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The hygiene and quality of hare meat (*Lepus europaeus* Pallas) from Eastern Croatia

*Hygiene und Qualität von Fleisch von Feldhasen (*Lepus europaeus* Pallas) aus dem östlichen Kroatien*

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Summary

This research was conducted over two seasons (winter and spring) and comprised a total of 71 samples of hares shot in the eastern region of Croatia, and was aimed at examining the quality and hygiene of the meat. The carcass dressing percentage in relation to the total average weight of the shot hares was 66.54%. Chemical tests showed the following average contents in the meat of the hares: water 75.34%, protein 23.19%, fat 1.12%, ash 1.16%. Of the 71 samples examined, as many as 44 (61.97%) did not meet the requirements of the rules on microbiological food standards. In 19 samples (26.76%) this was due to the high level of aerobic mesophilic bacteria, 2 samples (2.82%) contained *Staphylococcus aureus* and 37 samples (52.11%) were not satisfactory because they contained enterobacteria. In terms of heavy metals in kidney and meat samples, 17 (23.94%) did not meet the provisions of the rules on toxins, metals, metaloids and other harmful substances which may be found in food. Of these 15 kidney samples, 88.24% contained cadmium and 2 samples (11.76%) mercury in amounts greater than the permitted concentration. Tests on liver samples from the hares (n = 71) did not show organophosphorus pesticides nor organochlorinated pesticides or polychlorinated biphenyls.

Keywords: *Lepus europaeus* Pallas, chemical composition, microbiological quality, heavy metals, pesticides

Zusammenfassung

Diese Studie wurde über die Dauer von zwei Jahreszeiten (Winter und Frühjahr) durchgeführt, wobei insgesamt 71 im östlichen Teil Kroatiens erlegte Feldhasen im Hinblick auf die Fleischqualität und -hygiene untersucht wurden. Die Schlachtausbeute in Bezug auf das durchschnittliche Gesamtgewicht der erlegten Hasen betrug 66,54 %. Die chemischen Untersuchungen ergaben folgende durchschnittliche Gehalte: Wasser 75,34 %, Protein 23,19 %, Fett 1,12 %, Asche 1,16 %. Von den 71 untersuchten Proben erfüllten 44 (61,97 %) nicht die Anforderungen der kroatischen Vorschriften über mikrobiologische Kriterien in Lebensmitteln. Die Ursache hierfür war bei 19 Proben (26,76 %) das Überschreiten der Grenzwerte für die aerobe mesophile Gesamtkeimzahl, 2 Proben (2,82 %) enthielten *Staphylococcus aureus* und 37 Proben (52,11 %) wurden auf Grund des Gehalts an Enterobacteriaceen als nicht zufriedenstellend eingestuft. Im Hinblick auf den Gehalt an Schwermetallen in Fleisch- und Nierenproben wichen 17 Proben (23,94 %) von den Anforderungen der kroatischen Vorschriften über Toxine, Metalle, Metalloide und weitere Schadstoffe in Lebensmitteln ab. Hierbei überschritten 15 Nierenproben die erlaubten Grenzwerte für Kadmium und 2 Proben wiesen Gehalte an Quecksilber oberhalb der Grenzwerte auf. Bei Untersuchungen an Leberproben der Hasen konnten weder Organophosphor- oder Organochlor-Pestizide noch polychlorierte Biphenyle nachgewiesen werden.

Schlüsselwörter: *Lepus europaeus* Pallas, chemische Zusammensetzung, mikrobiologische Qualität, Schwermetalle, Pestizide

Introduction

The numerical status of the population of the European brown hare (*Lepus europaeus* Pallas) in Eastern Slavonia has seen significant variations over the past twenty years. Regular monitoring by members of hunting associations has revealed greater or lesser oscillations in a constant fall in the number of hares. The reasons for this situation are usually said to be the uncontrolled use of pesticides in fields, various forms of pollution (industrialization, exhaust fumes), the expansion of agricultural property, intensive treatment of soil using agricultural machines, etc. Apart from these reasons, man also has an important influence (breeders, hunters), which by inappropriate management can cause great harm to the hare population (Kolar, 2003; Šelmić, 1984).

