

IMPROVING THE CONDITION OF EUROPEAN HARE THROUGH NUTRITION

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ABSTRACT

The objective of the article is to evaluate the effects of a newly designed granulated mixture enriched with Bohemian knotweed (*Reynoutria x bohemica*) on European hare (*Lepus europaeus*) kept at closed farms. The positive influence of knotweed on the microbiome in the digestive system and better usage of the fodder were proven based on biochemical and haematological analysis of blood. Lower manifestation of pathogenic organisms is also expected. Finally, the positive influence on higher weight gains in baby hares was proven, which improves their condition. The results can be used in practice at closed farms breeding European hare focused on releasing bred young hares into open hunting grounds where it is possible to obtain a monetary contribution for the releasing of hares from a grant of the Ministry of Agriculture of the Czech Republic in the field of hunting. Furthermore, the results can be used for feeding hares in open hunting grounds.

Keywords: European hare; granulated feeding mixture; Bohemian knotweed; biochemical analysis of blood; haematological analysis of blood

INTRODUCTION

The nutrition of animals is the part of the overall regimen of animal care that requires expert knowledge of animals' biology and their physiological needs, as well as proper administration of fodder according to time and the needs of animals. Proper nutrition is an inseparable part of animal care and one of the key factors for achieving good condition and constitution and increasing the breeding quality of animals at closed farms and in the wild.

Kučera *et al.* (2006) rank hares among steppe and forest-steppe species that are predominantly found on field and forest edges in the cultural landscape. Hell & Slamečka (1999) state that hare is an exclusive herbivore which feeds mainly on plant communities that are found in meadows and pastures. Based on the energetic balance, the need for fresh mass of hare was determined as 0.6–0.8 kg. Hares prefer a diet consisting of a large number of herbs, field weeds, and agricultural crops (e.g. beet, cabbage, oilseed rape, pea, winter crops

and permanent grassland). They also like woody plants (e.g. grapevine and fruit trees), which constitute about 5 % of hares' diet. When there is sufficient food, hares are quite choosy, whereas in times of scarcity, they may suffer from a lack of quality food due to the monotrophic diet, which can lead to death. A sudden change in the species' structure of food may be the cause, for instance, due to the harvest of crops or tillage. The hare is an animal that is characterised by a relatively small active radius, and such a sudden change may be fatal for it due to the hectare areas that are routinely farmed at the same time. When looking for appropriate food, it goes a maximum of 1 km from its territory, which does not have to be enough to acquire a more varied diet. Moreover, it is necessary to realise that for digesting, hares use caecotrophy, which is consumption and repeated digestion of mushy excrement (caecotropes) which contains, among others, microorganisms that help in digesting plant food.

To understand all the contexts related to nutrition and animal feeding, it is necessary to state that our landscape and environment have changed significantly during the evolution. Natural biotopes are giving way to agriculture, which forces animals to adapt to constantly changing conditions. Large-area agricultural management to obtain higher yields has created a territory with very limited and poor sources of food and shelter from a landscape that was once rich and lively. The rapid process of cereal, forage and root crop harvest on large areas with subsequent tillage and cultivation of land causes a drastic change in the availability of natural food for animals.

For Bohemian knotweed (*Reynoutria × bohemica*), it is possible to assume similar positive effects that were described by several authors for Japanese knotweed (*Polygonum japonicum*, syn. *Reynoutria japonica*), which is also ranked among invasive plants. For resveratrol and piceid contained in the knotweed, antioxidation properties were proven, and piceid seems to be more effective than resveratrol (Jacob *et al.*, 2014). Lawless (2010) states that daily administration of resveratrol improves energetic metabolism in horses by acting on mitochondria, which serve as a 'power station' of the body. In vitro tests on laboratory animals and clinical studies in human medicine show antibacterial effects (Chan, 2002; Docherty *et al.*, 2001), antifungal effects (Adrian *et al.*, 1997; Bavaresco *et al.*, 2001, 2003; Filip *et al.*, 2003; Jung *et al.*, 2005; Schulze *et al.*, 2005), antiparasitic effects (Anantaphruti *et al.*, 1982), anti-cancer effects (Ferry-Dumazet *et al.*, 2002; Roman *et al.*, 2002; Ulrich *et al.*, 2005; Wolter *et al.*, 2004; El-Mowafy & Alkhalaf, 2003; Lu *et al.*, 2008; Muto *et al.*, 2007; Fu *et al.*, 2007; Pecere *et al.*, 2000; Jeong *et al.*, 2010; Pezzuto, 2008), and effects against vascular and other so-called diseases of civilisation, even those related to overweight and diabetes (Kerem *et al.*, 2006; Dal-Pan *et al.*, 2010; Brasnyo *et al.*, 2011). Maděra *et al.* (2021) found positive effects of Bohemian knotweed in pig diet, especially on lipid metabolism.

