NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 51, 2018 (2): 86–90

ISSN 13379984

EFFECT OF HUMIC SUBSTANCES ON THE REPRODUCTION PARAMETERS OF FARMED BROWN HARE : SHORT COMMUNICATION

T. SLÁDEČEK*, R. JURČÍK, J. SLAMEČKA, Ľ. ONDRUŠKA

NPPC – Research Institute for Animal Production Nitra, Lužianky, Slovak Republic

ABSTRACT

The aim of the study was to evaluate the effect of humic acids on the reproduction parameters of farmed brown hares and the possibility of their utilisation as prevention of gastrointestinal tract diseases when feeding green fodder. Animals were divided into three groups, 4 breeding pairs per group. Brown hares in the control group were fed granulated feed mixture without additives. The hares in experimental groups were fed granulated feed mixture with HUMAC Natur additive, purchased from Humacon, at 1 % concentration. The experimental group 2 was fed also green fodder during the entire experiment (April - September) in addition to the granulated feed mixture. In the experimental group 1, the ratio of live born leverets was significantly higher by 19.54 % ($P \leq 0.05$) and the ratio of weaned leverets was higher by 9.74 % compared to the control group, but no significant differences were found. The significantly highest mortality (12.50 %; $P \leq 0.001$) before weaning (28 days of age) was determined in the experimental group 1. In the experimental group 2 was the highest ratio of weaned leverets (89.19; no significant differences was found) and no increase in mortality, as a result of feeding green fodder, was determined.

Key words: brown hare; *Lepus europaeus*; reproduction; humic substances

INTRODUCTION

Rapid decrease in the population numbers of wild brown hare in Europe led to expansion of its farming as one of the possible methods of strengthening the decimated populations in the intensely managed agricultural environment. However, due to the hare's behavioural characteristics as a wild game animal, brown hares farmed in cages are under increased stress and therefore also express increased sensitivity to various pathogens. Most often, losses are caused by proliferation of coccidia in the digestive track, enterocolitis and decreased resistance to bacterial infections due to stress.

As a preventive measure, fodder feed and hay were excluded in favour of feeding exclusively granulated feed mixtures. Despite this, coccidiosis is present in the farming systems and many breeders choose to treat with anticoccidial medicaments. Many farms in fact feed the hares granulated feed mixtures with added anticoccidial medicaments throughout the year. However, it is necessary to point out that the primary objective of brown hare farming should be production of animals that can adapt after release to the new environment with natural sources of nutrition. When animals from farms that use feed mixtures with added medicaments are used for restocking, mortality is high already shortly after release. One of the causes is the digestive system not adapted to the large quantities of green fodder and the collapse of "virgin" organism's immunity system.

In recent years, humic substances are ever more frequently utilized in animal nutrition. Humic substances are among the most widespread

***Correspondence:** Email: tomas.sladecek@vuzv.sk
Tomáš Sládeček, NPPC – Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic
Tel.: +421 37 6546 615

Received: February 2, 2018
Accepted: May 9, 2018

organic compounds. They belong to natural organic compounds, which are created by chemical and biological decomposition of organic matter, mainly plants and animals. Humic compounds are naturally present primarily in sediments, soils, peat, brown coal, lignin and some other materials (Veselá *et al*., 2005).

At present, the prevalent opinion among scientist is that humic substances have their origin in lignin. Biodegradation of lignin creates polyphenols, which likely play key role in the creation process of humic substances and are considered their precursors (Peña-Méndez *et al*., 2005).

Due to their high absorption capacity and ability to bind microbial toxins, fungal toxins and other toxic compounds, such as ammonia and polychlorinated biphenyl (PCB), dioxins, etc. humates were in the past used primarily for reduction of ammonia in stables for large livestock (Ndayegamiye and Cote, 1989; Shi *et al*., 2001).

Humic acids have significant influence on decreasing the amount of pathogens in the digestive track and function as prevention and treatment for coccidiosis in livestock. Effective utilization of antibacterial, antiviral and anticarcinogenic impact of humates in veterinary practice and animal nutrition is well-described (Thiel *et al*., 1977; Huck*et al*., 1991; Yamada *et al*., 1998; Joone *et al*., 2003).

