The lowest content was found in the spring (6.61 mmol.l^{-1}). The authors also found differences among the months, where the highest value was in February ($13.63 \text{ mmol.l}^{-1}$) and the lowest in March (5.15 mmol.l^{-1}). The average concentration for the whole year was 8.10 mmol.l^{-1} (fig. 1). The concentration of sodium ranged from 142 mmol.l^{-1} in January to $158.2 \text{ mmol.l}^{-1}$ in December (fig. 1). A significant ($p < 0.05$) difference in the content of this parameter was also between summer and autumn. The lowest concentration was detected in the autumn ($145.21 \text{ mmol.l}^{-1}$) and the highest in summer ($155.33 \text{ mmol.l}^{-1}$) with an average value of $148.71 \text{ mmol.l}^{-1}$. The ratio Na : K decreased from spring to winter. In the spring it was 22.6 : 1, in the summer 21.84 : 1, in the autumn 16.37 : 1 and in the winter 15.01 : 1.

The average concentration of glucose in blood plasma of hare ranged during the whole year from 8.99 mmol.l^{-1} in spring to 5.72 mmol.l^{-1} in winter, with an average value of 6.56 mmol.l^{-1} . The highest content was found in June ($10.64 \text{ mmol.l}^{-1}$) and the lowest in September, 5.66 mmol.l^{-1} (fig. 2). The average year concentration of urea in a observed period was 5.00 mmol.l^{-1} . The highest amount was found in spring (6.07 mmol.l^{-1}) and the lowest in autumn, 4.68 mmol.l^{-1} (fig. 2). Analysis of cholesterol in blood plasma found an average annual value of 1.53 mmol.l^{-1} . The interval of values ranged from 0.81 mmol.l^{-1} in December to 2.61 mmol.l^{-1} in October. The highest cholesterol concentration was measured in the spring (1.93 mmol.l^{-1}) and the lowest (1.22 mmol.l^{-1}) and in summer (1.22 mmol.l^{-1}), where a significant ($p < 0.05$) difference was found (fig. 2). The concentration of total proteins in the blood plasma reached the average value of $56.49 \text{ mmol.l}^{-1}$. The highest value was in autumn ($61.67 \text{ mmol.l}^{-1}$) and the lowest in summer ($51.03 \text{ mmol.l}^{-1}$). A significant difference was recorded in the spring ($57.45 \text{ mmol.l}^{-1}$) and summer. When comparing individual months the highest content of total proteins ($65.89 \text{ mmol.l}^{-1}$) was in October and the lowest in August ($45.40 \text{ mmol.l}^{-1}$) (fig. 2). In the case of total lipids (fig. 2) values from 0.67 g.l^{-1} in June to 2.31 g.l^{-1} in November were measured, with an average value for the whole year of 1.41 g.l^{-1} . A significant difference was calculated between spring (1.46 g.l^{-1}) and summer (0.91 g.l^{-1}). The concentration of bilirubin ranged at intervals of $2.64 \mu\text{mol.l}^{-1}$ in September to $5.33 \mu\text{mol.l}^{-1}$ in May (fig. 2). The highest amount of bilirubin was found in the spring ($4.25 \mu\text{mol.l}^{-1}$) and the lowest in autumn ($3.70 \mu\text{mol.l}^{-1}$). The average concentration presented a value of $3.97 \mu\text{mol.l}^{-1}$.

The average concentration of ALT was $1.94 \mu\text{kat.l}^{-1}$ and ranged from $0.40 \mu\text{kat.l}^{-1}$ in February to $4.32 \mu\text{kat.l}^{-1}$ in October. Depending on the season the highest concentration was in the spring ($2.53 \mu\text{kat.l}^{-1}$) and the lowest in summer ($1.43 \mu\text{kat.l}^{-1}$), where a significant

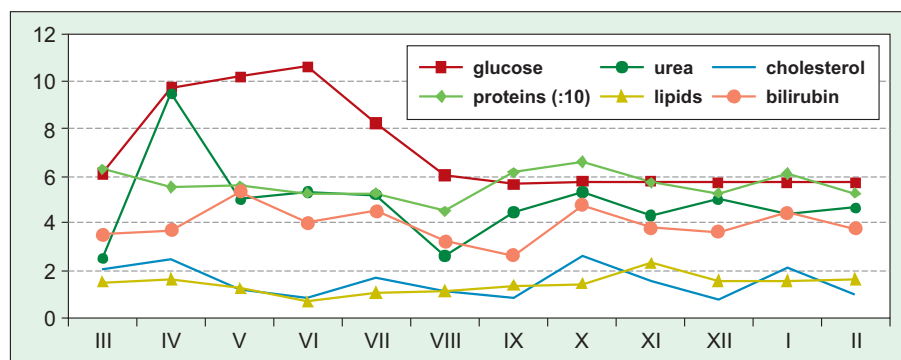


Fig. 2. Dynamics of the concentration of glucose, urea and cholesterol in blood plasma of brown hare during the year

