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Effect of Aloe-plus preparation supplement on hematological and immunological blood parameters and performance of turkey hens

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Abstract: It has long been considered that aloe has many beneficial effects for humans and animals, exhibiting antibiotic, antihistaminic, anesthetic, antiinflammatory, and immunostimulant properties. The aim of the present study was to evaluate the influence of supplemental aloe preparation on some immunological and hematological indices of turkey hen blood. The study involved 160 medium-heavy BUT-9-type 6-week-old female turkey hens, administered Aloe-plus plant preparation at the following varying concentrations: Group 1, control without aloe addition; Group 2, daily doses of 0.35 mL/kg body weight; Group 3, daily doses of 0.70 mL/kg body weight; and Group 4, daily doses of 1.40 mL/kg body weight. The preparation was supplied as a drinking water additive. The study results indicate that the aloe supplement did not contribute to a significant improvement of the rearing performance of the birds. However, the feed conversion rate proved to be lower in all of the experimental groups compared to the control. Aloe supplement at a daily level of 0.70 mL/kg body weight significantly increased the blood parameters of nonspecific immunity (percentage of phagocyting cells, phagocytic index, percentage of nitro blue tetrazolium-reducing cells, and lysozyme activity). In conclusion, it seems meaningful to include Aloe-plus preparation at a daily dose of 0.70 mL/kg into turkey hen diets.

Key words: Turkey hen, Aloe-plus, blood index, rearing performance

Introduction

The improvement of animal health status and production efficiency, as well as high-quality animal products tailored to satisfy consumer expectations, necessitate the development of new feed additives (1-6). Research has focused on various herbs possessing immunostimulating and antioxidant properties, and among them, *Aloe arborescens* has proven to be a viable choice (7-9). This plant's leaves are widely used to produce a broad range of preparations. For example, Biostymina, a water extract from aloe, is very effective in the prophylaxis and treatment of bronchopneumonia in calves, as well as in piglets with cachexia syndrome (10,11). Aside from inducing a nonspecific immune reaction, this preparation contributes to improved weight gain and blood parameters (12,13). In veterinary phytotherapy, aloe-based preparations are employed as cholekinetics that enhance digestion and boost appetite (14,15). Biostymina is also applicable during the rearing stage of slaughter turkey hens and has been shown to promote higher weight gains in birds at a lower feed

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utilization rate (6). Results have confirmed the high efficiency of Bioaron C, an aloe and black chokeberry syrup with a vitamin C addition. This implies that a wide variety of other preparations with an aloe component, such as Aloe-plus and many others with an immune-modulating function recommended in human medicine, may be useful in both the prophylaxis and therapeutic practices in animal diseases. Aloe-plus is a plant-source preparation in which the synergistic action of active substances in the aloe extract, with a trans-resveratrol addition (strong antioxidant from Japanese knotweed) and vitamin C, is used. A high vitamin C content is of great importance, as it is a prerequisite for the appropriate functioning of the immune system (e.g., it stimulates neutral granulocyte production, their phagocytic and killing activities, and the complement activation) (16). Although turkeys are able to synthesize ascorbic acid, this process may be limited in high-density housing; thus, signs of ascorbic acid deficiency are likely to occur.

The aim of the present study was to assess the influence of Aloe-plus preparation on some red and white blood cell indices, nonspecific cell and humoral immunity, and the results of rearing (body weight gains, feed conversion, and survivability) of turkey hens.

Materials and methods

Animals and experimental design

The experiment involved 160 medium-heavy BUT-9-type 6-week-old female turkey hens, maintained in litter-bed pens from 6 to 15 weeks of age. The birds were fed standard balanced diets ad libitum (Provimi, Poland). The dietary ingredients and compositions are given in Table 1. The turkey hens

Table 1. Ingredients and composition of the standard diets.

