

Analysis of the individual condition of hares (*Lepus europaeus* Pall.) based on the body weight, KFI index, and pathological changes in selected internal organs

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Summary

The study aimed to demonstrate the relationship between the presence of pathomorphological changes in the liver, heart, and kidneys of hares *Lepus europaeus*, and their individual condition expressed by body weight and the KFI index. The material for the study consisted of 70 hares harvested during two consecutive hunting seasons, 2021/2022 and 2022/2023, in two hunting districts in the western part of the Lublin Upland. Forty hares were examined in 2021/2022, and 30 were examined in 2022/2023. Pathomorphological changes were detected in 89% of the hares, and 14% of the hares with pathomorphological changes suffered from a parasitic invasion of the liver. In the group of hares with pathomorphological lesions, the poorest individual condition expressed by the body weight and kidney fat index (KFI) was found in individuals with a parasitic infection of the liver. This may indicate that parasites play a crucial role in the deterioration of the individual condition of hares and may have a negative impact on the survival and reproduction of the entire population.

Keywords: *Lepus europaeus*, health status, histopathological examination, individual quality

A clear decline in the hare population, also affecting most of Poland, has been observed in Europe for over 50 years (6, 10, 13, 32). The downward trend is recorded in most European countries, and the causes of this phenomenon have not yet been clearly identified. The most common causes of the decline in the hare population include the intensification of agriculture leading to the loss of basic habitats, urbanization, pollution of water, air, and soil, and the ongoing climate change, which has a negative impact on population processes, including reproduction. Another important factor is a considerable increase in predation by foxes, raccoon dogs, and birds of prey. Equally important is poaching and hunting-related damage, as well as the impact of synanthropic predators (18, 19, 21-23, 27, 28, 35, 36, 38, 44, 45). Hare populations are also severely affected by the spread of infectious diseases and parasitic infestations, which deteriorate their

health and are often the direct cause of death (11, 29, 34, 40, 41).

In hunting practice, when managing wildlife populations, there is a need to constantly monitor the nutritional status of animals by means of simple and easily accessible indicators. The kidney fat index (KFI) was first proposed by Riney (42) as a measure of the physical condition of cervids, as it provides immediate results and is very easy to calculate. It is especially widely used in ungulates, but is also useful for the assessment of the *Lepus* species (3, 16, 18). This parameter is an easy-to-obtain tool for monitoring the population status and provides important information on the dynamics of changes in individual quality traits, which has a direct impact on hunting management (8). Body weight and fat reserves are strongly dependent on environmental factors, such as climatic conditions, food base, and population density, as well as the

physiological condition of the organism (affected by diseases and parasitic infections) (2, 33).

The aim of the study was to determine the health status and individual condition of hares harvested by hunters in the western part of the Lublin Upland, where the population of this species is sufficiently large for hunting. The research hypothesis assumed a relationship between pathological lesions in the liver, heart, and kidneys of hares and their individual condition expressed by body weight and KFI index values.

Material and methods

The research material consisted of 70 hares harvested by hunters during two consecutive hunting seasons, 2021/2022 and 2022/2023, in two hunting districts in the western part of the Lublin Upland. Forty hares were examined in 2021/2022, and 30 individuals of this species were examined during the 2022/2023 season. In the hunting areas where the animals were harvested, there were no reports of hares dying of natural causes or clinical signs of any diseases characteristic of this species. Density rates in these areas are sufficiently high for sustainable hunting every year. The study included animals that had been culled in accordance with the Polish hunting law, so no consent of an ethics committee was required to conduct the research. The animals selected for the study, were the first hares culled during the hunt on a given day, which ensured sampling randomness.

Since young hares become sexually mature between eight and twelve months of age, the animals were divided into two age groups: juveniles under 1 year of age and adults over 1 year of age, in order to make appropriate comparisons. The age of the animals was first determined in the field according to an age index (20) and then, definitively, from the weight of dried eye lenses (26). The sex of the hares was determined based on secondary sexual characteristics, which clearly and unambiguously distinguish males from females. In the field, the body weight of each hare was determined immediately after harvesting, and then cooled carcasses were transported for further analyses to the Department of Pathomorphology and Forensic Veterinary Medicine, University of Life Sciences in Lublin.

