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Influence of differentiated rations of 1,2,4-triazole derivative on the level of chosen blood indicators and rearing efficiency of slaughter turkey-hens

Wpływ różnych dawek pochodnej 1,2,4-triazolu na poziom niektórych wskaźników krwi oraz efektywność odchowu indyczek rzeźnych

Summary. The experiment was carried out on 300 one-day-old Big-6 turkey-hens which for sixteen weeks administrated 1,2,4-triazole derivative [3-(2-pyridyl)-4-phenyl-1,2,4-triazole-5-carboxylic acid] as additive to drinking water. The aim of the present study was to estimate the influence of the tested compound on some parameters of blood and effectiveness of rearing of turkey-hens. After 5, 9, 13 and 16 weeks blood was taken from 60 turkey-hens (from each group) in order to carry out hematological, immunological and biochemical examinations of the blood. An essentially lower content of total protein, glucose and increased AST and ALT activity, percentage of phagocytosing cells and NBT were noticed in the blood revealing the possibility in turkey-hens which received 1,2,4-triazole additive. The body weight of the group with 1,2,4-triazole derivative was higher compared to the weight of the control. The feed conversion ratio was higher in this group as compared to control group. The survivability of the experimental groups was better than in the control.

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 improving health [Hegngi *et al.* 1999, Koncicki *et al.* 2006] and performance of animals [Engstad and Raa 1999] with using different additives. Due to multi-directional pharmacological activity of amidrazones, e.g. 1,2,4-triazole derivative, the possibility to use the compound as an additive stimulating the animals' immunity has gained some interest [Truchliński *et al.* 2000]. Recent studies have revealed that this synthetic com-

pound stimulates the immune reactions and thus possesses antiviral, antifungal, anti-infectious and analgesic properties [Truchliński *et al.* 2000, Modzelewska and Szczesniak 2001, Modzelewska-Banachiewicz and Kamińska 2001]. A successful study performed by Truchliński *et al.* [2000], confirming the lack of toxic action of 1,2,4-triazole derivative on living organisms, makes the compound qualified to tests involving slaughter turkey-hens. Earlier, 1,2,4-triazole derivative had not been on farm animals. In the present study executed on a sample of this substance the influence on indices of blood and effectiveness of rearing turkey-hens Big-6 was shown.

The aim of the study was to determine the influence of the tested compound on immunological, hematological and biochemical indices of blood and performance of turkey-hens.

MATERIAL AND METHODS

The study material consisted of clinically healthy one-day-old Big-6 turkey-hens maintained for 16 weeks of rearing. The experiment used 300 birds randomly divided into 4 experimental groups with three replicates of 25 each. Poultry had continuous veterinary control for the whole rearing period. All tests were previously accepted by Local Ethic Commission. Uniform standard full-dose mixtures were applied to all groups during rearing in accordance to the program that included 5 feeding stages (Table 1). The 1,2,4-triazole derivative (3-(2-pyridyl)-4-phenyl-1,2,4-triazole-5-carboxylic acid) was administrated with drinking water once a week beginning from the first day of rearing. Group I was the control, without 1,2,4-triazole derivative addition. Experimental groups II, III and IV were administered with a solution at concentrations of 50, 75, and 100 µg of 1,2,4-triazole derivative per 1 dm³ of water, respectively. The other days of the week birds did not receive the additive. Feed and water were provided ad libitum. Synthetic 1,2,4-triazole derivative (produced at Department of Organic Chemistry, Pharmacological Faculty, Medical University in Lublin) is a powder, the intended amount of which was dissolved in absolute ethanol (0.5 ml) and adjusted with water to achieve the calculated concentration.

