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Original article

Effect of humic-plant feed preparations on biochemical blood parameters of laying hens in deep litter housing system

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Abstract

An influence of various humic-plant feed additives based on some herbs (nettle, chamomile, yarrow, perforatum), lucerne and humic materials on biochemical indices of Lohmann Brown (LB) layers blood plasma was estimated. Hens were housed in deep litter system, 20 birds in a group. Four groups were formed: control (C – standard feeding), and experimental, supplemented with preparations: E-1 herbal-humic, E-2 humic-herbal and E-3 – humic-lucerne. Hens were placed in the pens on the 16th week of life, addition of preparations with standard food mixture started at the 22nd wk and lasted until 66th wk of life. Blood for analyses was collected four times in the following periods: 27, 37, 54 and 65th wk of life. The applied humic-plant preparations to a limited degree affected the values of examined biochemical parameters in serum: total protein (TP), albumins (Albs), glucose (Glu), urea, triacylglycerols (TAG), total cholesterol (TCh), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). It is difficult to determine based on these study, which preparation is one the most active biologically, however is seems that humic-lucerne preparation affected the examined blood parameters to the highest degree. The reference values ranges in hens blood serum LB hens were proposed for: TP (43-65 g/l), Albs (15-22 g/l), urea (0.5-1.2 mmol/l), Glu 10-15 mmol/l), TCh (2.2-4.5 mmol/l), TAG (10-24 mmol/l), AST (4-12 U/l), ALT (150-280 mmol/l) and ALP (190-350 U/l).

Key words: humic-plant preparations, hen, blood, biochemical parameters

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Introduction

Intensifying trends of animal production "ecologization", prohibition of an application of antibiotic growth stimulators or meat-and-bone meals (excluding fish meals) favor an increased interest in an application of herbs and special plants (Czech et al. 2012, Saeid et al. 2013, Zanu et al. 2013) as well as other natural raw materials including humic ones (some species of brown coal, peat, humus) as the sources of biologically active substances for production of special feed additives and biopreparations (Dobrzański et al. 2007, Trziszka et al. 2011, Bubel et al. 2014). These may considerably modulate animal metabolic processes and act in immunostimulating and preventive manner (Yildiz et al. 2008, Eevuri and Putturu 2013, Rząsa et al. 2014).

Herbs or medical plants used in poultry feeding are already well examined in chemical aspects, but physiological significance has not been fully recognized so far. It is known that various interactions may occur between fodder components and bioactive components of special plants (incl. herbs), thus they are not always effective as feed additives (Wallace et al. 2010). For example Mansoub (2011b) noted more profitable performance and eggs quality in laying hens fed with lucerne extracts, with no changes in blood parameters (biochemical and immunological).

It is also known, that an application of various phytobiotics or mineral-organic supplements may change physicochemical composition of eggs, both yolk and white as well as shell, which is highly significant in an assessment of their quality and technological value (Dolińska et al. 2011, Opaliński et al. 2013, Grela et al. 2014).

Blood biochemical parameters reflect the physiological state of the animal resulting from nutrition manner and fodders quality, pathogenic factors activity, welfare level or breeding technology (Pavlik et al. 2007, Ognik and Sembratowicz 2012).

These raw materials (humic, herbs, lucerne) have been already used in laying hens feeding in a form of humic-plant preparation. To a limited degree they affected macro- and microelements content in the eggs, and blood serum mineral profile of hens (Bubel et al. 2013a,b). An effect of these biopreparations dietary application on the basic of biochemical parameters of hens' blood has not been recognized.

The aim of the study was to determine the influence of various mineral-organic feed additives based on herbs, lucerne and humic raw materials on biochemical blood serum parameters in laying hens.

Materials and Methods

The study was conducted on Lohmann Brown (LB) laying hens in an experimental hen-house with floor housing system on straw bedding (5 hens/m^2). Environmental conditions (air temperature 18-23°C, relative humidity 65-75%, light-day – 14 hours of light, intensity of 10-15 lx) were according to technological and hygienic requirements, consistent to manufacturer's recommendations (Management Guide 2010). Five groups were formed:

– C (control group) – standard feeding.
– E-1 (experimental group) – herbal-humic preparation (P-1) additive.
– E-2 – humic-herbal preparation (P-2) additive.
– E-3 – humic-lucerne preparation (P-3) additive.
– E-4 – humic preparation (P-4) additive.