In relation to this, in this study, we conducted laboratory experiments over two seasons (winter and spring) on a total of 71 hares, 41 in winter and 30 in spring. The tests covered an assessment of the quality of the meat (chemical composition, energy value, carcass dressing percentage) and the health and hygienic quality of the meat and organs (microbiology, heavy metals, organophosphorus and organochlorine pesticides and polychlorinated biphenyls).

Material and Methods

The hares' bodies were brought to the laboratory by members of hunting associations, whole and undamaged, apart from shot wounds in various parts of the body. In 30 samples of hares during the summer season, the age was established using the Pintur et al. (2005) method as follows: after extraction, the eye lenses were fixed in formalin for 3 days and then dried in a thermostat at 37°C for 72 h, under normal pressure. After they were dried, they were weighed on a precise analytical scale (Ohaus, Pine Brook, NJ, USA) to 1 mg precision. Interpretation of the results was conducted according to Table 1.

TABLE 1: Determination of age using the method of Pintur et al. (2005)

Eye lens mass (g)	Age of hare
0.045–0.100	3 months
0.100–0.200	3–6 months
0.200–0.280	6–12 months
0.280–0.310	1–2 years
0.310–0.370	2–3 years
More than 0.370	Older than 3 years

Before the beginning of the tests, each body was weighed and the total mass of the body was thus established. Since we did not have information about the weight of the live animals as a basis for calculating the carcass dressing percentage we used the total body weight of the shot hare. Then, the hares were skinned and eviscerated and the organs (lungs, heart, liver, spleen and kidneys) were weighed individually. After the organs were weighed, all the organs and the digestive tract were removed and the metacarpal and metatarsal parts of the legs were removed, and the body was weighed once more.

Chemical analysis

Chemical tests included establishing the quantity of water, fat, protein, calcium and phosphorus. The tests were carried out according to the Rules on the Methods of Chemical Analysis and Super-analysis of Meat, Fat and Oil Products (Pravilnik o metodama obavljanja kemijskih analiza i superanaliza proizvoda od mesa, masti i ulja, OG RoC no. 53/91). All samples of hare meat ($n = 71$) for chemical tests were put into special glass phials with 150 g in weight.

The energy values were calculated according to the formula:

$$\text{Energy value (kJ/100 g)} = [(\% \text{ fat} \times 9.3) + (\% \text{ protein} \times 4.2) + (\% \text{ carbohydrates} \times 4.1)] \times 4.186$$

Bacteriological analysis

For microbiological tests, the surface of the meat of the thigh was charred, and then a sterile sample of 100 g was taken from deep within. In total, 20 g of the sample was homogenized in 180 ml saline solution, in a stomacher for 2 minutes. After this, a series of solutions were made in 1 or 0.1 ml solution, on a selective or non-selective surface. The total number of bacteria was established using the HRN ISO 4833:2003 method, sulphite reducing clostridia were determined according to HRN ISO 15213:2004, *Salmonella* spp. using the HRN ISO 6579:2003 method, and *Listeria* (*L.*) *monocytogenes* was examined by the HRN ISO 11290-1 method. To find enterobacteria, 1 ml of basic solution was inoculated in a EE stock cube (Oxoid, Basingstoke, Hampshire, England) incubated 24 h at 37°C and then streaked on VRBG agar (Oxoid) and incubated at 37°C for 24 h. In order to determine the coagulase positive staphylococci 1 ml of basic solution was inoculated in Giolitti Cantoni broth, incubated at 37°C for 24 h and then transferred onto Baird Parker agar (Merck, Darmstadt, Germany). Suspect colonies were confirmed by coagulase test (Bactident Coagulase, Merck).

Analysis of heavy metals

For the analysis of heavy metals, pesticides and polychlorinated biphenyls, the following samples were taken from each wild hare: one kidney, part of the liver and a part of the muscles weighing 150 g.

The samples were packed in PVC bags, frozen and sent to the laboratory for analysis. The amount of cadmium, lead, arsenic and mercury was determined using atomic absorption spectrophotometry (AAS). Since on the market of certified reference materials (CRM) there were no lyophilised preparations with a known quantity of metal in the muscles, liver and kidneys of hares, during the evaluation we used CRM beef lyophilised liver (CRM 185 R) and pork lyophilised kidney (BCR 186 N). The quantity of cadmium and lead in the organism and the meat of the hares was determined by the AAS flame technique (atomic absorption spectrophotometer ATI UNICAM 929 with a hydride connection UNICAM FI 90) after dry burning of the samples (Sapunar-Postružnik, 1993). For analysis, duplicate samples were prepared with an obligatory control test. In order to prepare the working standards of the solution, a ready-made standard solution was used with a concentration of 1000 mg/l. In order to determine the lead content, working solutions of 1, 3 and 5 mg/l were prepared. Since lead shows linearity up to as much as 20 mg/l, as a standard we only used one of the prepared solutions depending on the quantity of lead in the sample. To measure cadmium, working stan-

