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IMPACTS OF HUMIC ACIDS IN NUTRITION ON HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF BROWN HARES

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ABSTRACT

Humic substances are natural organic substances resulting from the decomposition of mainly plant but also animal residues. The objective of our study was to test if the humic acids can negatively/positively affect brown hares health status. As the main indicators for this evaluation, we chose haematological and biochemical blood tests. In this study, we used 24 brown hares (12 males and 12 females) in the age of 12-24 months. As part of the 6-month experiment, we planned three blood samplings (at the beginning of the experiment and then after three and six months). Animals were divided into three groups at the beginning of the experiment (n = 8/group): CG (control group, 0-11KKZ standard diet without additives), EG1 group (experimental group 1, received 0-10KK/D standard feed + 1% of Humac Natur AFM – humic acids), and EG2 group (experimental group 2, received 0-10KK/D standard feed + 1% of Humac Natur AFM – humic acids, enriched with green feed – clover-grass mixture). We found a statistically significant difference between the control group and the EG2 group in the RBC parameter ($P < 0.05$) after three months of consumption. We found a statistically significant decrease in urea levels in both experimental groups compared to the control group ($P < 0.001$), as well as a decrease in urea concentration in the EG2 group compared to the EG1 group ($P < 0.01$); decrease in cholesterol levels in the experimental groups compared to the control, and between EG1 and the control group, this decrease was statistically significant ($P < 0.05$); and we recorded a statistically significant increase of triglycerides in the EG2 group compared to the other groups ($P < 0.01$). Based on the obtained results, we can evaluate the use of 1% humic acid as a feed additive as safe for feeding hares.

Keywords: bioactive substances, health status, feed additives, biomarker, blood

INTRODUCTION

In recent times, the trend has been to use various organic compounds in animal feed that would otherwise be unused. They are mainly products considered to be by-products or waste products from various activities of industry and agriculture **(Wadhwa *et al.*, 2015; Alao *et al.*, 2017; Kolláthová *et al.*, 2020; Juráček *et al.*, 2021; Socas-Rodríguez *et al.*, 2021)**. Humic substances are natural organic substances resulting from the decomposition of mainly plant but also animal residues. Humic substances are subject to further decomposition with difficulty and are contained in large quantities in soil, peat, coal and some waters **(Steelink, 1964; Hayes, 1997)**. Humic substances are mainly used for plant nutrition. Although it is not a classic type of fertilizer, humus enables an easier intake of nutrients, stimulates the formation of root hairs, thanks to which the plant absorbs water and nutrients better, supports photosynthesis and improves soil properties **(Steelink, 1964; Jindo *et al.*, 2012)**. They stimulate plant growth to a degree comparable to the phytohormone auxin **(Scaglia *et al.*, 2016)**.
The above-mentioned properties subsequently resulted in the possible use of humic acids in animal nutrition. First, these substances were tested mainly on poultry, where the use recorded quite decent results. Many studies talk about improved growth, carcass traits, higher and better-quality egg laying, or a positive effect on reproduction **(Avci *et al.*, 2007; Ozturk *et al.*, 2012; Arafat *et al.*, 2015; Arpášová *et al.*, 2016)**. Relatively few studies have been conducted on mammals. Tests on rats, rams and pigs are known **(Galip *et al.*, 2010; Vucskits *et al.*, 2010; Wang *et al.*, 2020)**, and these studies focus more on the health side than economic interests and production. Several studies were carried out on freshwater fish, where the authors were interested in both economic characteristics and the health status of individuals after the application of certain concentrations of humic acids **(Sharaf and Tag, 2011; Arif *et al.*, 2019; Yılmaz *et al.*, 2018)**. Basically, we can talk about studies that either deal with monitoring the impact of a natural bioactive substance on quality indicators as well as increasing the production of economically interesting parameters for breeders; or studies of this type are supplemented with selected individual health parameters, such as the body's immune response, selected haematological or biochemical parameters, or markers of oxidative stress.
In general, it would be appropriate and necessary to determine the possible negative/positive effect of natural substances, where we include humic acids, on the health status of the individual in commonly used concentrations. The objective of our study was to test if the humic acids can negatively/positively affect wild animal (brown hares) health status. As the main indicators for this evaluation, we chose haematological and biochemical blood tests. We evaluated the subsequent analyses within individual samplings as well as by factorial ANOVA.