Each group consisted of 20 hens, which were placed in the pens at 16th week of life, addition of preparations with standard feed mixture started at the 22nd week and lasted up to the 66th week of life.

Feed mixture (FM) from industrial fodder plant aimed at commercial stocks of laying hens was applied in hens feeding. Concentration of crude protein in FM amounted to 17.5% and metabolizable energy level was 11.9 MJ/kg. The content of basic nutrients in FM is presented in Table 1. Groups E-1, E-2 and E-3 were given humic-plant additives, marked as P-1, P-2 and P-3 preparation, respectively. Group E-4 was supplemented with humic preparation P-4 (without plant additives). Their share in FM was 3%, while the control group (C) was fed with FM without any feed additives (preparations). Humic-plant preparations were manufactured based on humic raw materials and dried herbs using exothermal reaction of calcium oxide (CaO) hydration (Bubel et al. 2010). In turn, humic preparation was a mixture of calcium, "soft" brown coal (humodetrynite) and peat of sedge-alder wood type. Humic raw materials were of Polish origin (Tronina Comp.), calcium was obtained from Lhoist Opolwap Comp., herbs from Astex Comp., while lucerne from Eko-Rol Comp. Characteristics of these preparations (P-1 – P-4) are presented in Table 2. The mixture as well as the preparations after exothermal processing were subjected to analysis concerning basic nutrients content according to standard laboratory procedures used in fodders assessment (Horwitz and Latimer 2011).

Blood samples were collected four times, from the left asilic vein (*vena basilica*) from 12 hens selected randomly from each group according to the following scheme:

Table 1. Content of basic nutrients in standard feed mixture for laying hens.

Component	Unit	Content
According to laboratory analysis		
Dry matter	(%)	89.5
Crude protein	(%)	17.5
Metabolizable energy *	MJ/kg	11.9
Crude ash	(%)	12.2
Crude fiber	(%)	3.96
Crude fat	(%)	4.61
Calcium	(g/kg)	33.0
Total phosphorus	(g/kg)	6.39
Value declared by the producer of mixture		
Vitamin A	(IU/kg)	10500
Vitamin D_3	(IU/kg)	2500
Vitamin E	(mg/kg)	100.0
L-lysine	(%)	0.870
Methionine	(%)	0.379
3-phytase	FTU/kg	500,0000

* calculated by method of Smulikowska and Rutkowski (2005)

was determined using colorimetric test (Biuret method), albumin (Albs) – colorimetric test (method with bromocresol green) (Gornall et al. 1949). Total cholesterol (TCh) was examined using enzymatic-photometric test (Deeg and Ziegenhorn 1993). Concentration of glucose (Glu) in the serum was marked acc. to Burrin and Price (1985), triacylglycerols (TAG) and urea by enzymatic tests (Talke and Schubert 1965, Bucolo and David 1973), respectively. Analysis of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed using kinetic method at 37°C according to IFCC (International Federation of Clinical Chemistry) recommendations, while alkaline phosphatase (ALP) by photometric kinetic test (Moss and Henderson 1999). All analyses were done using Pentra 400 biochemical analyzer manufactured by Horiba ABX (France), with reagents from the same company.

Constant observation of the birds was performed, production parameters (number and weight of laid eggs, fodder intake) were registered, and also

Table 2. Composition and chemical characteristics of feed preparations.

Raw material (%)	Type of preparation			
	P-1 herbal-humic	P-2 humic-herbal	P-3 humic-lucerne	P-4 humic
Ground burnt lime	59.3	41.7	41.7	58.4
Dried herbs*	32.2	22.6	–	–
Dried lucerne	–	–	22.6	–
Humodetrynite ("soft" brown coal)	8.47	35.7	35.7	20.8
Peat of sedge–alder wood type	–	–	–	20.8
Nutrients content **:				
Dry matter	73.0	75.8	72.7	71.4
Crude ash	52.8	44.7	45.0	64.3
Crude protein	4.52	4.85	4.75	1.42
Crude fiber	13.3	13.4	12.9	9.15
Crude fat	0.309	0.269	0.440	0.420
pH	12.7	12.7	12.6	12.8

* composition: nettle, chamomile, yarrow, perforatum – for 8.08 % of each kind of herbs in P-1 and for 5.65 % of each kind of herbs in P-2

** according to laboratory analysis

– I series (sampling) – 5th week of experiment (27 wks old- peak of laying).