standard solutions were prepared of 0.1, 0.3 and 0.5 mg/l. Cadmium shows linearity up to 2 mg/l, so as with lead, only one concentration standard was used, depending on the size of the sample. The quantity of mercury was determined using the AAS method with cold steam, after wet burning the sample (Hatch and Ott, 1968). The sample was prepared by the process of wet destruction of the organic substances in a closed system (due to the volatile nature of mercury at room temperature), where the sample was oxidized by sulphur and nitric acid. To determine arsenic, the sample was prepared by dry burning with oxidation using powerful oxidants, which makes it possible to obtain the complete quantity of arsenic in the ash (Skurikhin, 1989). Measurements were taken with AAS UNICAM 929 with a hydride connection UNICAM FI 90.

Analysis of pesticides

The quantity of chlorinated pesticides in the samples was determined using gas chromatography (ATI UNICAM 619 with electron absorption detector ECD). The samples were prepared according to Long et al. (1991). Organophosphorus pesticides were also examined by gas chromatography, with quantification in a linked system of gas chromatography-mass spectrometry (GC-MS). The samples were prepared according to the protocol of Richardson et al. (1993). To determine pyrethroids, the samples were prepared using the EPA-821-R-00-018/2000 method and determined by gas chromatography-mass spectrometry (ATI UNICAM 610 with mass spectrometry).

Analysis of polychlorinated biphenyls (PCB)

Determination of the total polychlorinated biphenyls (PCB) was conducted using gas chromatography (ATI UNICAM 619 with electron absorption detector ECD) using the protocol of Long et al. (1991).

Results and Discussion

The total average mass of the shot hares ($n = 71$) was 4011 g and the weight of the prepared hares was on average 2669 g, amounting to 66.54%. From Table 2 it is clear that in the winter period a lower average body weight was recorded, as well as a lower weight for the skinned hares, the prepared hares, the organs lungs and heart, the skin, the inedible organs and other inedible parts. In relation to spring, in winter the liver, the spleen, the kidneys and the total edible organs weighed more. It should be pointed out that when we weighed the hare samples from the spring-summer period we noticed that in them the skin with the subcutaneous tissue accounted for the

largest part, as much as 14.11%, of the total weight of the hare, but at the same time in those samples the hare meat contained the lowest fat content (only 0.86%).

In spring, the hares were aged from 3 months to over 3 years. Only 3 of them (10.71%) were aged up to one year, and fell into the category of younger hares, 25 (89.29%) were aged over one year, and in 2 it was not possible to determine the age. The structure of the captured hares in terms of age in our research is significantly different from the results by Pintur et al. (2005) who in their research found as many as 50.4% younger hares.

As is visible from the results of the chemical tests, the quantity of fat and protein was significantly higher in the winter season (Tab. 3). Regardless of the season, the quantity of water and minerals and the energy values were equal. Comparing our results with literature data, important differences are visible, especially in energy values and the percentage of water and fat. The difference in the quantity of fat is particularly significant (4% in comparison with 1.18% in our results). According to the figures of Kulier (1996), in the meat of wild hares there is 72% water, 4% fat, 22% protein, 0.013% calcium, 0.210% phosphorus and the energy value is 530 kJ/100 g. In the results we received for 30 samples of meat in the spring period, the average percentage of fat was 0.86%. Certainly the reason for this significant difference in the percentage of fat in the meat should be sought in the quantity of available food according to the season in which the hares were shot. The larger quantities of fat in the meat in winter indicate the fact that with a lack of food, hares accumulate greater quantities of fat in their muscles, and in the spring and summer months, when there is sufficient food, the fatty tissue is stored to a greater extent below the skin.