The objective of the article is to evaluate the effects of a newly designed granulated mixture enriched with Bohemian knotweed (*Reynoutria x bohemica*) on European hare (*Lepus europaeus*) kept at closed farms.

MATERIAL AND METHODS

During a two-year period, a group of 14 European hares, to which the granulated feed mixture with the addition of knotweed was administered, and a control group of 8 European hares, to which the same feed mixture without the addition of knotweed was administered were tested. Throughout the whole experiment, breeding pairs of hares were kept together in special cotes for hares. The number of baby hares born to each breeding pair was monitored, and the birth weight and weight at 5 days of each baby hare were noted. At the end of the

calendar year (after the end of the reproductive season and before the start of a new reproductive season), a veterinarian took blood from a vein in the auricle from all the alive individuals. Subsequently, the blood samples were biochemically analysed at Medila s.r.o. by MVDr. Štěpán Vencl, a veterinarian specialised in haematology. Values of ALT, AST, ALP, GMT, urea, creatinine, total protein, albumin, A/G ratio, globulins, glucose, triglycerides, cholesterol, CK, bilirubin T and bilirubin P were determined biochemically; and values of leukocytes, erythrocytes, haemoglobin, haematocrit, MCV, MCH, MCHC, neutrophiles, eosinophiles, basophiles, lymphocytes and monocytes were determined haematologically. The group fed with the addition of knotweed and the group fed without the addition were evaluated separately using statistical methods.

RESULTS AND DISCUSSION

Significantly different results between the group of hares fed a mixture with the addition of knotweed and the group of hares fed a mixture without the addition of knotweed were found in the content of urea and creatinine and the CK value. No statistically significant results were found in other monitored biochemical and haematological parameters, and therefore they are not described in detail here. For an overview, however, we present the range of measured values from biochemical and haematological analysis of blood from the entire period of the experiment in the following tables.

Table 1: The range of biochemical values measured within the monitored groups of hares

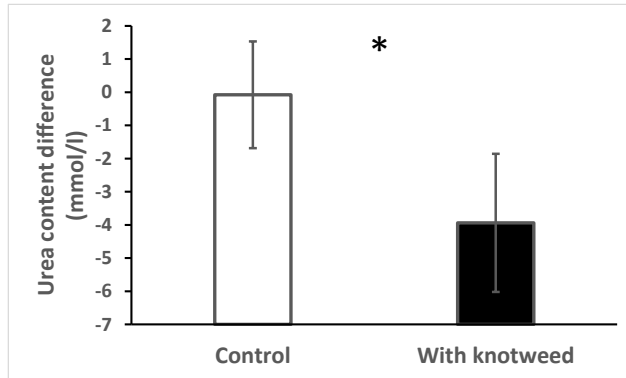
Biochemical value	Unit	The group with knotweed		The group without knotweed	
		Min.	Max.	Min.	Max.
ALT	μkat/l	0.51	2.92	0.70	1.14
AST	μkat/l	0.51	1.89	0.52	1.74
ALP	μkat/l	0.35	2.71	0.45	1.63
GMT	μkat/l	0.09	0.38	0.12	0.34
Urea	mmol/l	4.00	8.60	6.80	10.80
Creatinine	μmol/l	61.00	90.00	70.00	103.00
Total protein	g/l	45.00	55.60	45.30	55.00
Albumin	g/l	35.90	41.60	36.50	42.60
Globulins	g/l	7.20	14.40	7.20	13.40
A/G ratio		2.86	5.50	3.10	5.29
Glucose	mmol/l	8.68	14.95	9.75	16.20
Triglycerides	mmol/l	0.48	1.42	0.65	1.10
Cholesterol	mmol/l	0.36	0.71	0.37	0.63
CK	μkat/l	6.96	17.36	6.57	15.13
Bilirubin T	μmol/l	<1.8		<1.8	
Bilirubin P	μmol/l	<1.8		<1.8	

Table 2: The range of haematological values measured within the monitored groups of hares