The objective of our study was to evaluate the effect of humates, supplemented in granulated feed mixture as the only feed and in combination with green fodder, on the reproduction parameters of farmed brown hare.

Table 1. Composition of the feed mixture used in control and experimental groups

Protein (%)	15.00
Fibre (%)	17.00
Ash (%)	10.00
Ca (%)	0.80
P (%)	0.50
Na (%)	0.14
vit. A (I.E. kg^{-1})	8000
vit. D2 (I.E. kg^{-1})	800
vit. E ($mg.kg^{-1}$)	40

MATERIAL AND METHODS

The experiment was conducted at a registered brown hare farm (reg. number - SK-FCH-NR- 468) at the NPPC – Research Institute for Animal Production Nitra.

Brown hares (*Lepus europaeus*, Pall.), kept in outdoor cages throughout the year were used in the experiment. Duration of the experiment was 6 months (began: 1.4.2017, finished: 30.9.2017).

Breeding pairs in the first or second reproduction year were selected for the experiment, due to variability of reproduction parameters increasing significantly in older animals.

The breeding pairs were kept in standard cages for brown hare farming sized 2 x 1.8 m. The leverets were weaned into cages of the same size at the age of 28 days. As the cages were situated outdoors, the reproduction cycle was influenced by the climatic conditions, which might have influenced the reproduction parameters as compared to other seasons.

Total of 12 breeding pairs of brown hare were included into the experiment and divided into 3 groups, 4 pairs per group:

- Control group: feed mixture with no additive
- Experimental group 1: 1.00 % additive in the feed mixture
- Experimental group 2: 1.00 % additive in the feed mixture + green fodder

Granulated feed mixture was supplemented in storage feeders *ad libitum* to all groups. During the entire experiment, the hares in the experimental groups were fed experimental feed mixture with added feed supplement – HUMAC Natur (Humacon, s.r.o., Košice, Slovakia) with high ratio of humic acids at 1 % concentration. Nutrient composition of the feed mixture is presented in Table 1.

The evaluated commercial feed additive (Humacon, s.r.o., Košice, Slovakia) is a brown and black powder on the basis of oxihumolit. It is biologically and pharmacologically highly active 100 % natural substance. Usually, it is applied in small doses to feed mixtures for all animal species. Application of this additive prevents diseases (especially diarrhea) or corrects existing disorders due to its wide-range effect on the organism as a whole. It has detoxical, antiseptic and fungicidal effects. The active ingredients in the additive are

humic acids, fulvic acids, minerals, trace elements and carboxymethylcellulose complex with humic acids. Composition of the additive is presented in Table 2.

In the experimental group 2, green fodder was fed at least 6 times a week at dose of 0.5 - 1.00 kg depending on the number of reared leverets. Due to the necessity to use fresh green fodder, green fodder was not supplemented when the weather conditions (rainy days) prevented it. Selected as green fodder was a mixture of red clover, white clover, alfalfa, *Lotus corniculatus*, meadow fescue, red fescue, orchard grass and perennial ryegrass.

During the entire experiment, the animals had *ad libitum* access to drinking water supplied through stainless steel drinker.

In the experiment, selected reproduction parameters were monitored: ratio of live born leverets (%), mortality before weaning at 28 days of age (%), and ratio of weaned leverets (%).

Statistics

Statistical analysis of the results was performed using χ^2 test and the t-test with the level of significance at P-values of less than 0.05; 0.01 and 0.001. The result are presented as means ± standard deviation. The statistical package SAS 9.1 (SAS, 2003) was used for the analysis.

Table 2. Composition of the HUMAC Natur additive in 100 % dry mater

Humic acids (%)	62.00
Unbound humic acids (%)	48.00
Fulvic acids (%)	9.00
Minerals and trace elements (%)	9.00

RESULTS AND DISCUSSION

Of the total of 12 breeding pairs included in the experiment, one pair from the control group was excluded due to the female's infertility. Infertility in one animal in a pair is encountered on average in 5 to 10 % of farmed brown hares. Similar ratio of infertile animals was determined in the evaluation of farms in 2016 (Sládeček *et al.*, 2016).