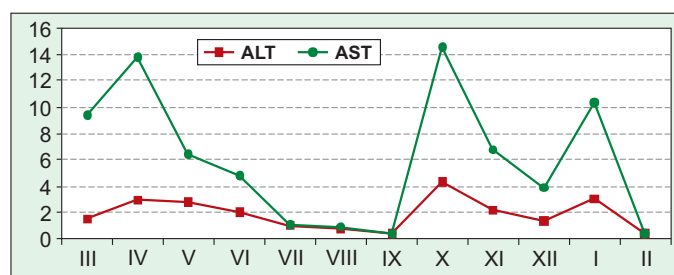


Fig. 3. Dynamics of the concentration of ALT and AST in blood plasma of brown hare during the year

difference ($p < 0.05$) was detected (fig. 3). The average concentration of AST during the year was $1.94 \mu\text{kat.l}^{-1}$ ($0.40 \mu\text{kat.l}^{-1}$ in September – $14.57 \mu\text{kat.l}^{-1}$ in October). The highest content was recorded in spring ($9.95 \mu\text{kat.l}^{-1}$) and the lowest in summer ($2.74 \mu\text{kat.l}^{-1}$), where a significant difference was found (fig. 3).

Plasma biochemical parameters might be altered by different factors such as feeding, season, sex, milking, pregnancy, age or environmental stress (21, 28). The physiological responses of the animals to environmental stress during the winter and summer, along with their energy balance, shows that seasonal heat and cold stress have profound effects on serum biochemical parameters (3, 20, 31, 33).

The concentration of calcium in the plasma of hares varied moderately in individual seasons. The average concentration was 3.16 mmol.l^{-1} . A higher value (3.40 mmol.l^{-1}) was published in rabbits (17). We recorded the highest concentration of calcium during the winter, which is in accord with previous observation (32). In spring and autumn lower amounts were probably connected with higher water content and lower nourishment and mineral stuffs in plants. In case of starvation the production of digestive fluid decreases, which also decreases resorption of calcium. It was concluded that the group of hares shot in February had higher Ca than those shot in December (33). It seems that high population density combined with a lack of suitable food leads to poor condition. The higher concentration of calcium in cold stress conditions was also present in sheep (22) and in rabbits (5).

The average magnesium concentration of 1.40 mmol.l^{-1} was analogously described (32) as measured

during the experiment. A similar result of magnesium in rabbits (1.22 mmol.l^{-1}) as a very near relation of hares was published (17). A low content of magnesium in the winter was found. It could be created by the highest lipolysis as a consequence of energy deficiency when Mg^{2+} is redistributed from extracellular liquid to adipocytes. The differences in Ca and Mg are probably due to diet.

The content of plasma phosphorus was relatively steady (2.19 mmol.l). Lower values (1.88 mmol.l^{-1}) were found in rabbits (17). The authors' highest concentrations in summer (2.34 mmol.l^{-1}) were previously confirmed (32). The lowest spring concentration (2.02 mmol.l^{-1}) was probably caused by a low concentration of phosphorus in young grass. The intensity of phosphorus changes decreases with age but increases again in the reproduction period. Rabbits kept at a lower temperature grew faster, consumed more feed and had a higher feed conversion ratio than those at the higher temperature (5). Hence they had higher serum calcium, phosphorus and magnesium levels. The differences in comparison with the authors' values may be due to the condition the rabbits were kept in as well as more sources of food, and the wild living conditions of hares. Some authors found differences in phosphorus levels in wild deer that may be due to the influence of seasonal and nutritional variation (24).

The concentration of sodium was constant and ranged from $145.21 \text{ mmol.l}^{-1}$ (autumn) to $153.33 \text{ mmol.l}^{-1}$ (summer) with an average value of $148.71 \text{ mmol.l}^{-1}$. Sodium is important for maintaining an acid-base balance. Its decline relates with water decrease and its retention with water suppression in the body. With a reduced supply in food the content of sodium in the body increases (6). The highest concentration of birch extract caused severe sodium losses via the urine by European hares that disrupted their sodium balance (9).