MATERIAL AND METHODS

Experimental design

In this study, we used 24 brown hares (12 males and 12 females) in the age of 12-24 months. All hares were fed a standard chow diet (0-11KKZ for rabbits and hares) on *ad libitum* basis to the beginning of the experiment. As part of the 6-month experiment, we planned three blood samplings (at the beginning of the experiment and then after three and six months). Animals were divided into three groups at the beginning of the experiment (n = 8/group): CG (control group, 0-11KKZ standard diet without additives), EG1 group (experimental group 1, received 0-10KK/D standard feed + 1% of Humac Natur AFM – humic acids), and EG2 group (experimental group 2, received 0-10KK/D standard feed + 1% of Humac Natur AFM – humic acids, enriched with green feed – clover-grass mixture). The composition of feed mixtures was: nitrogenous substances (150-175 g/kg), crude fat (35-90 g/kg), crude fiber (160-170 g/kg), ash matter (60-90 g/kg), lysine (9 g/kg), calcium (7.5-12 g/kg), sodium (1.5 g/kg), phosphorus (4.5-7.0 g/kg), vitamin A (6900-9800 IU/kg), vitamin D_3 (1000-1870 IU/kg), iron (74-110 mg/kg), manganese (108-120 mg/kg), zinc (79-90 mg/kg), copper (14 mg/kg), selenium (0.25-0.28mg/kg), iodine (1.1 mg/kg). The animals were housed in individual cages per pair. The dimensions of the cage were: floor plan 2 x 1.8 m, height 1 m. The cage was divided into 3 parts. The front feeding part was 2 x 1 m in size, the two rear parts (asylum part) were 0.8 x 1 m in size. The structure of the cage was wooden, the floor was made of plastic grates and the roof was thermally insulated. The conditions of animal care, manipulations and use corresponded with the instructions of the Ethics Committee of the Slovak University of Agriculture in Nitra.

Blood sampling

All animals were healthy and in good health condition. We carried out the control sampling on March 13th, followed by the sampling on June 14th and the final sampling on September 18th. Blood samples were taken by a qualified veterinarian from *vena auricularis centralis* and placed into two tubes. Samples for biochemical assessment were placed into tubes without additive, and tubes containing EDTA as an anticoagulant, were used for the haematological analysis. Coagulated blood samples were centrifuged at 1006 x *g* for 20 min and obtained blood serum was stored at -20 °C until further analyses at the Institute of Applied Biology (SUA in Nitra).

Blood haematology and serum chemistry analysis

Haematology parameters (WBC - total white blood cell count, LYM - lymphocytes count, MID - cell population of middle dimensions including monocytes and eosinophils, GRA - granulocytes count, LYM % - lymphocyte percentage, MID % - cell population of middle dimensions including monocytes and eosinophils percentage, GRA % - granulocytes percentage, RBC – red blood cell count, HGB - haemoglobin, HCT - haematocrit, MCV - mean corpuscular volume, MCH - mean corpuscular haemoglobin, MCHC - mean corpuscular haemoglobin concentration, RDWc – red cell distribution width (%), PLT – platelet count, PCT % - platelet percentage, MPV - mean platelet volume, PDWc - platelet distribution width) were determined using the haematology analyser Abacus Junior VET (Diatron®, Wien, Austria) **(Kovacik *et al.*, 2017)**.
The blood serum parameters (Ca – calcium, P – phosphorus, Mg – magnesium, TP – total proteins, glucose, urea, AST - aspartate aminotransferase, ALT - alanine aminotransferase, Chol – cholesterol, TG – triglycerides) were measured using DiaSys commercial kits (Diagnostic Systems GmbH, Holzheim, Germany) using the Randox RX Monza (Randox Laboratories, Crumlin, UK) semi-automated chemistry analyser. The content of albumin (Alb) was measured using an ALB BioLa Test (PLIVA-Lachema, Brno, Czech Republic) commercial kit using the Genesys 10 (Thermo Fisher Scientific Inc., Waltham, MA, USA) spectrophotometer **(Kovacik *et al.*, 2019)**. The serum globulin (Glob) level was calculated by subtracting the serum albumin level from the total protein level. The albumin/globulin ratio was calculated using the follow formula: A/G ratio = Alb / (TP - Alb) **(Kovacik *et al.*, 2020)**.