Table 3 shows the results for reproduction parameters in each group. The total number of litters in the control group was 12, on average 4 ± 0.00 litters per breeding pair. Similar number of litters was determined in experimental group 1, where the average number of litters per breeding pair was 4 ± 0.00 as well. The lowest number of litters was determined in the experimental group 2: total of 15 litters, which represents 3.75 ± 0.50 litters per breeding pair on average.

In the control group, 37 leverets were born during the experiment, at average 12.33 ± 3.06 per breeding pair. Of the total number of born leverets, 21.62 % were dead born. In the experimental group 1, 48 leverets were born, 12 ± 1.63 per breeding pair at average, with a large number of live born leverets (97.92 %). Compared to the control group, the number of dead born leverets was lower by 19.54 % ($P \leq 0.05$).

Similarly, lower number of dead born leverets was determined also in the experimental group 2, where from the total of 37 born leverets only 3 leverets were dead born (8.11 %). Other authors also determined lower mortality on different species of animals. Árvayová *et al.* (2012) determined in experiments on rabbits lower mortality before weaning by 0.76 % in the experimental group fed

Table 3. Comparison of the reproduction parameters among the groups

	Total number of litters	Number of born			Mortality before weaning (%)	Weaned leverets (%)	Significance of differences
		Total (pc)	Live born (%)	Dead born (%)			
Control	12	37	78.38	21.62	2.70	75.68	
Exper. 1	16	48	97.92*	2.08***	12.50***	85.42	χ^2 test
Exper. 2	15	37	91.89	8.11**	2.70	89.19	

$^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$

feed mixture enriched with humates. Increase in live weight gain by 5-7 % and decrease in mortality by 3-5 % was determined by Stepchenko *et al.* (1991) in broiler chickens (up to 22 days old) after application of peat-based supplements at 0.25 % concentration in feed.

The highest mortality (12.50 %; $P \leq 0.001$) before weaning (28 days of age) was determined in the experimental group 1, where losses appeared in four litters with higher number of leverets. Mortality during rearing in the control group and the experimental group 2 was at the same level of 2.70 %, which represents one leveret dying in each group during the experimental period.

Additive of Humacid 60 (natural humic substances) at 0.5 % concentration, applied to weaned piglets at the age of 25 to 70 days, had a positive influence on weight gain and decrease of mortality rate and added at 0.7 % concentration to complete feed mixtures for broiler chickens, it increased the growth intensity, while decreased feed consumption and mortality rate (Vaško *et al.*, 2007).

The evaluation of the number of weaned leverets out of the total number of born leverets shows positive impact of the tested additive on this parameter. While in the control group the ratio of weaned leverets was only 75.68 %, in the experimental group 1 this ratio was higher by 9.74 % and in the experimental group 2 - higher by 13.51 %, however these results were not significantly different.

Significant effect of humate application was noticed in relation to higher number of live born leverets in the experimental group 1 ($P \leq 0.05$) and lower number of dead born leverets in experimental group 1 ($P \leq 0.001$) and experimental group 2 ($P \leq 0.01$).

According to Dabovich *et al.* (2003) a humin acid product Promax has nutraceutical properties in that it stimulates neutrophil activity, which may protect against bacterial pathogens and reduce mortality during acute bacterial infection. Islam *et al.* (2005) found that humin acid has many beneficial effect like antibacterial, antiviral and anti-inflammatory in animals, improves immune system, stress management and reduces odour in faeces. It also has positive effect on liver functioning. Ultimately reduces mortality and increases growth in poultry. Edmonds *et al.* (2014) describes that mortality was numerically reduced (-36 %) from broilers fed the diets supplemented with humin acid compared with control diet.

In our experiment, we have not confirmed a positive effect of the application of humates in relation to mortality before weaning.

CONCLUSION

The results of our study show positive influence of humic substances on the reproduction parameters of farmed brow hare. In the experimental groups supplemented with humates (HUMAC Natur) the ratios of both live born leverets and weaned leverets was higher than in the control group.

The results suggest the possibility of successful feeding with green fodder in combination with complete granulated feed mixtures with humic additive. The highest ratio of weaned leverets was in the experimental group 2. In this group no increased mortality due to enterocolitis or coccidiosis, as a result of feeding green fodder feed, was determined.

ACKNOWLEDGEMENT

This work was supported by the Slovak Research and Development Agency under the contract no. APVV-15-0474 and APVV-0044-12.