– II series – 15th week (37 wks old – after peak of laying).

– III series – 32nd week (54 wks old – late phase of laying).

– IV series – 43rd week (65 wks old – final phase of laying period).

In total, 240 blood samples were collected from all the groups for laboratory biochemical analysis. Serum was extracted and stored at -20°C. Total protein (TP)

physicochemical and sensory features of the eggs were analyzed in particular phases of laying period, but these results will be presented in another publication. Generally, the results obtained (laying rate, feed intake, feed conversion) were typical for Lohmann Brown laying hens (Al-Khalifa and Ragheb 2013, Grela et al. 2014) with a tendency of more profitable results in group E-3.

The results of the study were analysed statistically calculating the mean values and standard deviations (analysis of variance). The significance of differences

between the C and experimental three groups (E-1 – E-4) were assessed using Duncan's test in Statistica ver. 8. software. The significance level of $P<0.05$ was accepted.

Results

The results of biochemical examinations of hens' blood serum in various phases of their laying period are presented in Tables 3 and 4. Total protein content was in range of 43.1-67.9 g/l. Significant differences between C and E-1- E-4 groups were noted in blood taken in the I sampling (27th week of life), and between C and E-3 in the 37th week of life (II sampling). Albumins concentration was in the range of 14.0-22.1 g/l. Significant differences between C and E-1 – E-4 groups were noted in the 27th and 65th week of hens life. An attention should be paid to significant increase in Albs concentration in the 27th week in experimental groups compared to C, and the reverse tendencies in the 65th (IV sampling). Glucose level was in the range of 8.89-15.7 mmol/l. Statistical differences between C and E-3 and E -4 groups were noted in the I sampling, between C and E-2 and E-3 in the 37th and 54th week of hens life, and between C and E-2, E-3, E-4 in the III sampling, and between C group and other groups in the 65th week of life of hens. Urea concentration was in the range of 0.510-1.21 mmol/l. In all series it was generally lower in experimental groups than in the control one, but significant differences were noted between C and other groups (incl. E-2) in the 37th wk, and between C and others groups in the final experiment (sampling IV). Total cholesterol concentration was in the wide range of 2.12-4.55 mmol/l. Statistically significant differences were noted only in the I sampling (27th week of hens life), between group C and E-3. Triacylglycerols content was in the range of 9.53-23.9 mmol/l. Statistically significant differences were only noted in the 27th week of hens life, between group C and E-3.

Alanine aminotransferase activity was in the range of 3.42-11.9 U/L. No statistically significant differences between the groups were demonstrated. Attention should be paid to over two-fold increase in ALT activity in the final laying phase (IV sampling) in all the groups. Aspartate aminotransferase activity was in the range of 154-275 U/L. Statistically significant differences were noted between groups C and E-1 – E-4 in the I sampling, between C and E-2 in the 37th week and between C and E-3 in the 65th week (IV sampling). Alkaline phosphatase activity was in the range of 183 – 351 U/L. Statistical differences between C and E-1, E-2 and E-4 group were noted at the beginning of experiment (I sampling), between C and E-3 in the 37th week (II series), and between C and E-3 and E-4 in the 65th week (IV sampling). High dispersion of the results in groups E-3 and E-4, as well as between last two samplings (late and final phase of laying period) should be emphasized.

Discussion

It is difficult to confront the obtained results of biochemical analysis with reference values, since so far no such values have been elaborated (lack of data in veterinary literature). In turn, there are quite many data concerning laying hens blood parameters in various systems of housing and feeding (including fodder phyto-additives application).