During the autopsy, attention was paid to the macroscopic appearance of internal organs: the head, neck, chest cavity, and abdominal cavity. Sections of the liver, heart, and kidney were taken for histopathological examination. The material was fixed in 10% buffered formalin, pH 7.2, and exposed to increasing concentrations of alcohol solutions, acetone, and xylene. Each stage of this procedure was performed using a Leica TP-20 tissue processor. A Leica SR-200 slide microtome was used to prepare 4 μ m thick tissue sections, which were then placed on glass slides. For histopathological analysis, the samples were stained with hematoxylin and eosin (HE). The slides were assessed under a Nikon Eclipse E-600 light microscope. Parasite-induced lesions were diagnosed based on macroscopic and histopathological features. Only liver sections with obvious macroscopic lesions visible under the liver capsule were taken for the parasitological analysis.

The kidney fat index (KFI) was calculated to assess the individual condition of the hares. Kidneys dissected from

carcasses were weighed with an accuracy of 1 g on a SECA 856 scale. Next, perirenal fat was removed, and the kidneys were re-weighed. Based on the measurements, the kidney fat index was calculated as the ratio of the weight of the kidney with the fat to the weight of the kidney without the fat. The inclusion of kidney weight in index calculations made it possible to compare KFI values between animals with different body sizes (3).

Statistical analysis. Mean body weight values and kidney fat index (KFI) values were compared between animals with or without pathological lesions. The differences between the means were assessed using the *t*-test for independent variables. The normality of the distribution of the traits analyzed was assessed using the Shapiro-Wilk test. Statistical analyses were performed with the Statistica 13.1 program.

Results and discussion

During the histopathological evaluation, some organs of the animals exhibited more than one lesion, which may indicate a more advanced and complex effect of the causative factor. In total, the organs of 89% of the hares had one or more pathological lesions. The study results demonstrated that pathological lesions were more frequent in the kidneys (45% of all pathological lesions) and the liver (41%) than they were in the heart muscle (14%).

Of all 13 pathological changes identified in the analyses, the four most frequently detected lesions are presented and described in this study. The most frequently observed were histopathological lesions indicating nephritis (acute and interstitial). Damage to the glomerular basement membrane in the kidneys led to a significant increase in the thickness of glomeruli. The ongoing chronic process led to mesangial cell proliferation. The acute phase of inflammation was characterized by hyperemia in glomerular capillaries, accumulation of inflammatory exudate composed of lymphocytes and plasma cells under Bowman's capsule, as well as swelling and necrosis of vascular endothelial cells (Fig. 1).

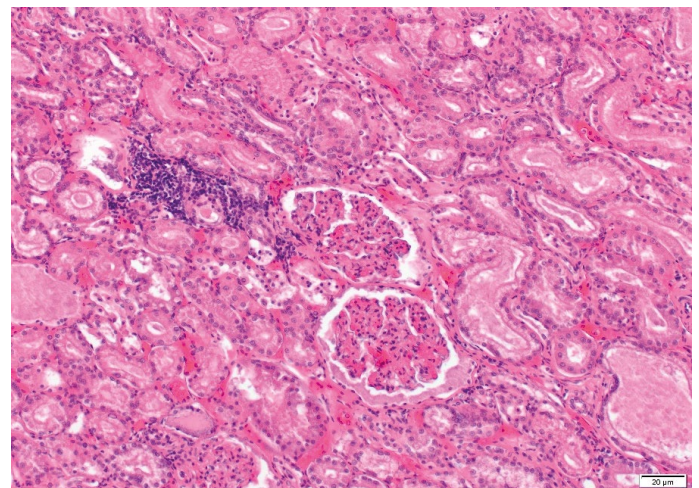


Fig. 1. Acute glomerulonephritis with transudate and inflammatory infiltration composed of lymphocytes and plasma cells. H-E staining. Magnification $\times 100$

Liver congestion was the second most frequent change. The histopathological examination revealed disturbances in the trabecular hepatocyte system,

