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**A note on the effect of garlic, coneflower preparation
and 1,2,4-triazole derivative on the hematological
and immunological indices of blood of Big-6 turkey-hens**

Wpływ zastosowania dodatku czosnku, preparatu z jeżówki oraz pochodnej
1,2,4-triazolu na hematologiczne i immunologiczne wskaźniki krwi indyczek

Summary. The experiment was carried out on 1-day old Big-6 turkey-hens which for sixteen weeks were administered coneflower preparation, garlic and 1,2,4-triazole derivative as additives to drinking water. The aim of this study was to determine the influence of the tested compounds on some hematological and immunological parameters of blood turkey-hens'. The results showed that the examined substances did not significantly affect the hematological indices, i.e. the level of haematocrit, the content of hemoglobin, the mean corpuscular value, the mean corpuscular hemoglobin and its concentration. After the intake of the tested supplements the percentage of phagocytosing heterophils and phagocytic index decreased.

Key words: 1,2,4-triazole derivative, indices of blood, turkey-hens Big-6

INTRODUCTION

One of the most active research areas in recent years has focused on finding new additives stimulating immune reactions and improving performance of animals [Fritz *et al.* 1994, Świącicka-Grabowska *et al.* 1998, Faruga and Pudyszak 1999, Faruga and Jankowski 2000, Majewska 2001]. The results of our earlier pilotage studies dealing with testing the garlic, coneflower preparation and 1,2,4-triazole derivative [Truchliński *et al.* 2006a] pointed to the influence on blood hematological and immunological indices caused further studies upon the evaluation additives on indices levels of slaughter turkey-hens [Truchliński *et al.* 2006b]. A system of 1,2,4-triazole was applied for the production

of pharmaceutical preparations for humans and animals as well as components of plant protection means [Negwer and Scharnow 2001, Baturite and Ruksenas 2002]. Due to the multi-directional pharmacological activity of amidrasones, e.g. 1,2,4-triazole derivative, the possibility to use the compound as an additive stimulating the animals' immunity has gained some interest.

The present studies revealed that this synthetic compound stimulates the immune reactions and thus possesses antiviral, antifungal, anti-infections and analgesic properties [Modzelewska-Banachiewicz 2000, Truchliński *et al.* 2000, Modzelewska and Szczepniak 2001]. In available literature, there are reports on the effects of the compound on metabolic indices in experimental animals [Baturite and Ruksenas 2002] but there are no studies on its using for farm animals rearing. Successful tests of Truchliński *et al.* [2000] indicating the lack of toxic action of 1,2,4-triazole derivative to living organisms qualify that substance for experiments involving slaughter turkey-hens.

The aim of the study was to evaluate the influence and comparison of garlic extract, coneflower preparation and synthetic 1,2,4-triazole derivative on application haematological and immunological indices of turkey-hens' blood.

MATERIAL AND METHODS

The experiment material comprised clinically healthy 360 one-day old heavy Big-6 turkey-hens. The birds were allocated to four feeding groups, each with three replicates of 30 turkey-hens, and were maintained for 16 weeks on rearing. All tests were previously accepted by the Local Ethic Commission. The turkey-hens in each groups were fed identical granulated standard feeds in a 5-stage system, IB-1, IB-2, IB-3, IB-4 and IB-5. Feed composition and nutrient value are presented in Table 1. The tested additives were given into drinking water. The birds in group I (control) did not receive additives. The turkey-hens of group II were given natural poultry immune stimulant Echinovit C at a dose of 0.25 ml/dm³ of water (P.W. Mikita, Poland), prepared from coneflower (*Echinacea*) and enriched in synthetic vitamin C. The birds of group III were fed fresh and ground garlic (*Allium sativum*) at a dose of 0.25 g/dm³ of water, prepared directly before

Table 1. Nutritive value (g/kg) and periods of feeding standard feeds, g/kg
Tabela 1. Wartość pokarmowa mieszanek pełnoporcjowych dla indyczek