Ingredient (feeding period)	Grower 1 (6-9 weeks)	Grower 2 (10-13 weeks)	Finisher 1 (14-17 weeks)
Corn, %	25.0	25.0	20.0
Wheat, %	30.6	36.8	56.6
Soybean, %	33.5	28.0	15.0
Meat and bone meal, %	5.00	5.00	4.0
Soya oil, %	2.00	2.00	1.2
Limestone, %	0.70	0.50	0.50
Cytromix Plus, %	0.20	0.20	0.20
Farmix*, %	3.00	2.50	2.50
	Nutrient composition (calculated)		
Crude protein %	23.0	19.5	17.0
ME, kcal/kg	2900	2950	3000
Lysine, %	1.45	1.25	1.05
Methionine + cysteine, %	0.95	0.85	0.75
Tryptophan, %	0.25	0.21	0.18
Threonine, %	0.92	0.79	0.67
Calcium, %	1.20	1.15	1.10
Phosphorus, %	0.65	0.55	0.50
Sodium, %	0.15	0.15	0.15

*Farmix, a mineral and vitamin premix, provided the following per kilogram of diet: 3,000,000 IU of vitamin A, 900,000 IU of vitamin D_3, 10,000 mg of vitamin E, 500 mg of vitamin K_3, 700 mg of vitamin B_1, 2000 mg of riboflavin, 1200 mg of vitamin B_6, 6 mg of vitamin B_{12}, 400 mg of folic acid, 72 mg of biotin, 15,000 mg of niacin, 120,000 mg of choline, 4200 mg of calcium pantothenicum, 30,000 mg of Mn, 18,000 mg of Zn, 12,000 mg of Fe, 3000 mg of Cu, 200 mg of I, 60 mg of Se, 40 mg of Co, and 15 g of Ca.

were randomly assigned into 4 groups comprising 40 birds (2 replications, 20 birds each). The test diets consisted of an aloe preparation administered as a drinking water additive. Varying concentrations of aloe were administered through the drinking water. Aloe-plus preparation is a water extract made of *Aloe arborescens*, with trans-resveratrol (an antioxidant obtained from Japanese knotweed) and a vitamin C addition. Group 1 received drinking water without the dietary supplement. The birds in Groups 2-4 were supplied with a commercial Aloe-plus preparation: Group 2, a daily dose of 0.35 mL/kg body weight (bw); Group 3, a daily dose of 0.70 mL/kg bw; and Group 4, a daily dose of 1.40 mL/kg bw. To calculate the daily dose, the birds were randomly weighed every day, and on the grounds of the weight results, the preparation dose was increased proportionally to the body weight growth. The analyzed supplement was added to the drinking water in consistency with the norms for 28 days. It was administered in the morning, first dissolved in 1 L of water and then diluted with drinking water according to the birds' daily needs. The 4-week treatment period of the Aloe-plus application was followed by a 2-week recovery period, with the turkey hens receiving only pure water. The recovery time was selected on the basis of immunostimulators and antioxidant use, assuming that an intermittent therapy is preferred to a continuous one and prolonged treatment may induce immunosuppression (17). After this 2-week recovery time, the same aloe supplement usage was repeated again for another 4 weeks. On days 28, 42, and 70 of the experiment (i.e. at 9, 11, and 15 weeks of age), blood samples were taken from the wing vein for immunological and hematological evaluations.

Chemical analysis

The hematological assay included hematocrit (Ht), determined using the microhematocrit method; hemoglobin (Hb), determined using the colorimetric method according to Drabkin; and white blood cells (WBCs), determined using the chamber method (18,19). Natt & Herrick's solution was used to stain the leukocytes. The percentage of WBCs was measured by staining the blood smears after using Pappenheim's method (18). The nonspecific cell and humoral immunity was assessed on the basis of the phagocytic activity of the leukocytes against the 209P *Staphylococcus aureus* strain, expressed as the percentage of phagocyting cells (%PC), as well as the phagocytic index (PI), absorption ability, and a test of nitro blue tetrazolium (NBT) reduction by heterophils according to the procedure by Park et al. (20); and lysozyme activity in the blood serum by means of diffusion in agarose gel (21). The vitamin C content in the Aloe-plus preparation was measured colorimetrically in a reaction with 2,6-dichlorophenolindophenol according to the method of Omaye et al. (22). Some mineral elements were analyzed by means of the atomic absorption spectroscopy technique using a Unicam 939 apparatus (AA Spectrometer Unicam). All of the analyses were performed in triplicate.