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

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

Table 2. Hematological and biochemical parameters of blood of turkey-hens
Tabela 2. Wskaźniki hematologiczne i biochemiczne krwi indyczek

Index	Blood taking	Group				SEM
		I	II	III	IV	
Ht, %	1	33.79	33.49	33.9	33.68	0.13
	2	34.28	33.94	34.49	33.99	0.15
	3	35.5	34.74	35.21	35.85	0.16
	4	37.61	36.61	37.15	36.79	0.19
Hb, g/dl	1	12.04	11.98	11.89	12.03	0.09
	2	12.2	12.95	11.85	12.0	0.13
	3	12.95	13.00	12.5	12.83	0.10
	4	13.15	13.14	13.28	13.12	0.10
RBC, 10 ¹² /l	1	2.04	2.01	2.03	2.02	0.01
	2	2.15 ^b	2.22 ^{ab}	2.24 ^a	2.20 ^{ab}	0.02
	3	2.27 ^{ab}	2.32 ^a	2.31 ^a	2.25 ^b	0.01
	4	2.29 ^c	2.44 ^a	2.37 ^b	2.43 ^{ab}	0.02
Total protein, mg/dl	1	3.45	3.54	3.49	3.39	0.45
	2	3.76	3.7	3.63	3.74	0.46
	3	7.43 ^a	6.56 ^b	6.41 ^b	5.64 ^c	0.53
	4	8.17 ^a	7.91 ^b	7.88 ^b	7.98 ^b	0.57
Glucose, mmol/l	1	10.69	9.58	9.69	9.85	1.70
	2	13.87 ^a	12.48 ^b	12.35 ^b	12.34 ^b	1.68
	3	15.19 ^a	14.61 ^b	14.13 ^c	14.44 ^b	1.56
	4	13.29	12.85	12.76	12.59	1.54
LDH, IU/l	1	234.4	225.8	262.7	258.4	1.54
	2	382.7	393.5	383.5	383.3	1.36
	3	428.7	393.8	445.8	462.8	1.35
	4	2285.2	2322.5	2338.4	2294.8	1.42
AST, IU/l	1	183.4	163.2	178.5	199.8	1.34
	2	228.4 ^{ab}	215.4 ^b	220.8 ^{ab}	242.8 ^a	1.2
	3	271.8 ^b	268.4 ^b	288.7 ^{ab}	292.8 ^a	1.27
	4	578.4 ^c	595.3 ^b	635.8 ^{ab}	661.3 ^a	1.42
ALT, IU/l	1	8.43	9.15	9.48	10.01	0.98
	2	10.88 ^b	11.54 ^b	12.32 ^a	13.42 ^a	0.88
	3	14.2 ^b	14.98 ^b	15.32 ^{ab}	16.10 ^a	0.69
	4	38.8 ^b	37.82 ^b	39.42 ^{ab}	42.10 ^a	1.54

^{a, b, c} Statistically significant differences at $p \leq 0.05$

SEM – standard error for mean values

Blood for tests was taken from the wing vein from 60 turkey-hens of each group in 5th, 9th, 13th, and 16th weeks of rearing. Hematological tests included the determination of: hematocrite (Ht) level, concentration of hemoglobin (Hb), red and white blood cells (RBC, WBC) with the use of semi-automatic Coulter analyzer and leukogram were de-

terminated in blood stained with smears applying Pappenheim's method. In reference to immune tests, the following parametres were determined: leucocyte phagocytic activity using standard *Staphylococcus aureus* 209P Oxford strain and the value was expressed as a percentage of phagocytosing cells as well as phagocyte index by means of standard methods [Kandafer-Szerszeń 1993]. Tetrazolic salts reduction test (NBT) was applied to evaluate the ability of neutrophilic granulocytes in peripheral blood to kill phagocytosed microorganisms [Park *et al.* 1968]. Lysozyme activity in peripheral blood serum was determined with the turbidymetric technique using *Micrococcus lysodeicticus* strain after re-calculation onto mg/l units [Zucker *et al.* 1970]. The content of glucose, total protein, AST (aspartate aminotransferase), ALT (alanine amonotransferase) and LDH (lactate dehydrogenase) activity were determined with tests Cormay. Feed conversion ratio (FCR), consumption of water and survival were monitored during the whole experiment (Table 5). Body weight was estimated at the end of 5th, 9th, 13th, 16th weeks of rearing.