Total protein level in blood serum was quite variable in particular periods of hens life. The highest TP values were noted in laying period final phase, which probably resulted from decreased demand on egg white production, and thus growth was noted in TP concentration in blood serum in all the groups. Popiela et al. (2013) revealed no TP concentration changes in LB hens (40.52-42.43 g/l) in case of different mixtures composition but with similar energy, protein and mineral components level. The results obtained suggest absence of an effect of applied feed additives (herbs, lucerne, humic raw materials) on TP concentration in blood serum. It may be also concluded from the study by Nobakht et al. (2012) that the mixture of three various herbs (*Melissa*, *Tanacetum* and *Ziziphora)* in Hy-line laying hens does not cause any significant changes in the level of immunity parameters, like heterophils and lymphocytes.

Applied feed additives generally did not affect also the Albs concentration in blood serum, however a significant decrease in all experimental groups compared to the control one was noted in the final phase of production. It is difficult to explain, especially that proteins content in blood depends on fodder quality, alimentary tract efficiency, liver and kidneys state (Kłyszejko-Stefanowicz 2005). Zanu et al. (2013) observed a significant Albs concentration decrease (17.8-15.5 g/l) in 30-38 wks-old Lohmann strain hens after 10% *Cassava* leaf meal introduction to their diet, while Kralik et al. (2006) observed a narrow Albs range in Hy-line laying hens (18.08-18.37 g/l).

These results point to some differentiation in Glu concentration in particular groups, however mean values were similar. Glu level in 34 wks-old LB layers was 13.39 and 14.21 mmol/l with 5 and 10% extruded amaranth grains supplementation, respectively (Popielaet al. 2013), while in 62-74 wks-old Hy-line strain they ranged from 9.556 to 14.36 mmol/l, without an effect of herbal mixture kind in the fodder (2%)

Table 3. Values of basic biochemical indices (mean ±SD) in blood serum of LB hens in period of 27th-65th week of life (mean ±SD).

Parameter	Sampling	Group				
		C	E-1	E-2	E-3	E-4
Total protein (g/l)	I	43.1 ± 6.5	51.5 ± 7.4*	50.2 ± 9.9*	49.8 ± 6.9*	53.6 ± 4.6*
	II	48.3 ± 6.0	52.0 ± 5.5	52.5 ± 4.8	47.3 ± 3.4	52.6 ± 4.4
	III	54.5 ± 4.2	53.8 ± 6.0	53.1 ± 6.1	43.5 ± 13.4*	51.2 ± 7.7
	IV	67.9 ± 7.3	60.6 ± 8.8	64.2 ± 13.6	59.7 ± 12.5	64.7 ± 12.2
Albumin (g/l)	I	14.0 ± 1.4	17.7 ± 2.2*	15.9 ± 2.0*	16.3 ± 1.8*	17.0 ± 0.6*
	II	18.0 ± 1.5	17.4 ± 1.6	17.6 ± 1.4	17.5 ± 1.3	18.1 ± 1.0
	III	17.8 ± 1.8	18.3 ± 1.2	17.6 ± 1.6	17.9 ± 1.1	18.2 ± 1.2*
	IV	22.1 ± 1.2	18.7 ± 2.3*	18.3 ± 2.0*	18.2 ± 2.5*	18.6 ± 2.5*
Glucose (mmol/l)	I	9.75 ± 1.15	9.72 ± 0.89	10.0 ± 1.6	12.2 ± 1.1*	12.0 ± 0.77*
	II	14.9 ± 1.1	14.1 ± 1.0	13.2 ± 0.8*	13.7 ± 0.6*	14.2 ± 0.96
	III	12.2 ± 0.9	15.3 ± 1.2	14.2 ± 0.9*	14.7 ± 1.3*	15.7 ± 1.51*
	IV	8.89 ± 2.58	11.2 ± 1.3*	11.5 ± 1.5*	12.1 ± 0.9*	10.6 ± 1.55*
Urea (mmol/l)	I	1.02 ± 0.98	1.07 ± 0.81	0.581 ± 0.170	0.652 ± 0.451	0.620 ± 0.334
	II	0.733 ± 0.191	0.551 ± 0.071*	0.710 ± 0.231	0.600 ± 0.140*	0.591 ± 0.070*
	III	0.651 ± 0.220	0.570 ± 0.190	0.500 ± 0.121	0.510 ± 0.170	0.575 ± 0.120
	IV	1.21 ± 0.630	0.732 ± 0.201*	0.841 ± 0.151*	0.652 ± 0.151*	0.696 ± 0.281
Total cholesterol (mmol/l)	I	2.12 ± 0.71	3.11 ± 1.04	2.22 ± 1.32	3.58 ± 1.67*	2.52 ± 0.48
	II	3.28 ± 0.66	3.22 ± 0.70	3.13 ± 1.02	3.62 ± 0.84	3.59 ± 0.86
	III	3.51 ± 1.14	4.04 ± 1.47	3.38 ± 0.98	3.76 ± 0.97	3.69 ± 1.34
	IV	4.11 ± 1.08	3.82 ± 1.77	3.94 ± 1.46	4.55 ± 2.72	4.44 ± 1.87
Triacylglycerols (mmol/l)	I	10.6 ± 4.2	16.3 ± 8.0	9.53 ± 7.9	16.9 ± 6.4*	9.77 ± 3.1
	II	16.8 ± 5.4	15.0 ± 5.5	17.8 ± 8.1	13.9 ± 5.0	18.1 ± 6.2
	III	17.0 ± 8.3	19.3 ± 10.0	16.9 ± 7.9	18.4 ± 7.8	18.8 ± 9.3
	IV	19.7 ± 10.3	18.2 ± 11.8	19.9 ± 9.2	23.9 ± 10.7	20.0 ± 12.0