The data illustrating the content of the minerals and vitamin C in the aloe preparation are presented in Table 2. Importantly, they show a slightly higher vitamin C concentration compared to that stated by the manufacturer (30.0 mg/mL), which suggests that a part of it originates from the aloe extract. It is a common view that aloe leaf juice is a valuable source of ascorbic acid and many other vitamins and minerals.

Growth performance parameters

The following production indices were analyzed: body weight (at the start of experiment and afterwards, at 9, 11, and 15 weeks of age), feed intake, and feed conversion. Based on the production results, the index of rearing effectiveness (WEO) was calculated (23).

Table 2. Content of the mineral elements and vitamin C in the Aloe-plus preparation.

Aloe extract	Macroelement (mg/L)				Microelement (mg/L)			Vitamin C (mg/mL)
	Na	K	Ca	Mg	Fe	Cu	Zn	
Aloe-plus	32.4	243.6	38.43	3.66	0.044	0.108	0.34	35.12

$$WEO = \frac{\text{Mean body weight after rearing (kg)} \times \text{liveability (\%)} \times 100}{\text{Day of rearing} \times \text{feed conversion (kg/kg)}}$$

Statistical analysis

The obtained results were analyzed statistically [standard error of means (SEM), standard deviation (SD)] by ANOVA, using Statistica 6.0 software (StatSoft). $P \leq 0.01$ and $P \leq 0.05$ were considered significant.

Results

The findings of the immunological analysis revealed a significant increase in the %PC and PI values (Table 3) compared to the control, which was found in Group 3 (supplemental Aloe-plus at 0.70 mL/kg bw) and Group 4 (Aloe-plus at 1.40 mL/kg bw). Growth was reported at week 4, as well as at week 10. Regarding the PI and %PC, the highest NBT test results were recorded in Group 3 (Aloe-plus at 0.70 mL/kg bw). The highest percentage of NBT-positive cells was noted in Group 3 at 6 weeks (15.1%). In comparison to Group 1, the mentioned value appeared to be significantly higher ($P \leq 0.05$). As compared to Group 2 (Aloe-plus at 0.35 mL/kg bw) and Group 4 (Aloe-plus at 1.40 mL/kg bw), the percentage of NBT-reducing cells was slightly higher, but the rise was not statistically significant. At 9 and 11 weeks of age, an approximately 2-fold elevation of the killing activity of heterophils in the stimulated version of the NBT test was observed in all of the groups, which implies the appropriate functioning of the immune system in the examined birds. The outcomes of the study have shown that the aloe preparation supplement (Groups 3 and 4) considerably affected the lysozyme content in the blood serum of the turkey hens. The highest activity of the enzyme (1.53 μg/mL in Group 3 with Aloe-plus at 0.70 mL/kg bw, and 1.54 μg/mL in Group 4 with Aloe-plus at 1.40 mL/kg bw) was recorded in those groups at week 10 (15 weeks of age). The activity was significantly ($P \leq 0.05$) higher than in Group 1 (1.34 μg/mL).

Table 3. Level of blood immunological parameters in the turkey hens.