Statistical analysis

The obtained data were subjected to statistical analysis using the STATGRAPHICS Centurion© (StatPoint Technologies, Inc., Warrenton, VA, USA). The data were checked for normality using a Kolmogorov-Smirnov normality test before the statistical analyses. The effect of the humic acids on the haematology and serum biochemistry parameters was analysed using the analyses of variance (ANOVA) followed by Tukey's multiple comparison test (the means and standard errors are reported). All obtained data was also analysed using factorial ANOVA, with effects of the group (different diet), duration of administration and gender (*P* values are reported). The results of the analyses were considered significant at $P < 0.05$; $P < 0.01$ and $P < 0.001$.

RESULTS

The data obtained by blood and blood serum analyses after three months of the experiment are presented in Tables 1 and 2. Based on the statistical analysis, we found a statistically significant difference between the control group and the EG2 group in the RBC parameter ($P < 0.05$) for the haematological examination. During the biochemical examination, we noted a statistically significant increase in the cholesterol content in the EG2 group compared to the EG1 group ($P < 0.05$). No significant differences in other parameters were observed between the control and experimental groups.
Data obtained by blood and blood serum analyses after six months of the experiment are presented in Tables 3 and 4. Based on the statistical analysis, we found no statistically significant difference between the control group and the experimental groups for the haematological parameters. In biochemical analyses, we noted several statistically significant differences. We recorded a statistically significant decrease in urea levels in both experimental groups compared to the control group ($P < 0.001$), as well as a decrease in urea concentration in the EG2 group compared to the EG1 group ($P < 0.01$). We noted a decrease in cholesterol levels in the experimental groups compared to the control, and between EG1 and the control group, this decrease was statistically significant ($P < 0.05$). Triglyceride levels were relatively balanced in the control and EG1 groups, but in the EG2 group we recorded a statistically significant increase compared to the other groups ($P < 0.01$). The other monitored markers were relatively balanced.
We then subjected the entire set of results obtained during the entire length of the experiment to a multifactor ANOVA. We used group, duration, and gender as main effects. We also observed statistical dependence for interactions between observed main effects. The results of this analysis are presented in Table 5. We recorded a statistically significant influence of the main effect "group" for RBC, PDWc, urea, ALT, cholesterol, and triglycerides. We recorded a statistically significant influence of the main characteristic "duration" for WBC, MID, GRA, MCH, MCHC, RDWc, PLT, PCTCa, glucose, AST, ALT, and triglycerides. For the last main effect "gender", we noted an influence on the parameters WBC, MID, GRA, LYM%, RBC, HGB, HCT, MCHC, RDWc, Ca, Mg, urea, TP, albumin, globulin, and A/G ratio. By analysing the interactions of the monitored main effects, we noted the impact on the parameters LYM%, GRA%, MCV, MCH, MCHC, RDWc, MPV, Mg, urea, TP, AST, ALT, triglycerides, globulin, and A/G ratio, as presented in table 5.