Specification	Feed mixtures and period of feeding (weeks of life)				
	1-2 starter	3-5	6-9	10-12	13-16
Crude protein	244.4	219.6	209.4	188.3	150.2
ME, MJ/kg	11.46	11.74	12.19	12.59	13.10
Crude fibre	28.6	27.7	27.2	27.1	27.1
Lysine	18.2	17.1	15.8	13.4	11.8
Methionine	5.9	5.0	5.0	4.4	4.0
Methionine + Cysteine	9.9	9.0	8.9	7.9	7.0
Tryptophan	3.5	2.9	2.8	2.5	1.9
Ca	13.9	12.4	11.7	10.6	9.5
Availablie P	7.7	6.8	6.0	5.7	4.8

administration. The birds of group IV were given 1,2,4-triazole derivative (3-(2-pyridyl)-4-phenyl-1,2,4-triazole-5-carboxylic acid) at a dose of 25 $\mu\text{g}/\text{dm}^3$ of water. Earlier, this compound was dissolved in ethanol (0.5 ml), then adjusted with water to achieve the expected concentration. All additives were given twice in week beginning from the first day of rearing. The 1,2,4-triazole derivative was produced by means of chemical synthesis at the Department of Organic Chemistry Pharmaceutical Faculty Medical University in Lublin. On the 6th, 10th and 14th weeks of the experiment blood samples were taken for the hematological and immunological tests. Hematological analysis was conducted on 30 birds each group using a semi-automatic Coulter hematological analyzer. Hematological tests included the determination of haematocrit (Ht), hemoglobin (Hb), the number of white and red blood cells (WBC, RBC), the mean corpuscular value (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC). The percentage of WBC was determined in blood smears stained with Papanheim's method. The blood smears were prepared and stained according to May-Grünwald-Giemsa. The analysis of the phagocytic activity of heterophils were performed with the use of *Staphylococcus aureus* phagocytosis test and with the use of nitrotriazolium blue (NBT) reduction test [Park *et al.* 1968]. Serum lysosyme levels were determined with the turbidimetric method [Zucker *et al.* 1970]. Body weight, feed conversion ratio (FCR), consumption of water and survival were monitored during the whole experiment (Table 5). After 16 weeks of rearing, turkey-hens were starved for 12 hours. Slaughter and dissection of 120 birds (each of 30 birds in group) were made in accordance with recommendations of Faruga and Jankowski [2000].

RESULTS AND DISCUSSION

The results of blood analysis are presented in Tables 2, 3 and 4. Some hematological values of turkey-hens' blood differed from those presented by Koncicki and Krasnodębska-Depta [2005]. These differences referred to all studied groups and perhaps they resulted from different feeding, breed and turkey-hens' maintenance technology. Analysis of blood showed that Echinovit and garlic additions caused a significant ($p \leq 0.05$) increase of white blood cells number. There were no significant differences among the groups in leukogram. The percentage of phagocytosing cells was higher in all groups with additives in 6th and 14th weeks of rearing as compared to control. Addition of supplements caused a significant ($p \leq 0.05$) increase of phagocytosing index in 6th week of rearing. The analysis of blood showed that only garlic and 1,2,4-triazole derivative addition caused the increase of this indicator after 10th week of application. In 14th – this height was only in the group with garlic. No significant influence of additives on red blood cells, hemoglobin and haematocrit content was observed. No significant changes were affirmed in the mean corpuscular value, the mean corpuscular hemoglobin and the mean corpuscular hemoglobin concentration.

The present results indicated that garlic and coneflower preparation exerted the strongest and 1,2,4-triazole derivative slightly weaker influence on the immune response. Therefore, these results confirm other the findings suggesting that natural immunostimulants are more effective than synthetic or processed ones [Święcicka-Grabowska *et al.* 1998]. The present results indicate that biologically active substances contained in garlic, coneflower and 1,2,4-triazole derivative stimulated the humoral and cellular re-

Table 2. Hematological and immunological parameters of blood of turkey hens, 6th week of rearing
 Tabela 2. Hematologiczne i immunologiczne wskaźniki krwi indyczek, 6. tydzień odchowu