Index	Week of life	Feeding groups				
		1 (control)	2	3	4	SEM
Lysozyme, mg/L	9	1.10 ± 0.10	1.12 ± 0.12	1.22 ± 0.08	1.21 ± 0.07	0.02
	11	1.15 ± 0.10	1.20 ± 0.13	1.25 ± 0.17	1.27 ± 0.18	0.06
	15	1.34^{b} ± 0.13	1.45^{ab} ± 0.11	1.53^{a} ± 0.15	1.54^{a} ± 0.2	0.04
Phagocytic index (PI)	9	4.46^{b} ± 0.37	4.54^{ab} ± 0.34	5.2^{a} ± 0.38	5.12^{a} ± 0.35	0.05
	11	4.56 ± 0.20	4.54 ± 0.30	4.50 ± 0.50	4.60 ± 0.51	0.02
	15	5.30^{b} ± 0.37	5.36^{ab} ± 0.66	6.08^{a} ± 0.85	5.70^{a} ± 0.7	0.09
Phagocyting heterophils, % (PC)	9	29.4^{b} ± 1.14	29.8^{b} ± 2.38	33.0^{a} ± 2.91	32.4^{a} ± 2.6	0.48
	11	31.3 ± 1.57	32.0 ± 2.11	30.7 ± 1.85	31.5 ± 1.80	1.26
	15	49.4^{b} ± 2.30	52.4^{ab} ± 6.10	53.8^{a} ± 4.54	53.4^{a} ± 4.3	1.30
NBT-positive heterophils, % resting version	9	12.8 ±1.92	14.0 ± 1.87	14.6 ± 1.67	13.4 ± 1.81	0.86
	11	13.0^{b} ± 1.78	14.1^{ab} ± 1.34	15.1^{a} ± 0.74	14.3^{ab} ± 1.4	0.96
	15	24.9 ± 0.91	24.6 ± 3.78	27.4 ± 2.60	27.5 ± 2.87	0.12
NBT-positive heterophils, % stimul. version	9	29.6 ± 2.96	30.0 ± 3.16	31.8 ± 2.77	29.8 ± 1.92	0.99
	11	34.5^{ab} ± 2.18	32.9^{b} ± 1.68	36.6^{a} ± 3.50	35.8^{ab} ± 1.5	0.95
	15	44.6 ± 7.23	42.8 ± 5.67	46.4 ± 5.77	45.4 ± 3.64	0.88

a, b: means in the same row with different superscripts differ significantly at $P < 0.05$; SEM: standard error of the mean; n = 16.

The analysis of hematological indices in the turkey hens (Table 4) showed a substantially higher Hb level ($P \leq 0.05$) in 15-week-old birds with the highest dose of Aloe-plus in Group 4 (9.99 mmol/L) when compared to Group 1 (8.82 mmol/L) and Group 2 (8.98 mmol/L). No statistically confirmed differences among the groups regarding Ht, WBC, and percentage of heterophils, lymphocytes, and eosinophils were recorded. Taking into account the monophil share, its lowest load was found at 11 weeks of age in the turkey hens from Groups 2 (0.6%) and 4 (0.8 %). A basophil percentage significantly lower than that in the control group was determined only at 15 weeks of age in Group 2 with Aloe-plus at a daily dose of 0.70 mL/kg bw.

During the experimental period, all of the birds were in good physical condition and no mortality was recorded. The body weights of the birds in all of the groups at 9 and 15 weeks of age did not differ significantly (Table 5). Some differences among the groups occurred only at 11 weeks of age. The turkey hens receiving Aloe-plus at 0.70 mL/kg bw (Group 3) were considerably ($P \leq 0.05$) heavier compared with the control, while the feed conversion in this

Table 4. Level of blood hematological indices in the turkey hens.