Table 1 The effect of humic acids diet on the haematology parameters of the brown hares after three months of consumption

Parameters	Diet						*P* - value
	Control		EG1		EG2		
	Mean	SEM	Mean	SEM	Mean	SEM	
WBC (10^9 L^{-1})	6.80	0.89	7.75	1.26	6.40	0.80	*0.6304*
LYM (10^9 L^{-1})	1.30	0.23	0.96	0.26	1.42	0.35	*0.2270*
MID (10^9 L^{-1})	0.63	0.11	0.59	0.09	0.41	0.06	*0.2407*
GRA (10^9 L^{-1})	5.86	0.82	6.19	1.43	4.56	0.92	*0.5476*
LYM (%)	25.54	5.32	19.68	7.41	27.58	9.00	*0.0945*
MID (%)	8.85	0.92	8.43	1.23	6.99	1.12	*0.4700*
GRA (%)	65.60	2.38	71.88	8.03	65.42	9.84	*0.1734*
RBC (10^{12} L^{-1})	**10.59***	0.25	10.06	0.37	**9.36***	0.29	***0.0339***
HGB (g/L)	187.19	2.41	177.53	8.82	166.46	7.38	*0.1215*
HCT (%)	57.22	0.57	54.42	2.23	52.05	2.05	*0.1469*
MCV (fL)	54.18	1.11	54.06	1.10	55.53	0.85	*0.5456*
MCH (pg)	17.71	0.28	17.59	0.39	17.74	0.33	*0.9492*
MCHC (g/L)	327.10	2.33	325.46	4.01	319.54	4.27	*0.3241*
RDWc (%)	16.45	0.43	16.86	0.75	17.27	0.51	*0.6143*
PLT (10^9 L^{-1})	566.82	75.96	520.58	63.24	680.36	67.21	*0.2643*
PCT (%)	0.40	0.05	0.37	0.05	0.47	0.04	*0.4530*
MPV (fL)	7.14	0.18	7.13	0.22	6.93	0.13	*0.6813*
PDWc (%)	35.47	0.66	35.48	0.84	33.81	0.41	*0.1495*

Legend: bold values are significant; * the means within a row with *sign differ significantly ($P < 0.05$)

Table 2 The effect of humic acids diet on the serum chemistry parameters of the brown hares after three months of consumption

Parameters	Diet						*P* - value
	Control		EG1		EG2		
	Mean	SEM	Mean	SEM	Mean	SEM	
Ca (mmol/L)	3.32	1.25	2.91	0.54	2.82	0.32	*0.8995*
P (mmol/L)	2.38	0.17	4.35	1.95	4.23	2.15	*0.6561*
Mg (mmol/L)	2.18	0.28	2.04	0.16	2.02	0.20	*0.8746*
Urea (mmol/L)	11.22	0.90	9.08	0.65	9.51	0.91	*0.1826*
TP (g/L)	55.99	1.59	57.94	3.30	59.56	2.18	*0.5977*
Glucose (mmol/L)	9.57	0.93	8.55	0.59	10.01	0.39	*0.3224*
AST (µkat/L)	1.73	0.13	2.44	0.23	2.37	0.37	*0.1437*
ALT (µkat/L)	0.47	0.03	0.62	0.07	0.64	0.05	*0.0874*
Cholesterol (mmol/L)	1.76	0.17	**1.32***	0.09	**2.10***	0.26	***0.0297***
TG (mmol/L)	2.17	0.13	2.14	0.17	2.16	0.09	*0.9816*
Albumin (g/L)	34.50	1.61	37.76	3.48	38.44	2.87	*0.5695*
Globulin (g/L)	21.49	2.71	20.17	5.19	21.12	3.08	*0.9690*
A/G ratio	1.79	0.22	3.93	1.30	2.43	0.65	*0.2134*

Legend: bold values are significant; * the means within a row with *sign differ significantly ($P < 0.05$)

Table 3 The effect of humic acids diet on the haematology parameters of the brown hares after six months of consumption