Item	Groups				SEM
	1	2	3	4	
Hematological indices					
Ht, l l ⁻¹	0.34	0.31	0.34	0.34	0.18
Hb, mmol l ⁻¹	7.13	7.11	7.08	7.13	0.13
RBC, 10 ¹² l ⁻¹	2.22	2.23	2.16	2.18	0.06
MCV, fl	151.85	139.01	157.41	155.96	1.65
MCH, pg	51.76	52.19	52.73	52.75	0.25
MCHC, g l	337.94	375.48	335	338.23	1.24
WBC, 10 ⁹ l ⁻¹	20.98 ^b	26.31 ^a	26.15 ^a	20.51 ^b	0.46
Lymphocytes, %	52.18	50.86	49.38	50.88	1.28
Heterophils, %	40.28	40.77	41.55	40.95	1.28
Monocytes, %	2.95	3.12	3.14	3.46	0.22
Bazophils, %	3.14	3.52	3.35	3.25	0.11
Eozynophils, %	1.45	1.73	1.58	1.46	0.28
Immunological indices					
NBT – positive heterophils, %	31.43	31.43	31.43	30.57	0.69
Phagocytting heterophils, %	33.43 ^b	46.00 ^a	46.86 ^a	44.86 ^a	0.95
Phagocytic index	5.09 ^c	5.39 ^b	5.46 ^{ab}	5.76 ^a	0.07
Lysozyme, mg l ⁻¹	1.17	1.12	0.97	0.97	0.02

^{a, b}Mean values in the rows with different letters were significantly different at $p \leq 0.05$

SEM – standard error for mean values

Table 3. Hematological and immunological parameters of blood of turkey hens, 10th week of rearing
 Tabela 3. Hematologiczne i immunologiczne wskaźniki krwi indyczek, 10. tydzień odchowu

Item	Groups				SEM
	1	2	3	4	
Hematological indices					
Ht, l l ⁻¹	0.36	0.37	0.35	0.34	0.02
Hb, mmol l ⁻¹	9.42	10.11	9.59	9.00	0.12
RBC, 10 ¹² l ⁻¹	2.29	2.29	2.24	2.26	0.05
MCV, fl	157.21	161.57	156.25	150.44	1.65
MCH, pg	66.29	71.14	68.37	64.16	0.58
MCHC, g l	421.67	440.27	441.43	426.47	1.23
WBC, 10 ⁹ l ⁻¹	22.00 ^b	26.21 ^a	28.32 ^a	21.14 ^b	0.63
Lymphocytes, %	51.14	52.84	50.34	49.82	1.39
Heterophils, %	40.38	38.05	41.65	40.87	1.38
Monocytes, %	3.42	3.65	3.65	3.48	0.28
Bazophils, %	3.45	3.95	3.98	3.88	0.10
Eozynophils, %	1.61	1.51	1.29	1.95	0.35

Continuance Tab. 3

Immunological indices					
NBT – positive heterophils, %	35.14 ^a	32.14 ^b	33.86 ^{ab}	33.29 ^{ab}	0.94
Phagocytting heterophils, %	48.00 ^{ab}	46.71 ^b	51.00 ^a	52.14 ^a	1.04
Phagocytic index	5.09 ^b	5.43 ^{ab}	5.83 ^a	5.9 ^a	0.12
Lysozyme, mg l ⁻¹	1.12	1.10	1.21	1.32	0.06

^{a, b}Mean values in the rows with different letters were significantly different at $p \leq 0.05$
SEM – standard error for mean values

Table 4. Hematological and immunological parameters of blood of turkey hens, 14th week of rearing
Tabela 4. Hematologiczne i immunologiczne wskaźniki krwi indyczek, 14. tydzień odchowu