Item	Week of life	Feeding groups				
		1 (control)	2	3	4	SEM
Hb, mmol/L	9	8.25 ± 0.46	8.50 ± 0.47	8.56 ± 0.52	8.81 ± 0.31	0.09
	11	7.94 ± 1.14	8.50 ± 1.05	8.13 ± 0.99	8.13 ± 1.04	0.22
	15	$8{,}82^{bc}$ ± 0.59	8.98^{b} ± 0.73	9.56^{ab} ± 0.54	9.99^{a} ± 0.42	0.16
Ht, %	9	32.8 ± 2.86	33.7 ± 1.82	33.5 ± 2.29	32.2 ± 3.27	0.54
	11	34.2 ± 1.30	33.4 ± 1.63	34.2 ± 1.78	34.7 ± 1.78	0.35
	15	33.5 ± 2.02	33.1 ± 2.48	34.2 ± 3.04	33.4 ± 1.84	0.49
WBC, 10^9/L	9	24.7 ± 5.05	26.4 ± 5.2	25.7 ± 5.35	23.4 ± 4.84	1.10
	11	27.8 ± 6.14	29.7 ± 3.61	28.6 ± 5.58	28.2 ± 3.39	1.0
	15	30.4 ± 3.02	28.6 ± 5.08	29.3 ± 4.43	30.7 ± 5.05	0.93
Leukogram, %						
Heterophils, %	9	47 ± 5.87	45.6 ± 6.5	43 ± 3.5	46.6 ± 4.7	1.14
	11	27.8 ± 6.14	29.7 ± 3.61	28.6 ± 5.58	28.2 ± 3.39	1.0
	15	45.0 ± 4.06	48 ± 2.0	47.8 ± 7.82	46.8 ± 4.86	1.08
Lymphocytes, %	9	49.4 ± 4.5	49.6 ± 5.7	53 ± 2.54	47.6 ± 4.5	1.02
	11	42.2 ± 5.11	40 ± 6.32	43 ± 5.65	42 ± 5.24	1.17
	15	51 ± 3.31	49.8 ± 1.48	49.2 ± 7.22	50.8 ± 6.37	1.07
Monocytes, %	9	1.2 ± 0.83	1.8 ± 0.44	1.2 ± 0.83	2 ± 0.70	0.16
	11	1.8^{a} ± 0.44	0.6^{b} ± 0.54	1.2^{ab} ± 0.83	08^{b} ± 0.44	0.16
	15	1.2 ± 1.09	0.6 ± 0.54	1.0 ± 0.70	08 ± 0.44	0.16
Basophils, %	9	1.2 ± 0.83	1.8 ± 1.09	1.4 ± 1.14	2.8 ± 1.64	0.28
	11	0.8 ± 0.44	0.6 ± 0.54	1.2 ± 0.83	1.2 ± 1.09	0.17
	15	2.0^{a} ± 1.41	0.6^{b} ± 0.54	1.4^{ab} ± 0.89	1.0^{ab} ± 1.0	0.23
Eosinophils, %	9	1.2 ± 0.44	1.2 ± 0.83	1.4 ± 0.54	1.0 ± 0.70	0.13
	11	0.6 ± 0.54	1.0 ± 0.70	0.8 ± 0.44	1.2 ± 0.83	0.14
	15	0.8 ± 0.44	1.0 ± 0.70	0.6 ± 0.54	0.6 ± 0.54	0.12

a, b, c: means in the same row with different superscripts differ significantly at $P < 0.05$; SEM: standard error of the mean; n = 16.

Table 5. Rearing results of the turkey hens.

Item		Feeding groups				SEM
		1 (control)	2	*3*	*4*	
Body weight, kg	6 weeks	1.76 ± 0.04	1.77 ± 0.06	1.74 ± 0.06	1.73 ± 0.01	0.01
	9 weeks	3.58 ± 0.12	3.6 ± 0.18	3.69 ± 0.26	3.72 ± 0.24	0.04
	11 weeks	4.83^{b} ± 0.29	4.95^{ab} ± 0.22	5.45^{a} ± 0.50	5.13^{ab} ± 0.43	0.09
	15 weeks	7.84 ± 0.37	7.69 ± 0.25	7.85 ± 0.31	7.93 ± 0.55	0.08
Feed conversion, kg/kg (5-15 weeks of life)		2.58	2.50	2.47	2.49	-
WEO points		394.7	399.5	412.9	413.6	-

a, b: means in the same row with different superscripts differ significantly at $P < 0.05$; SEM: standard error of mean; n = 40; WEO: index of rearing effectiveness.

group was the lowest, at 2.47 kg/kg. Generally, the feed conversion ratio was lower in all of the groups administered the aloe preparation additive as compared to the control group.