REFERENCES

ÁRVAYOVÁ, M. – POSPÍŠILOVÁ, D. – SUPUKA, P. – ONDRUŠKA, Ľ. – HANUSOVÁ, E. – HANUS, A. 2012. Vplyv laktobacilov a humínových látok na mikrobiálne zloženie obsahu čreva u prepelíc a králikov. *Spravodajca Bioveta SK*, 2012, č.1, s. 10–12.

DABOVICH, L.A. – HULBERT, L. – RUDINE, A. – KIM, S. – MCGLONE, J.J. 2003. Evaluation of nutriceutical effects on pig immunity: Effects of Promox. 2003 *Southern Section ASAS meeting*. Pork Industry Institute, Department of Animal and Food Science, Texas Tech University, Lubbock, TX 79409.

EDMONDS, M.S. – JOHAL, S. – MORELAND, S. 2014. Effect of supplemental humic and butyric acid on performance and mortality in broilers raised under various environmental condition. *The Journal of Applied Poultry Research*, vol. 23 (2), 2014, p. 260–267.

HUCK, J.A. – PORTER, N. – BUSHED, M.E. 1991. Effect of humates on microbial activity. *Journal of General Microbiology*, vol. 137, 1991, p. 2321–2329.

ISLAM, K.M.S. – SCHUHMACHER, A. – GROPP, J.M. 2005. Humic Acid Substances in Animal Agriculture. *Pakistan Journal of Nutrition*, vol. 4 (3), 2005, p. 126–134.

JOONÉ, G.K. – DEKKER, J. – VAN RENSBURG, C.E.J. 2003. Investigation of the Immunostimulatory Properties of Oxyhumate. *Zeitschrift für Natur Forschung Redaktion*, vol. 58 c, 2003, p. 263–267.

NDAYEGAMIYE, A. – COTE, D. 1989. Effect of long-term pig slurry and solid cattle manure application on soil chemical and biological properties. *Canadian Journal of Soil Science*, vol.69, 1989, p. 39–47.

PEÑA-MÉNDEZ, E. M. – HAVEL, J. – PATOČKA, J. 2005. Humic substances – compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine. *Journal of Applied Biomedicine*, vol. 3, 2005, p. 13–24.

SAS Release 9.1 SAS Institute Inc. Cary, USA, 2002-2003.

SHI, Y. – PARKER, D.B. – COLE, N.A. – AUVERMANN, B. W. – MEHLHORN, J.E. 2001. Surface amendments to minimize ammonia emissions from beef cattle feedlots. *Transactions of the American Society of Agricultural Engineers*, vol. 44, 2001, p. 677–682.

SLÁDEČEK, T. – SLAMEČKA, J. – JURČÍK, R. 2016. Reproduction parameters of brown hare farmed in Slovakia. *Slovak Journal of Animal Science*, vol. 49, 2016, p. 180.

STEPCHENKO, L.M. – ZHORINA, L.V. – KRAVTSOVA, L.V. 1991: The effect of sodium humate on metabolism and resistance in highly productive poultry (in Russian). *Nauchnye Doklady Vysshei Shkoly, Biologicheskie Nauki*, vol. 10, 1991, p. 90–95.

THIEL, K.D. – KLOCKING, R. – SCHWEIZER, H. – SPROSSIG, M. 1977. *In vitro* studies of the antiviral activity of ammonium humate against herpes simplex virus type 1 and type 2. Zentralbl. Bakteriol, vol. 239, 1977, p. 304–321.

VAŠKO, L. – SZANYI, G. – JENÍKOVÁ, J. 2007. Vplyv humínových kyselín na výrobu krmív, zdravie a úžitkovosť zvierat, *Status Veterinarius*, 5, 2007, p. 9–11

VESELÁ, L. – KUBAL, M. – KOZLER, J. – INNEMANOVÁ, P. 2005. Struktura a vlastnosti přírodních huminových látek typu oxihumolitu. *Chemické listy*, roč. 99, 2005, s. 711–717.

YAMADA, E. – OZAKI, T. – KIMURA, M. 1998. Determination and behaviour of humic substances as precursors of trihalomethane in environmental water. *Analytical Sciences*, vol. 14, 1998, p. 327–332.