Parameters	Diet						*P* - value
	Control		EG1		EG2		
	Mean	SEM	Mean	SEM	Mean	SEM	
WBC (10^9 L^{-1})	5.42	0.68	4.37	0.72	5.34	1.06	*0.6336*
LYM (10^9 L^{-1})	1.20	0.53	0.93	0.25	1.18	0.33	*0.8481*
MID (10^9 L^{-1})	0.54	0.09	0.26	0.09	0.37	0.13	*0.2337*
GRA (10^9 L^{-1})	3.67	0.62	3.17	0.57	3.77	1.14	*0.8613*
LYM (%)	20.75	9.38	21.73	5.78	31.05	10.6	*0.6626*
MID (%)	9.84	0.75	5.54	1.90	5.78	1.61	*0.1354*
GRA (%)	69.39	9.41	72.66	5.63	63.15	9.80	*0.7130*
RBC (10^{12} L^{-1})	10.39	0.29	10.27	0.36	9.61	0.21	*0.1637*
HGB (g/L)	185.51	4.16	188.09	5.87	178.44	5.16	*0.4045*
HCT (%)	56.73	0.99	57.09	1.50	55.05	1.32	*0.5094*
MCV (fL)	54.67	1.01	55.69	0.97	57.30	1.11	*0.2321*
MCH (pg)	17.86	0.25	18.32	0.26	18.55	0.30	*0.2473*
MCHC (g/L)	327.04	5.32	329.18	2.46	323.98	3.80	*0.6311*
RDWc (%)	18.03	0.81	18.34	1.19	17.06	0.65	*0.6039*
PLT (10^9 L^{-1})	511.06	71.04	623.17	192.58	418.44	90.55	*0.5554*
PCT (%)	0.34	0.03	0.48	0.18	0.30	0.06	*0.5099*
MPV (fL)	7.03	0.31	7.36	0.30	7.35	0.24	*0.6730*
PDWc (%)	34.90	1.10	34.73	0.59	35.49	0.64	*0.7658*

Table 4 The effect of humic acids diet on the serum chemistry parameters of the brown hares after six months of consumption

Parameters	Diet						*P* - value
	Control		EG1		EG2		
	Mean	SEM	Mean	SEM	Mean	SEM	
Ca (mmol/L)	1.66	0.29	2.34	0.19	2.99	0.63	*0.1461*
P (mmol/L)	2.36	0.21	4.51	1.55	1.96	0.14	*0.1605*
Mg (mmol/L)	2.19	0.20	2.13	0.13	1.56	0.35	*0.1867*
Urea (mmol/L)	**12.53^{A}**	0.43	**$9.20^{B,a}$**	0.66	**$6.47^{B,b}$**	0.33	***0.0000***
TP (g/L)	52.67	2.86	58.08	1.32	56.28	2.59	*0.2856*
Glucose (mmol/L)	13.45	1.42	12.54	1.52	13.50	0.86	*0.8321*
AST (µkat/L)	1.59	0.16	1.22	0.11	1.43	0.16	*0.2579*
ALT (µkat/L)	0.59	0.06	0.54	0.03	0.69	0.05	*0.0863*
Cholesterol (mmol/L)	**1.91***	0.36	**1.01***	0.04	1.26	0.14	***0.0160***
TG (mmol/L)	**2.35^{a}**	0.11	**2.42^{a}**	0.17	**3.35^{b}**	0.16	***0.0004***
Albumin (g/L)	36.30	0.72	37.64	1.19	35.65	1.48	*0.5042*
Globulin (g/L)	16.36	3.10	20.44	1.12	20.63	3.09	*0.4607*
A/G ratio	3.08	1.01	1.89	0.13	2.09	0.36	*0.2940*

Legend: bold values are significant; * The means within a row with *sign differ significantly ($P < 0.05$);
a,b The means within a row with different superscript letters differ significantly ($P < 0.01$);
A,B The means within a row with different superscript letters differ significantly ($P < 0.001$)