C – (control group); E-1 – herbal-humic preparation (P-1) additive, E-2 – humic-herbal preparation (P-2) additive, E-3 – humic-lucerne preparation (P-3) additive; E-4 – humic preparation (P-4) additive; Sampling: I in 27th, II – 37th, III – 54th and IV – 65th week of life; Mean values with asterisk (*) are significantly different from the control group (in the same rows) $P<0.05$

Table 4. Enzymes activity in blood serum (U/L) of LB hens in a period of 27th-65th week of life (mean ±SD).

Enzyme	Sampling	Group				
		C	E-1	E-2	E-3	E-4
Alanine aminotransferase (ALT)	I	4.11 ± 1.03	4.32 ± 1.12	3.42 ± 1.41	4.12 ± 1.02	3.42 ± 1.16
	II	4.83 ± 0.82	4.73 ± 1.24	4.32 ± 1.73	4.91 ± 1.50	5.10 ± 1.22
	III	3.80 ± 1.80	4.22 ± 1.32	4.20 ± 1.40	3.63 ± 0.60	3.34 ± 1.40
	IV	9.60 ± 5.72	9.61 ± 4.11	11.9 ± 8.82	10.9 ± 4.90	7.58 ± 3.35
Aspartate aminotransferase (AST)	I	154 ± 19	189 ± 29*	191 ± 36*	173 ± 25*	218 ± 37*
	II	172 ± 21	181 ± 13	198 ± 48*	182 ± 13	169 ± 24
	III	190 ± 31	220 ± 67	217 ± 50	199 ± 28	196 ± 22
	IV	275 ± 50	242 ± 55	253 ± 62	226 ± 37*	217 ± 30*
Alkaline phosphatase (ALP)	I	336 ± 82	192 ± 83*	231 ± 110*	277 ± 155	183 ± 130
	II	270 ± 92	246 ± 108	241 ± 100	233 ± 50*	249 ± 65
	III	229 ± 99	265 ± 93	228 ± 108	238 ± 63	270 ± 90
	IV	234 ± 119	247 ± 121	286 ± 140	351 ± 103*	189 ± 67*

For description, see Table 3

(Nobakht et al. 2012). Glu is involved in numerous metabolic processes, and its concentration in blood is precisely regulated by complex mechanisms (Braun and Sweazea 2008). Urea is a final product of proteins nitrogen transformation and may change over the laying period. It is formed in liver from ammonia and carbon dioxide, and is secreted by kidneys. This way, the organism neutralizes toxic activity of ammonia (Kłyszejko-Stefanowicz 2005). Lower urea concentration in blood of hens from experimental groups (E) may suggest an effect of applied feed additives on nitrogen transformations, however the mechanism is difficult to explain. For example, considerably higher values of this parameter were noted by Kredatus and Valent (1993) in Shaver Starcross-228 laying hens. In turn, an average urea concentration noted by Kralik et al. (2006) was 0.45 mmol/l, which is considerably lower compared to the results obtained in this study.
Total cholesterol (TCh) concentration was within quite narrow limits irrespective of additives used. Similar values were provided by Suchý et al. (2004), who noted TCh concentration in the range of 3.13-5.80 mmol/l in laying hens aged 25-50 wks, while Nobakht et al. (2012) in older Hy-line laying hens (62-74 wks- old) observed the range of 3.58-7.63 mmol/l depending on herbs dose in a diet. This results from the fact that high requirements on TCh essential for yolk substance formation occurs in a period of intense laying production, which may lead to a decrease in this parameter level in blood serum of laying hens (Pavlik et al. 2007, Popiela et al. 2013).