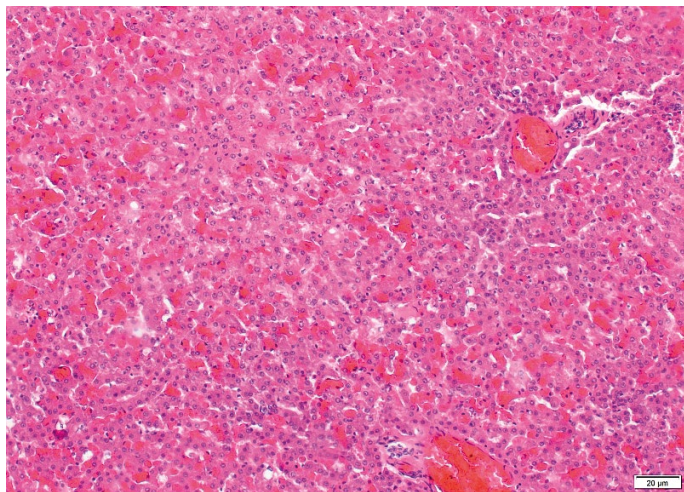


Fig. 2. Liver congestion with a few inflammatory infiltrations around the central vein. H-E staining. Magnification $\times 100$

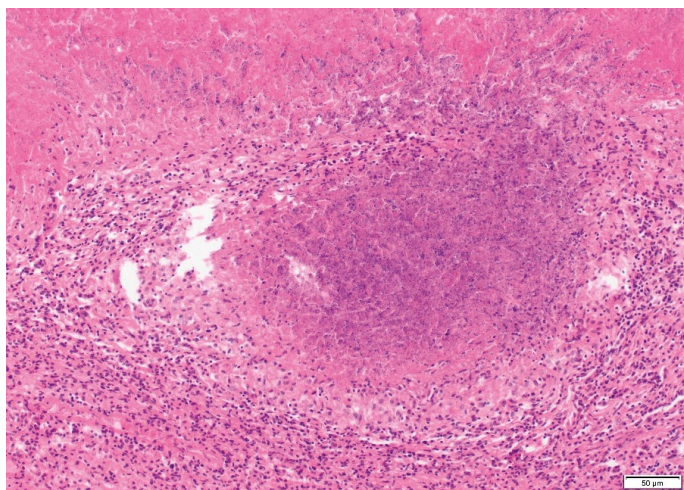


Fig. 3. Coagulative liver necrosis with inflammatory infiltrates composed of leukocytes, macrophages, and plasma cells and granulation tissue forming in the peripheral part. H-E staining. Magnification $\times 100$

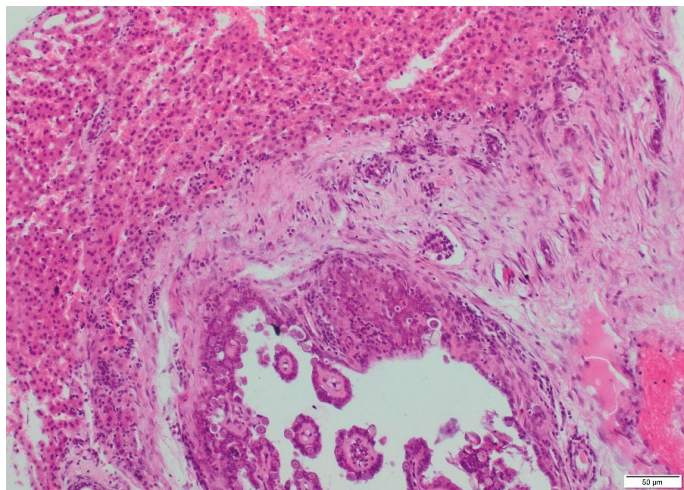


Fig. 4. Hepatic coccidiosis with hyperplasia of connective tissue and bile duct epithelium. Coccidia and fragments of dead cells (detritus) in the bile duct lumen. H-E staining. Magnification $\times 100$

dilatation of central veins, and hyperemia of central hepatic lobules, indicating serious disturbances in the blood outflow from this organ (Fig. 2). Moreover, the image of hepatic cell nuclei and cytoplasm was blurred.

Coagulative liver necrosis was detected in cells with pyknotic nuclei and fragmented cytoplasm (Fig. 3). Inflammatory infiltrate-forming cells accumulated around necrotic foci, indicating removal of dead tissue. Overgrowth of connective tissue was observed in the periphery, which indicates demarcation of necrotic areas. A relatively loose distribution of some hepatocytes was often observed around necrotic areas.