DISCUSSION

Our basic intention was a strict evaluation of the health status of individuals after the application of 1% humic acids as a feed additive. The main and very important health assessment is a haematological blood test. In both sampling, we can talk about the minimal influence of humic acids on the haematological parameters of the tested animals. We recorded a change in only one parameter, namely a decrease in RBC in experimental group 2. However, this change was also within the physiologically normal range. **Galip *et al.* (2010)** tested humic acid supplemented diets (5 g/day and 10 g/day) during 22 days in rams. Their results have a similar tendency to ours in several haematological parameters, such as a decrease in lymphocyte content, a partial increase in granulocytes, a decrease in erythrocyte content, or an increase in mean corpuscular haemoglobin (with no significant effect on blood haematology, except significant effect on eosinophils level). Other studies where the haematological examination was carried out were most often used on poultry, where the authors confirmed a significant influence again in a similar tendency; a decrease in haemoglobin, a decrease in the content of red blood cells, but in the case of white blood cells, the results were relatively uneven in the experimental groups **(Arafat and Khan *et al.*, 2017; Disetlhe *et al.*, 2018)**. **Miśta *et al.* (2012)** tested humic-fatty acid in New Zealand White rabbit. In this study, they monitored selected haematological and biochemical parameters of the animals after three and six weeks of application; experienced a statistically significant increase in RBC, HGB and HCT levels.

Biochemical examination of blood serum can be associated with many metabolic and health disorders of an individual. In our case, we took blood samples after three and six months, i.e. after enough time to show the positive but also possible negative effects of the set diet for the animals. After three and six months of humic acid administration, we noted a decrease in cholesterol levels. A similar effect is described by the authors of several studies (**Miśta *et al.*, 2012; Ozturk *et al.*, 2012; Kovacik *et al.*, 2020**). In addition to pure humic acids, the authors also tested their combination with blueberry leaf powder (**Kim *et al.*, 2019**). The result was again a decrease in total cholesterol in the experimental groups as well as an increase in high-density lipoprotein cholesterol (HDL) with a simultaneous decrease in low-density lipoprotein cholesterol (LDL), which is a significant positive finding.

A possible protective effect of humic acids against classic toxicants was also described in several studies. **Buchko *et al.* (2021)** tested the possible protective effect of humilid against chromium (Cr VI) in rats. Their results were quite interesting since this supplement initiated the normalization of haematological and biochemical parameters in exposed animals with a clear hepatoprotective and adaptogenic effect. The gastro protective effect of humic acids has been described in relation to induced ulcers (using ethanol and indomethacin) in rats (**Şehitoğlu *et al.*, 2022**). The authors confirmed the anti-ulcer activity of humic acid by macroscopic and histological examination of the number and severity of ulcers, mucosal edema, epithelial abrasion of mucosal tissue, infiltration of inflammatory cells and bleeding; also, they confirmed the healing effect on gastric tissues with ulcers and damage to the gastric mucosa, as well as a decrease in the level of inflammatory cytokines. The use of this additive in reproduction of farmed brown hares has been tested by the **Sládeček *et al.* (2018)**. Their results confirm the positive effects in this issue as well, as the ratio of live births and weaned leverets was higher than in the control group.

After an overall evaluation, we can talk about the possible safety of using this supplement in animal nutrition at presented concentration. When combining humic acid additions with clover-grass mixture (EG2), we recorded more contradictory results. Based on the evaluation of the results, we would not recommend such feeding. Basically, we can talk about a negative impact on haematological parameters (especially white blood cells) and cholesterol content, when combined standard feed + 1% of Humac Natur with green feeding. On the other hand, the group tested purely with humic acids showed a very good health status, and many previous studies on different species of animals refer to really positive effects from the stabilization of breeding, improvement of performance characteristics, as well as many proactive effects and improved reproductive characteristics.