Discussion

Supplementing Aloe-plus in the drinking water of turkeys caused a stimulation of nonspecific defense mechanisms. The values of the analyzed immunological indices (%PC, PI, percentage of cells reducing NBT, and lysozyme activity) appeared to be much higher in the group of turkey hens with the aloe extract additive at a daily dose of 0.70 mL/kg bw than in the control. It is noteworthy that the rise in parameters occurred at the final stage of the experiment (week 10 of observation), which was most likely caused by the longer administration period of the preparation. However, the elevation of some immune parameters was recorded at as early as after 4 weeks of aloe application. The 2-week break in the preparation inclusion into the drinking water usually resulted in more uniform values of the examined indices when compared to the control. A significant increase in lysozyme activity, PI, and %PC was also noted in the turkey hens receiving a double aloe amount (1.40 mL/kg bw). The obtained research findings indicate the positive influence of both doses of the aloe preparation (namely 0.70 mL/kg bw) on the phagocytic and killing activity of heterophils. Owing to the fact that a great number of active components in aloe possess potential immunotropic properties, it is challenging to determine whether its stimulating action resulted from the presence of plant source constituents or vitamin C supplementation. Ascorbic acid is essential for the appropriate course of phagocytic activity; it stimulates interferon synthesis and lymphocyte proliferation, thus playing an outstanding role (24,25). Undeniably, the active substances contained in Aloe-plus, i.e. polysaccharides and flavonoids, are in all probability responsible for the immunostimulating effects observed in the groups supplied with the preparation (26,27). The present research results are in agreement with those presented in scientific reports addressing the immunotropic properties of aloe-based preparations. Furowicz et al. (11) discussed the problem of the immunostimulating properties of Biostymina (aloe water extract). The authors reported that its application enhanced nonspecific cellular immune reactions in calves suffering from colibacillosis and bronchopneumonia. As Kołacz et al. (12) noted, Biostymina administration to piglets with cachexia symptoms (associated with immune system failure) produced a substantial improvement in immunological parameters. The aforementioned authors noted elevated macrophage phagocyting activity, growth of lymphocyte B population, and

increased IgG class antibody titer in the blood serum. Even better effects were reported in another study in which a combination of 5 herbs and aloe extract was applied. Sembratowicz et al. (6) found significant growth in the nonspecific immune parameters, i.e. %PC, PI, percentage of NBT-reducing cells, and lysozyme activity, in the blood of turkey hens given Bioaron C (aloe and black chokeberry extract). The stimulating influence of the water extract from aloe leaves on the human granulocyte system reported by Olechnowicz-Stępień et al. (26) also gives evidence of the immunostimulating properties of this plant. The curative properties are successfully and widely used in the prophylaxis of some cancer forms (namely skin) in laboratory animals (28,29).

An increase in the Hb levels recorded in the turkeys supplied with higher doses of aloe preparation in the late stage of the research appears to be difficult to interpret. It is generally known that some plant species may stimulate erythropoiesis, which is manifested by elevated red blood cell system parameters. However, the available literature offers scarce information on the erythropoietic properties of aloe. The changes visible in the WBC picture (i.e. in monocytes and basophils) in the birds receiving the analyzed preparation have not been addressed in the literature, either. Sembratowicz et al. (6) highlighted the growth in the total leukocyte counts and no differences in the WBC picture after aloe preparation application (Bioaron C and Biostymina) to the animals.

Importantly, the Aloe-plus supplement did not significantly influence the performance parameters of the turkey hens. Some better effects of the bird rearing stage observed in the experimental groups might result from more efficient digestion, boosted appetite, and the antimicrobial properties of aloe (15,30). Higher weight gains at lower feed utilization were noted throughout the Biostymina application period (6).

In conclusion, the administration of an Aloe-plus preparation at the daily doses of 0.70 mL/kg bw and 1.40 mL/kg bw contributed to the stimulation of nonspecific immunity in turkey hens, which was expressed by increased %PC, PI, percentage of NBT-reducing cells, and lysozyme activity in the blood serum. Bearing these points in mind, it seems meaningful to include Aloe-plus preparation at a daily dose of 0.70 mL/kg bw into turkey hen diets.

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