Also triacylglycerols (TAG) concentration was not affected by hens diet. Increase of TAG concentration in subsequent samplings attracts an attention, because laying rate and deposition in egg yolks is subject to a decrease. Similar tendencies were noted by Gyenis et al. (2006) in the study on Hy-Line Brown line hens. In turn, in Hy-line laying hens between 28th and 30th wk of life, the values in the range of 10.87-12.08 mmol/l were observed (Kralik et al. 2006).

Blood serum TCh and TAG in hens may be modified by some dietary components. An addition of Jerusalem artichoke (Yildiz et al. 2008), tamarind (Chowdhury et al. 2005) or powdered garlic (Yalçin et al. 2006) to the fodder causes a significant decrease in these lipid indices concentration. These relationships were not fully confirmed by Nobakht et al. (2012), in the study with an application of 2% dietary addition of herbs like *Melissa, Tanacetum* and *Ziziphora* in hens.

Aminotransferases (ALT and AST) are the most important enzymes from transferases group, they catalyze a range of reactions which are essential in animals and human metabolism, inter alia they synthesize amino acids and vitamins, are involved in glucogenesis process. No effect of examined preparations on ALT activity was noted. The results obtained are close to those obtained in another study on Lohmann Brown hens in which an average value of that enzyme on a level of 6.13 U/L was noted in the 55th week of life (Yildiz et al. 2008). The values of this parameter in Ross Brown hens were on a level of 16.4 U/L (Sahin and Kucuk 2001).

The level of next enzyme, i.e. AST was shaped quite differently, in the first sampling it increased significantly in the experimental groups, while in the last stage of the laying period it generally decreased in these groups (significantly in the groups with lucerne and humic addition) compared to the control group. These facts are difficult for interpretation, especially that the values were within the limits provided by other authors. This parameter in Ross Brown hens was on a level of 171.8 U/L (Sahin and Kucuk 2001), while higher values, 373.7 IU/L on average, are reported by Króliczewska et al. (2008) for Isa Shaver line laying hens. AST as well as ALT activity in serum may increase with an age of the hens.

Alkaline phosphatase (ALP) is a hydrolytic enzyme widely observed in animal tissues. This enzyme, operating in an optimum manner under alkaline pH, is observed in blood in various forms which are mainly derived from bones, bile ducts epithelium, intestines mucous membrane and kidneys (Kłyszejko-Stefanowicz 2005). An effect of applied preparations is not unequivocal, only a considerable decrease in this enzyme content in the first sampling in experimental groups compared to the control one deserves an attention. Moreover, high differences were noted within the groups, which is proved by high standard deviation of the mean values. Higher values of ALP level were noted by Garalevičienė (2003), i.e. in the range of 877.4-1030 U/L in Hisex Brown hens between 45th and 51st week of life. In turn, Gyenis et al. (2006) in the study on Hy-Line W-98 hens (Leghorn) noted about 4-fold decrease in ALP activity between 3rd and 30th wk of life, and then stable level of this parameter up to the 72nd week. Pavilk et al. (2007) noted the highest activity of this enzyme in the peak of laying period. The results obtained in this study did not fully confirm these tendencies.

The physiologically active components in applied plants (herbs) and humic substances have not yet been determined. Further studies are needed in order to determine their chemical and pharmacological characteristics.

Herbs and special plants have been used in poultry prevention and therapy for many years, and their effectiveness depends on physicochemical composition. Wallace et al. (2010) reviewed plant bioactives used in poultry in terms of health and production.