In the 71 samples examined, as many as 48 (67.60%) did not meet the provisions of the Croatian Rules on Microbiological Food Standards (Pravilnik o mikrobiološkim standardima za namirnice, OG RoC no. 46/94). The reasons for this were the finding of increased quantities of total bacteria in 19 samples (26.76%), the presence of *Staphylococcus (S.) aureus* in 2 samples (2.82%) and of enterobacteria in 37 samples (52.11%). In 8 samples, a large amount of bacteria and enterobacteria was found. In one sample a high number of bacteria and additionally *S. aureus* were determined and in one sample high enterobacteria counts were combined with the presence of *S. aureus*. Of 41 samples of hare meat shot during the winter, 35 samples (85.36%) did not meet the microbiological standards, since they contained a high number of total bacteria ($n = 9$, 21.95%) and enterobacteria ($n = 26$, 63.41%). Of 30 samples of hare meat collected in spring,

TABLE 2: Yield of meat and organs of brown hares during winter ($n = 41$) and spring ($n = 30$)

WINTER											
Statistical parameter	TW/g	WSH/g	WDH/g	L/g	H/g	Lv/g	S/g	K/g	EI/g	Skin/g	NEI/g
Mean	3829.27	3373.90	2603.66	65.43	46.02	106.83	5.95	62.58	294.75	455.36	475.48
%	100	88.10	67.99	1.70	1.20	2.79	0.15	1.63	7.70	11.89	12.42
SPRING											
Statistical parameter	TW/g	WSH/g	WDH/g	L/g	H/g	Lv/g	S/g	K/g	EI/g	Skin/g	NEI/g
Mean	4259.00	3657.66	2759.33	70.83	51.33	92.33	5.00	27.60	247.10	601.33	651.23
%	100	85.88	64.78	1.66	1.21	2.17	0.12	0.65	5.80	14.12	15.29

TW – total weight; WSH – weight of skinned hare; WDH – weight of dressed hare; L – lungs; H – heart; Lv – liver; S – spleen; K – kidneys; EI – edible innards; NEI – non-edible innards and other non-edible parts.

TABLE 3: Chemical composition of brown hare meat during winter ($n = 41$) and spring ($n = 30$)

WINTER							
Statistical parameter	Water %	Fat %	Protein %	Ash %	Calcium %	Phosphorus %	EV* kJ/100 g
Mean	75.45	1.31	23.77	1.16	0.013	0.236	439.81
x_{\min}	74.29	0.72	21.25	1.07	0.008	0.210	409.05
x_{\max}	77.11	1.93	27.54	1.34	0.028	0.250	483.99
SPRING							
Statistical parameter	Water %	Fat %	Protein %	Ash %	Calcium %	Phosphorus %	EV* kJ/100 g
Mean	75.18	0.86	22.39	1.16	0.015	0.230	434.15
x_{\min}	73.25	0.54	21.09	1.11	0.011	0.200	401.37
x_{\max}	76.84	1.76	23.57	1.22	0.026	0.270	467.66

*energy value.

a total of 16 (53.33%) did not meet the provisions of the Rules. In detail, 10 samples (33.33%) showed a high amount of total bacteria, in 2 samples (6.66%) *S. aureus* were detected, and in 11 samples (36.66%) enterobacteria. In none of the 71 samples of meat from shot hares *L. monocytogenes* or *Salmonella* spp. were found.

The total results of the tests for heavy metals in the meat and kidneys of the shot hares are shown in Table 4. According to the Rules on Toxins, Metals, Metalloids and other Harmful Substances in Food (Pravilnik o toksinima, metalima, metaloidima te drugim štetnim tvarima koje se mogu nalaziti u hrani, OG RoC no 16/05), the maximum levels permitted in meat and innards are 0.05 and 1.0 mg/kg for cadmium, 0.03 and 0.1 mg/kg for mercury, 0.1 and 0.5 mg/kg for arsenic and lead. Of the total 71 samples of kidneys and meat, in 17 (23.94%) concentrations of heavy metals above the permitted levels were found. The limits were exceeded in 15 kidney samples (88.24%) for cadmium and in 2 samples (11.76%) for mercury. Higher quantities of mercury were found in meat samples (0.039 mg/kg) and kidneys (0.326 mg/kg).