Table 5 Results of Multifactorial ANOVA analysis for the changes in the levels of monitored parameters among experimental group, duration of administration and gender of brown hares

Biomarker	Significance						
	Main effects			Interactions			
	group	duration	gender	group x duration	group x gender	duration x gender	group x duration x gender
WBC	ns	***0.0003***	***0.0012***	ns	ns	ns	ns
LYM	ns	ns	ns	ns	ns	ns	ns
MID	ns	***0.0089***	***0.0266***	ns	ns	ns	ns
GRA	ns	***0.0003***	***0.0022***	ns	ns	ns	ns
LYM%	ns	ns	***0.0492***	ns	***0.0102***	ns	ns
MID%	ns	ns	ns	ns	ns	ns	ns
GRA%	ns	ns	ns	ns	***0.0111***	ns	ns
RBC	***0.0499***	ns	***0.0000***	ns	ns	ns	ns
HGB	ns	ns	***0.0000***	ns	ns	ns	ns
HCT	ns	ns	***0.0002***	ns	ns	ns	ns
MCV	ns	ns	ns	ns	***0.0212***	ns	ns
MCH	ns	***0.0011***	ns	ns	***0.0011***	ns	ns
MCHC	ns	***0.0000***	***0.0017***	ns	ns	***0.0029***	ns
RDWc	ns	***0.0000***	***0.0010***	ns	ns	***0.0356***	ns
PLT	ns	***0.0131***	ns	ns	ns	ns	ns
PCT	ns	***0.0202***	ns	ns	ns	ns	ns
MPV	ns	ns	ns	***0.0184***	ns	ns	ns
PDWc	***0.0247***	ns	ns	ns	ns	ns	ns
Ca	ns	***0.0011***	***0.0478***	ns	ns	ns	ns
P	ns	ns	ns	ns	ns	ns	ns
Mg	ns	ns	***0.0076***	***0.0386***	ns	***0.0247***	ns
Urea	***0.0002***	ns	***0.0113***	***0.0066***	ns	ns	ns
TP	ns	ns	***0.0393***	***0.0038***	ns	ns	ns
Glucose	ns	***0.0000***	ns	ns	ns	ns	ns
AST	ns	***0.0004***	ns	ns	***0.0309***	ns	ns
ALT	***0.0003***	***0.0005***	ns	ns	***0.0001***	ns	ns
Cholesterol	***0.0141***	ns	ns	ns	ns	ns	ns
TG	***0.0024***	***0.0001***	ns	***0.0036***	ns	ns	ns
Albumin	ns	ns	***0.0017***	ns	ns	ns	ns
Globulin	ns	ns	***0.0001***	ns	ns	***0.0491***	***0.0494***
A/G ratio	ns	ns	***0.0012***	***0.0426***	ns	***0.0077***	***0.0129***

Legend: bold values are significant, ns - not significant.

CONCLUSION

Based on our results, we can talk about several conclusions. In the first case, we noticed a minimal impact on animal health after three or six months of feeding the supplement into the feed. Rather, we can talk about signs of positive effects (e.g., decrease in cholesterol content, balanced haematological parameters). In the second case, we cannot ignore the results of the factor analysis, where the effect of the time of administration of the additive is clear, as well as the effect of gender. In the latter case, we do not recommend combining prepared feeds containing humic acids with green feeding. In this experimental group, there are rather indications of a negative character. Based on the obtained results, we can evaluate the use of 1% humic acid as a feed additive as safe for feeding hares.

From the point of view of determining the effects of bioactive substances and the subsequent possible application in the physiology of animals, it is necessary to determine the protective or toxic effect, as well as their mutual interactions due to the elimination of health risks. Due to the wide variability of bioactive and bioprotective substances, it is difficult to include all active substances and examine their importance and effects. Therefore, we recommend continuing testing humic substances on other animal species as well as at higher concentrations.

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