They evaluated 36 plants and preparations composed of herbs, fruits and seeds. Most of them improve poultry performance, product quality (meat, egg), act as antibiotic growth promoters and improve digestive functions. The literature data concerning herbs and special plants themselves or extracts obtained from them on effect of birds blood hematological and biochemical parameters are sparse (Alloui et al. 2014).

Common nettle (*Urtica dioica* L.) contains a range of phytocompounds, including vitamins from C, K, B_2, B_6 groups and pantothenic acid, flavonoids, carotenoids, histamine, acetylcholine, silicic and formic acids, phytoncides and some mineral components, including iron (Mansoub 2011a). Chamomile (*Anthemis nobilis)* contains volatile oil (ca. 1.2%) containing lactones, nobilin, epinobilin, fatty acids esters. This plant also contains flavonoids, coumarin compounds, mucilage, choline, vitamin C, mineral compounds with high potassium content (Van Wyk and Wink 2008). Yarrow (*Achillea millefolium*) in turn, contains quite high amounts of volatile oil (ca. 1.5%) including azulene compounds, choline, flavonoids, tannins, betaine, bitted glycoside achilleine and mineral salts (especially manganese salts). Perforatum (*Hypericum perforatum* L.) contains anthranoid compounds, flavonoid glycosides (hyperoside, rutoside, quercetin). It contains moreover bactericidal catechin tannins and volatile oil (ca. 1%), choline, phytosterols, pectins, tannins, ascorbic acid, vitamin K and some mineral salts (Van Wyk and Wink 2008). Also lucerne preparations are used in poultry. Moreover, it contains high amount of protein, is rich in vitamins, minerals, carotenoids and saponins. Carotenoids are polyenoic terpenoids having trans-conjugated double bonds. They include carotenes (β-carotene and lycopene), which are polyene hydrocarbons and anthophylls (lutein, zeaxanthin, capsanthin, canthaxanthin, astaxanthin and violaxanthin) (Gaweł and Grzelak 2012).

Mansoub (2011a) observed that 1.5% dietary nettle addition in broiler chicken significantly decreases total serum cholesterol and triglyceride. Ognik and Sembratowicz (2012) using Aloes-plus preparation noted significant lysozyme and hemoglobin increase with monocytes and basophiles fraction decrease in turkey hens. Yalçin et al. (2006) noted significant decrease in serum triglyceride and total cholesterol in garlic powder-fed hens. Dietary cassava leaf meal 10% addition to laying hens diet caused significant decrease in total protein, albumen, globulin and phosphate serum concentration, while 5% dose did not result in such physiological reactions (Zanu et al. 2013). Thyme extract in laying hens has an antioxidant, immunomodulating, hypocholesterolemic and antilipidemic effect (Khan et al. 2012). Alfalfa extract significantly decreases total cholesterol and triglyceride, however it does not affect LDL and HDL fractions, glucose concentration and immunity system of hens (Mansoub 2011b). It may be thus concluded that poultry reactions on phytoadditives are variable, and blood indices are the criteria of their usefulness in these birds feeding. The components of applied humic-plant preparations are used in poultry feeding (Dobrzański et al. 2007, Eren et al. 2008, Gładkowski et. al 2011, Trziszka et al. 2011, Bubel et al. 2013a,b).

In the literature, there are no reference values for laying hens, they are not provided even in the specialist veterinary literature (Winnicka 2011, Mazurkiewicz 2012). Therefore the authors propose to accept the following values as the basis for determination of biochemical parameters of LB hens in litter housing system: TP (43-65 g/l), Albs (15-22 g/l), urea (0.5-1.2 mmol/l), Glu 10-15 mmol/l), TCh (2.2-4.5 mmol/l), TAG (10-24 mmol/l), AST (4-12 U/l), ALT (150-280 mmol/l) and ALP (190-350 U/l).

Conclusions

The applied humic-plant preparations affected to a limited degree the concentration of TP, Alb, glucose, urea and the activity of AST and ALP in blood serum of LB laying hens. Almost no effect of preparations on TCh, TAG and ALT was noted. It is difficult to conclude, which preparation is the most active biologically, however is seems that humic-lucerne preparation affected the examined blood parameters to the highest degree.

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