TABLE 4: Heavy metals (mean, mg/kg) in kidneys of brown hares during winter ($n = 41$) and spring ($n = 30$)

Season	Cadmium	Mercury	Arsenic	Lead
Winter	0.938	0.030	< 0.002	< 0.05
Spring	0.707	0.011	0.025	< 0.05

During the winter ($n = 41$) higher concentrations of cadmium were found in 10 kidney samples (36.36%). The average quantity of cadmium in the kidneys in the winter season was 0.938 mg/kg, and the values ranged from below the detection limit (< 0.005 mg/kg) to 9.588 mg/kg. Higher quantities of mercury were recorded in kidney samples and one meat sample. The average concentration of mercury ($n = 41$) in the kidneys was 0.030 mg/kg, and it ranged from below the detection limit (< 0.002 mg/kg) to 0.326 mg/kg. None of the kidney samples from the hares shot in winter contained lead or arsenic.

The tests on 30 kidney samples from hares shot in spring revealed increased levels of cadmium in 5 samples (16.66%). The average quantity of cadmium in the kidneys of the hares in this group was 0.707 mg/kg, with a range from below the detection limit (< 0.005 mg/kg) to 6.139 mg/kg. The average concentration of mercury in the kidneys of hares in this group was 0.011 mg/kg,

ranging from below the detection limit (< 0.002 mg/kg) to 0.057 mg/kg. Furthermore, in 10 kidney samples, arsenic was detected, but within the permitted level (the highest amount found was 0.109 mg/kg). None of the kidney samples contained lead. Furthermore, none of the meat samples from the hares contained cadmium, mercury, arsenic or lead in detectable quantities.

In contrast to our results, Massanyi et al. (2003) found higher concentrations of lead in kidneys in the winter period than in spring and summer. Similarly, these authors reported larger quantities of cadmium in winter, which was in agreement with our results. Kramarova et al. (2005) found that the highest concentrations of cadmium were found in the kidneys of game animals in the area of Slovakia and they ranged from 0.213 to 0.238 mg/kg, whilst the average quantity of cadmium was between 0.115 and 0.561 mg/kg. Their results are significantly lower than the results of our research, with average concentrations of cadmium in the kidneys of 0.840 mg/kg. Our results showed that the concentrations of cadmium in females (0.942 mg/kg for ten females) were significantly higher than in males (0.590 mg/kg for 20 males), which is in line with the results found by Massanyi et al. (2003). Their study showed higher total concentrations of cadmium than in our study, but the amounts were also much higher in females (1.464 mg/kg) than in males (1.384 mg/kg). The results of our research also agreed with those of Langgemach (1995) who determined the largest quantity of cadmium in the kidneys, less in the liver and the lowest amount in the muscles. It should be pointed out that we did not find cadmium in samples of the muscles of wild hares. This author emphasized that the population of hares is the best indicator for an assessment of contamination by heavy metals of the game population in the area examined. Furthermore, the results of our research for mercury in the kidneys of hares were almost three times lower (0.022 mg/kg) than the results gained by Massanyi et al. (2003) in their research (0.068 mg/kg). In terms of the relationship of the age of the hares and the findings of each form of pollution, it was established that in older animals the concentration of cadmium in the kidneys (0.783 mg/kg) was significantly higher than in younger animals (0.028 mg/kg). The results of our research are in line with the results obtained by Massanyi et al. (2003).

A total of 71 liver samples from hares were examined for organophosphorus pesticides and polychlorinated biphenyls. It should be emphasized that the data available from literature (Langlois and Langis, 1995) differ from our

results since we were not able to determine either organophosphorus pesticides or chlorinated pesticides or polychlorinated biphenyls above the level of detection. The results of our research showed the low level of pollution of the organs and meat of hares with these bio-residues in the area of the Vukovarsko-srijemska and the Osječko-baranjska counties. If the statement by Langgemach (1995) is correct that the wild hare population is the best indicator for an assessment of contamination by heavy metals and other pollutants, then we can presume that the situation in the area researched is satisfactory.

Conclusion

In terms of chemical composition, and in comparison with other types of meat, the meat of hares, in terms of its sensorial characteristics, the quantity of protein, the low quantity of fats and other basic chemical components along with the energy values found, may be considered to be recommendable concerning nutrition and diet. The reasons for the relatively unfavourable microbiological results and unsatisfactory hygiene of the hare meat in our opinion should primarily be ascribed to the disparity of the times of evisceration after shooting and the length of transport to the laboratory. The results of the toxicology analysis of the meat samples and the organs of the hares do not indicate significant environmental pollution of the hares' habitats with heavy metals, pesticides and polychlorinated biphenyls.

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