Haematological value	Unit	The group with knotweed		The group without knotweed	
		Min.	Max.	Min.	Max.
Leucocytes	$\times 10^9/l$	1.60	7.20	3.40	8.20
Erythrocytes	$\times 10^{12}/l$	9.28	11.40	9.00	10.18
Haemoglobin	g/l	183.00	211.00	180.00	201.00
Haematocrit	l/l	0.600	0.710	0.590	0.670
MCV	fl	58.77	69.47	63.85	68.93
MCH	pg	17.55	21.01	17.75	21.12
MCHC	g/l	284.85	314.52	268.66	316.13
Neutrophils seg.	$\times 10^9/l$	0.372	3.264	0.578	4.428
Neutrophils seg.	%	6.00	68.00	14.00	69.00
Eosinophiles	$\times 10^9/l$	0.000	0.124	0.000	0.064
Eosinophiles	%	0.00	5.00	0.00	1.00
Basophiles	$\times 10^9/l$	0.000	0.032	0.000	0.000
Basophiles	%	0.00	1.00	0.00	0.00
Monocytes	$\times 10^9/l$	0.000	0.252	0.034	0.246
Monocytes	%	0.00	7.00	1.00	3.00
Lymphocytes	$\times 10^9/l$	0.896	5.616	1.856	6.004
Lymphocytes	%	29.00	90.00	29.00	85.00

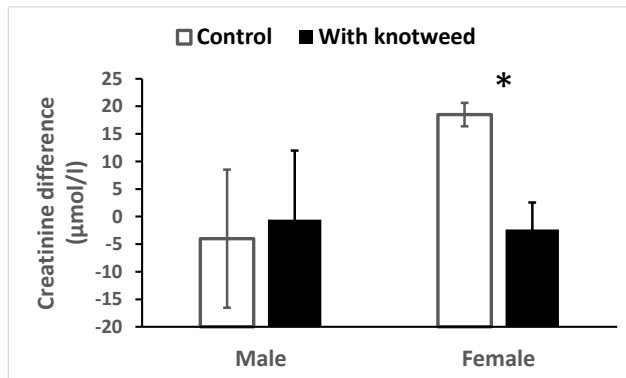
A statistically significant result was observed in the change of urea concentration during two years, where the values found in blood of hares fed on a feeding mixture with the addition of knotweed were lower than in hares fed normally (see Fig. 1). Due to the fact that urea is created as a metabiological product during the degradation of amino acids, where the created ammonia is bound and excreted in the form of urea, it is possible to state, based on the results of comparing the groups fed with and without knotweed, that fodder with the addition of knotweed contributes to better usage of proteins that were further metabolised and used by the organism. According to the chemical analysis of the fodders, it can be assumed that there is enough protein in the fodder, urea is rather unspecific for liver functions. However, if we were looking for pathologies, it can be seen in the following figure that the same group also has higher creatinine (see Fig. 2), which would indicate a possible limitation in kidney function, but the values are within the physiological standard. Marco *et al.* (2008) state that the range of the amount of urea for European hare is 11.31–21.19 mmol/l, which are higher values than we have observed in both monitored groups.

Fig. 1: The change of urea content during two years for hares fed on the feeding mixture with the knotweed addition and without the knotweed addition. Mean \pm S.D. Statistically different values at $P=0.05$ are marked with an asterisk.



The higher interannual increase of creatinine in the blood of female hares fed on the feeding mixture without the addition of knotweed compared to the female hares fed on the mixture with the addition of knotweed is statistically significant. The opposite is true for males; however, no significant results were found (see Fig. 2). The creatinine concentration in serum is directly proportional to the muscle mass of the organism; consequently, it is slightly higher in male hares and lower in baby hares and with lower volume of muscle mass.

Fig. 2: The change of creatinine content during the year 2019 in hares fed on the feeding mixture with the knotweed addition and without the knotweed addition according to sex. Mean \pm S.D. Statistically different values at $P=0.05$ are marked with an asterisk.



Higher values may also be found in the kidneys, for example, in the case of dehydration. The resulting values are relatively low, and therefore the physiology, the different age of animals and the state of their hydration may also have an impact here. Based on the results of the experiment, it can be assumed that a metabolism disruption occurred, probably caused by higher sensitivity to stress, in female hares fed on the mixture without the addition of knotweed, which may have affected the results. If we compare values we observed in individuals fed with the knotweed addition (61.00–90.00 $\mu\text{mol/l}$) or without the addition of knotweed (70.00–103.00 $\mu\text{mol/l}$) with the results of Marco *et al.* (2008), who found values in

the range of 87.4–140.6 $\mu\text{mol/l}$, our range of values is lower while the lowest is for individuals fed on the feeding mixture with the addition of knotweed.

A statistically significant result was also found for the interannual difference of CK values, where a lower value was found in the blood of hares fed on the feeding mixture without the addition of knotweed compared to the hares fed on the mixture with the addition of knotweed (Fig. 3). CK is an important enzyme in the energetic metabolism of organisms. It is necessary to take into account the influence of age, volume of muscle mass and physical activity. In pathologies, it usually increases with muscle damage (together with AST and other indicators). Together with the observed lower values of urea and better weight gain in baby hares, the pathology is not expected, though better management of nitrogenous substances is more likely. Furthermore, the effect of manipulation-capture from breeding cotes is possible here, which increases the muscle work before the blood collection, and therefore a higher amount of enzyme could be released into the blood.