The dominant histopathological lesions in the liver indicated hepatic coccidiosis. A substantial dilatation of the bile duct lumen combined with hypertrophy of the duct walls was detected in the liver (Fig. 4). The connective tissue of the ducts together with the epithelium formed papilla-like elevations. Their intensive growth led to a significant narrowing of the bile ducts and resulted in an almost complete loss of patency. Coccidia at various stages of development parasitizing the bile duct epithelium exerted pressure on cell organelles, leading to their deformation. The pressure resulted in the disintegration of bile duct epithelial cells and the release of parasites and exfoliated epithelium into the bile duct lumen, which in turn resulted in the formation of cellular “detritus”.

The comparison of the pathological changes detected in the samples with the mean body weight of the hares revealed that animals with the pathological lesions in the liver and kidneys also had the lowest body weight (Fig. 5). The lowest mean body weight was recorded in individuals with the parasitic invasion of the liver and interstitial nephritis in 2021 and in those with glomerulonephritis and liver congestion in 2022.

The mean values of the kidney fat index (KFI) were lowest for animals with pathogenic lesions of the liver and kidneys (Fig. 6). The lowest mean KFI value was noted in the case of liver parasitic invasion in both years.

The study did not show significant differences in the values of the kidney fat index (KFI) and the body weight between the sex and age groups of hares. Therefore, the influence of sex and age was disregarded in further analyses.

Table 1 presents the mean body weight values and fat reserves in hare carcasses in correlation with the presence or absence of pathological lesions. In both groups (with lesions and without lesions), the difference between the mean carcass weight and KFI was assessed. Parasitic liver invasion had the greatest impact on the decline in the mean values of the body condition parameters (carcass weight, KFI). The mean body weight of hares diagnosed with parasitic liver invasion was lower than that of animals with no infestation in both years, but the difference was not statistically significant. Comparison of fat reserves expressed by the KFI index showed a clear decrease

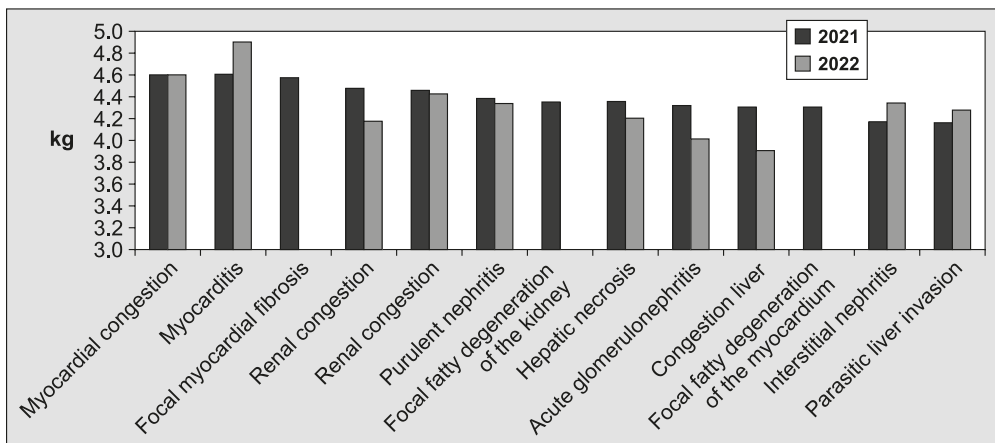


Fig. 5. Ranking of pathological lesions according to the mean body weight (kg) of the hares in 2021 and 2022