Item	Groups				SEM
	1	2	3	4	
Hematological indices					
Ht, l l ⁻¹	0.31	0.37	0.34	0.33	0.02
Hb, mmol l ⁻¹	10.3	9.98	10.05	9.57	0.10
RBC, 10 ¹² l ⁻¹	2.44	2.36	2.42	2.38	0.02
MCV, fl	127.05	156.78	140.49	138.66	1.23
MCH, pg	68.03	68.14	68.6	64.79	0.57
MCHC, g l	535.48	434.59	476.18	467.27	1.21
WBC, 10 ⁹ l ⁻¹	18.88 ^b	26.52 ^a	26.77	23.60	0.67
Lymphocytes, %	55.2	50.42	49.94	53.44	1.31
Heterophils, %	36.58	41.07	41.85	38.25	1.29
Monocytes, %	3.54	3.51	3.49	3.42	0.30
Bazophils, %	3.68	3.84	3.67	3.84	0.14
Eozynophils, %	1.00	1.16	1.05	1.05	0.29
Immunological indices					
NBT – positive heterophils, %	32.71	34.00	34.86	33.14	0.92
Phagocytting heterophils, %	37.57 ^c	43.43 ^b	45.00 ^b	52.86 ^a	0.14
Phagocytic index	4.93 ^b	5.04 ^b	6.09 ^a	5.46 ^{ab}	0.12
Lysozyme, mg l ⁻¹	1.21	1.12	1.14	1.25	0.08

^{a, b}Mean values in the rows with different letters were significantly different at $p \leq 0.05$
SEM – standard error for mean values

sponse of the organism [Trucker 2002]. It may be confirmed by the fact that values of non-specific immune indices, i.e. NBT test, phagocytting heterophils percentage and phagocytic index reached much higher levels in the experimental turkey-hens all groups (in 6th week of rearing). The study performed by Tucker [2002] revealed that plant secondary metabolites, including those from coneflower may inhibit the pathogenic bacteria

reproduction in the animal digestive tract. Feeding with plant metabolites probably caused the improvement of animals' health and the achievement of better production results.

Table 5. Body weight, kg, survival, %, feed conversion ratio, FCR, kg/kg, and consumption of water, dm³/head
Tabela 5. Masa ciała, kg, przeżywalność, %, wykorzystanie paszy i zużycie wody, dm³/szt. kg/kg

Specification	Groups				SEM
	I Control	II Echinovit C	III Garlic	IV 1,2,4-triazole derivative	
Body weight, kg 16 th week of rearing	9.20 ^b	9.65 ^b	9.80 ^a	9.40 ^b	0.84
FCR, kg/kg 0–16 th week of rearing	2.50	2.42	2.38	2.48	0.12
Consumption of water, dm ³ /head 0–16 th week of rearing	4.10	4.11	4.15	4.10	0.36
Survival, % 0–16 th week of rearing	88.89	91.12	96.67	94.45	

^{a, b} – Mean values in the rows with different letters were significantly different at $p \leq 0.05$
SEM – standard error for mean values

CONCLUSIONS

The present results suggest that application of garlic and Echinovit C caused the increase of white blood cells. After intake to 1,2,4-triazole derivative and garlic it was observed that phagocytic index was higher than control. The additives of all supplements increased the percentage of phagocytizing heterophils in 6th and 14th weeks of rearing. A decrease of results of NBT-test after intake to coneflower preparation in 10th week of rearing was observed. This study did not affirm the influence of the tested additives on hematological indices.

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Streszczenie. Doświadczenie przeprowadzono na 1-dniowych indyczkach typu ciężkiego Big-6, które przez 16 tygodni otrzymywały preparat z jeżówki, czosnek oraz pochodną 1,2,4-triazolu jako dodatek do wody pitnej. Celem podjętych badań było określenie wpływu tych dodatków na wybrane wskaźniki hematologiczne i immunologiczne indyczek rzeźnych. Uzyskane wyniki wskazują, iż dodatki nie wpłynęły istotnie na zawartość wskaźników hematologicznych krwi, tj. poziom hematokrytu, zawartość hemoglobiny, średnią objętość krwinek, średnią masę hemoglobiny oraz średnie stężenie hemoglobiny. Dodatek czosnku i Echinovitu C wpłynął na podwyższenie poziomu białych krwinek. Po podaniu testowanych dodatków stwierdzono również podwyższenie odsetka fagocytujących heterofili oraz indeksu fagocytozy.

Słowa kluczowe: pochodna 1,2,4-triazolu, wskaźniki krwi, indyczki rzeźne Big-6