The average concentration of potassium was 8.10 mmol.l^{-1} . The obtained values were similar to those reported (26). The highest winter concentration of 9.77 mmol.l^{-1} and the lowest spring of 6.61 mmol.l^{-1} were measured. The higher concentration of potassium in winter probably results from higher concentration of these elements in the water and diet (20). Changes in electrolyte values may be due to the influence of seasonal and nutritional variation (24).

The content of glucose ranged from 5.66 mmol.l^{-1} in September to $10.64 \text{ mmol.l}^{-1}$ in June, with an average amount of 6.56 mmol.l^{-1} . A higher concentration (7.15 mmol.l^{-1}) and the highest level were observed also in another study in the summer (32). In contrast to the above reports according to the literature the serum glucose concentration of sheep increased during a condition of cold stress (22). It is known, that Iranian sheep kept at a lower temperature consumed more feed than those at a higher temperature. A surprisingly lower value of 3.23 mmol.l^{-1} in hares was also found (26). This marked difference probably may relate to different methodical approaches applied in the chemical

analysis. Higher blood concentration levels in wild animals than domestic were found (24, 28). This may be associated with nervous temperament, higher metabolic rate and stress level (24).

The average annual concentration of urea was 5.00 mmol.l^{-1} . The highest amount was found in spring and the lowest in autumn and winter. The differences among seasons were insignificant. Similar results were previously published (32). Blood urea values may be affected by high protein food catabolism (28).

The cholesterol concentration is normally strictly regulated, showing only slight annual variation related to dietary changes (12). It is well known that cholesterol values increase with age and also different nutrition affects its concentration in serum (28). Plasma cholesterol has also been found to have a seasonal trend in which nutritional availability plays an important role (18). The average concentration of cholesterol during the year in our experiment was 1.53 mmol.l^{-1} and it ranged from 0.81 mmol.l^{-1} in December to 2.61 mmol.l^{-1} in October. The highest average concentration was in the spring and the lowest in summer. Lower values were detected in December (33). Degradation of cholesterol from the blood is realized in the liver by transformation to the bile acids and production of steroid hormones.

During the whole year a $3.97 \mu\text{mol.l}^{-1}$ average concentration of bilirubin was found. The highest values were found in the spring ($4.25 \mu\text{mol.l}^{-1}$) and the lowest in autumn. An increase of plasma bilirubin concentration in fallow deer was detected (28). The conclusion of this study was that it was partly due to muscular damage and myoglobin breakdown, that probably caused the elevation of the bilirubin concentration in the blood of continental animals, which were stressed before blood sampling.

An average level of total proteins of 56.49 g.l^{-1} was found, which is in agreement with previous findings (32). This differs from other results (26), where lower values were reported. The lowest concentration was found in the summer and the highest in autumn. Detected results are similar to other finding in rabbits (5) and in sheep (22), partly because of their adaptation to environmental stress and increased basic metabolism.

The concentration of total lipids during the whole year ranged from 0.67 g.l^{-1} in June to 2.31 g.l^{-1} in November, with an average year concentration of 1.41 g.l^{-1} . A significant difference between the spring and summer was detected. The concentration of total lipids in cold stress conditions was higher than in heat stress conditions (22).

The average concentration of AST for the year was $6.17 \mu\text{kat.l}^{-1}$. The highest concentration was in the spring ($9.95 \mu\text{kat.l}^{-1}$) and the lowest in summer ($2.74 \mu\text{kat.l}^{-1}$). It was discovered that sex does not influence AST values, but it could be influenced by the season (11, 13). High transaminase activity in adult animals is probably due to constant food supplementation, which results in a high rate of protein and glucose synthesis by the liver (28).

The highest average concentration of ALT was found in spring ($2.53 \mu\text{kat.l}^{-1}$) and the lowest in summer ($1.43 \mu\text{kat.l}^{-1}$) with the average value of $1.94 \mu\text{kat.l}^{-1}$. The authors explained that the increase of ALT values occurred with the increase of the whole metabolism demands (11).

In contrast to the authors' findings, the significantly higher values of AST and ALT observed in extremely hot conditions compared with cold conditions in sheep (22) and in rabbits (5). But the season had no significant effect on ALT and AST serum activities (3).

All evaluated blood biochemical parameters of brown hares during an annual evaluation were within the physiological range with relatively low differences caused by variations in food intake – changes and/or deficiency of available food.

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