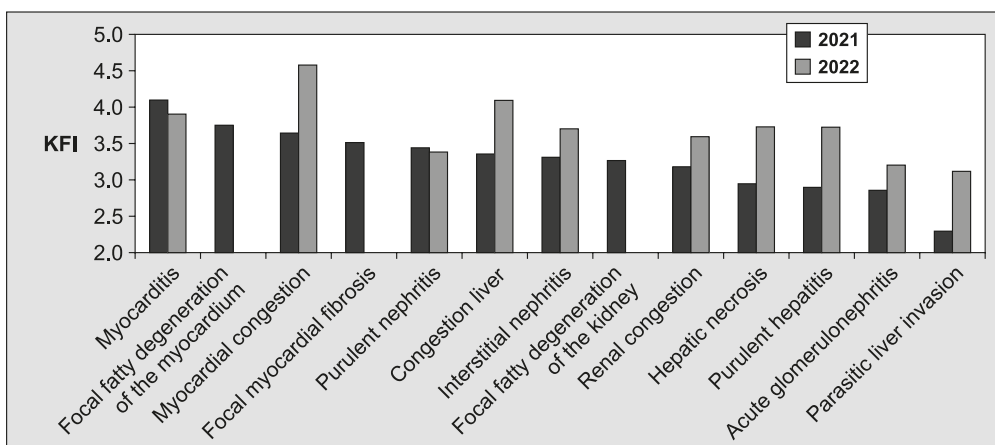


Fig. 6. Ranking of pathological lesions according to the average KFI values in 2021 and 2022

Tab. 1. Comparison of mean body weight, kidney fat index (KFI) values in correlation with the presence of liver parasites in the 2021/22 hunting season (*t*-test for independent variables)

Value	Year	Absence of pathological lesions of the liver [mean ± SEM]	Parasitic liver invasion [mean ± SEM]	<i>t</i> -value	<i>p</i> -value
Body weight (kg)	2021	4.38 ± 0.1	4.16 ± 0.1	1.211	0.233
	2022	4.34 ± 0.1	4.28 ± 0.3	0.393	0.697
KFI	2021	3.21 ± 0.1	2.29 ± 0.3	3.175	0.003*
	2022	3.73 ± 0.1	3.11 ± 0.3	2.003	0.055

Explanation: * – statistically significant at $p = 0.001$

in the value of this parameter in animals with parasitic infection. The difference was statistically significant in 2021 and close to significance in 2022 (Tab. 1).

One of the causes of the dynamic decline in the size of hare populations observed in recent years are various diseases, in addition to environmental factors and predation, which are currently becoming less important (25, 37, 43). However, due to the significant limitation or complete suspension of hunting in many regions of Poland and other European countries, there are no detailed research data on the etiology and prevalence of diseases in these animals. The available fragmentary studies are focused mainly on one or several diseases (7, 9, 12). Virological and molecular analyses of hares

from southern and eastern Poland conducted in 2014-2021 showed the absence of the EBHS virus decimating hare populations in recent years. Nevertheless, EBHS antibodies were detected in nearly 88% of hares (19). Acute glomerulonephritis with damage to the glomerular basement membrane and simultaneous accumulation of inflammatory exudates leading to further interstitial inflammation, characterized by significant stromal proliferation, is much more common in older hares than it is in younger animals (4, 30, 39).

E. stiedae is responsible for hepatic coccidiosis in hares. It causes chronic inflammation of bile ducts, which leads to dilatation of their lumen and hypertrophy of their walls. In the macroscopic image, the liver is enlarged and exhibits numerous white tumors, often merging into larger structures (15).

A study conducted on farm hares in Italy showed the presence of six species of coccidia of the genus *Eimeria* (5). Research conducted on wild hares in Bulgaria revealed infections by many parasites, with *Eimeria* coccidia as the most common species exerting an impact on the health of these animals. As reported by the author, the same phenomenon is noted in most European countries (34).

As demonstrated by Gál (24), there is a relationship between the KFI value and parasitic invasions, which confirms the

accuracy of assessing animal condition on the basis of the KFI index. It has been shown in a Portuguese study (14) that the presence of internal parasites deteriorates the condition of lagomorphs expressed by the KFI index. Studies of the effects of parasitic infections have shown that even a relatively low numbers of parasites may have a negative impact on individual condition and pregnancy, resulting in reduced survival and reproduction in the entire population (1, 33), especially when the presence of parasites is combined with a limited food base and predator pressure (31).

Pathological lesions were detected in 89% of the hares, with 14% of these individuals being affected by parasitic liver invasion. In the group of hares with

pathological lesions, the poorest individual condition expressed by the body weight and kidney fat index (KFI) was exhibited by animals with a parasitic liver infection. This may indicate that the presence of parasites plays a key role in the deterioration of the individual condition of hares and may exert a negative impact on the survival and reproduction of the entire population.

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