Fig. 3: The change of CK value during the year 2019 in hares fed on the feeding mixture with the knotweed addition and without the knotweed addition. Mean \pm S.D. Statistically different values at $P=0.05$ are marked with an asterisk.

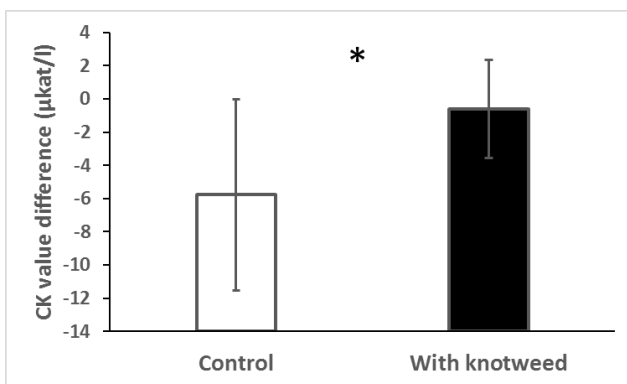
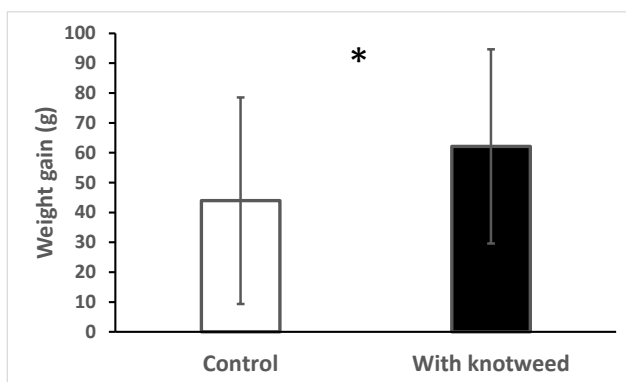


Fig. 4: The weight gain at 5 days after birth in baby hares from parents fed on the feeding mixture with the knotweed addition and without the knotweed addition. Mean \pm S.D. Statistically different values at $P=0.05$ are marked with an asterisk.



The final statistically significant result is the difference in the weight gain of baby hares from breeding pairs fed on the feeding mixture with the addition of knotweed compared to those from breeding pairs fed on the mixture without the knotweed addition (see Fig. 4).

This monitored parameter shows the proven manifestation of higher weight gain. The positive influence on the weight gain of baby hares from females fed on the feeding mixture with knotweed can be expected. Pregnant and lactating female hares have the highest demand for the protein content. If knotweed improved the protein usage, for example, by modifying the microbiome, this would be ideal since increasing the protein in hares is quite problematic due to the immediate reproduction of bacteria of the *Clostridium spp.* genus.

CONCLUSION

Following biochemical and haematological analysis, significant and different results between the group of hares fed on the mixture with the knotweed addition and the group of hares fed on the mixture without knotweed were observed in the urea and creatinine content and in the CK value. Based on the results, it is obvious that after the enrichment with knotweed, the feeding mixture has a positive influence on the microbiome in the digestive system and better usage of fodder in European hare. The lower manifestation of pathogenic organisms can be expected, and the positive influence on the weight gain of baby hares was proven, which improves their condition. For these reasons, the feeding mixture with the addition of knotweed is suitable for use mainly at closed farms that produce young European hare for releasing into the wild to improve the genetic variability in subpopulations or for the establishment of new subpopulations. However, it is also suitable for feeding *Leporidae* in open hunting grounds. Finally, the results obtained seem to indicate that it is suitable for use at backyard holdings and farms with rabbits, in which a similar effect can be expected. In conclusion, it can be stated that the observed results for hares fed on the feeding mixture with the addition of knotweed seem to be original, and therefore it was not possible to compare them with results of other authors.

Landscape ecological implication

Japanese knotweed (*Reynoutria japonica*), Sakhalin knotweed (*R. sachalinensis*) and their hybrid Bohemian knotweed (*R. × bohemica*) are considered some of the most dangerous invasive plant species in the Czech Republic (Mandák *et al.*, 2004) and in entire temperate zone of the Northern Hemisphere (Claude, 2017; Shaw *et al.*, 2009; Luque *et al.*, 2014). Utilisation of knotweed for fodder additive production could be way how to eradicate it at least partially from the landscape.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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