Table 3. Percentage content of white blood cells – leukogram
Tabela 3. Procentowa zawartość białych krwinek – leukogram

Index	Blood taking	Group				SEM
		I	II	III	IV	
WBC, 10 ⁹ /l	1	20.85	20.48	21.98	21.34	0.45
	2	28.75	29.4	28.73	27.85	0.46
	3	39.42	45.14	43.83	42.41	0.53
	4	59.05	56.4	54.53	53.12	0.57
Lymphocytes, %	1	64.95	64.95	65.37	64.05	1.70
	2	55.6	56.9	59.22	57.6	1.68
	3	45.1	43.86	45.5	43.5	1.56
	4	29.2	28.2	29.84	28.4	1.54
Heterophils, %	1	29.75	30.2	30.48	31.55	1.54
	2	39.3	38.1	36.2	38.9	1.36
	3	50.2	51.84	51.2	52.9	1.35
	4	65.9	68.2	66.36	68.3	1.42
Monocytes, %	1	2.6	2.3	2.1	2.2	0.15
	2	2.1	2.2	1.9	1.4	0.12
	3	1.8	1.9	1.9	1.8	0.09
	4	2.1	2.4	2.1	1.6	0.08
Bazofils, %	1	1.6	1.8	1.4	1.36	0.04
	2	1.8	1.6	1.3	1.3	0.03
	3	1.6	1.2	1.3	1.1	0.02
	4	1.1	0.8	0.8	0.7	0.05
Eozynophils, %	1	1.1	0.75	1.1	0.8	0.08
	2	1.2	1.2	1.2	0.8	0.05
	3	1.3	1.2	1.1	0.7	0.02
	4	0.6	0.4	0.9	1.0	0.06

Table 4. Level of selected indices of non-specific immune in the slaughter turkey-hens
 Tabela 4. Poziom wybranych wskaźników odporności nieswoistej indyczek rzeźnych

Index		Group				SEM
		I	II	III	IV	
NBT %	1	17.25 ^b	24.36 ^a	25.14 ^a	22.36 ^{ab}	0.36
	2	20.58 ^c	27.47 ^a	25.52 ^a	24.04 ^b	0.69
	3	19.45 ^b	27.74 ^a	26.89 ^a	23.86 ^{ab}	0.94
	4	28.32	27.12	28.43	28.44	0.92
Percent of phagocytosing cells	1	38.25	39.56	37.45	39.25	0.58
	2	44.18 ^c	52.33 ^a	51.48 ^a	49.95 ^b	0.95
	3	46.45 ^c	55.58 ^a	54.95 ^a	49.99 ^b	1.04
	4	49.25 ^c	59.88 ^a	58.93 ^a	54.28 ^b	1.14
Phagocytosis index	1	4.21	4.03	3.99	4.25	0.51
	2	4.58	5.21	5.15	5.18	0.07
	3	4.75	5.83	5.79	5.93	0.12
	4	4.78	5.98	5.89	5.83	0.12
Lisozyme mg/l	1	1.12	1.05	1.08	1.03	0.05
	2	1.15	1.29	1.28	1.25	0.02
	3	1.62	1.74	1.71	1.68	0.06
	4	1.42	1.41	1.42	1.42	0.08

^{a, b, c}Statistically significant differences at $p \leq 0.05$

SEM – standard error for mean values

Table 5. Result of rearing of turkey-hens
 Tabela 5. Efekty odchowu indyczek rzeźnych

Parameters	Group				SEM
	I	II	III	IV	
Body mass, kg					
5 th week of rearing	1.60 ± 0.03	1.57 ± 0.04	1.69 ± 0.04	1.58 ± 0.05	0.14
9 th week of rearing	4.98 ± 0.17	5.01 ± 0.10	4.95 ± 0.09	4.99 ± 0.13	0.20
13 th week of rearing	7.69 ± 0.17	7.88 ± 0.13	7.72 ± 0.13	7.78 ± 0.09	0.49
16 th week of rearing	8.96 ^c ± 0.47	9.49 ^a ± 0.24	9.47 ^b ± 0.23	9.24 ^b ± 0.36	0.67
FCR, kg/kg	2.60 ^a	2.40 ^b	2.39 ^b	2.48 ^{ab}	0.11
Consumption of water, dm ³ /head	4.10	4.12	4.15	4.09	0.36
Survivability, %	84.4	89.3	88.3	85.7	0.02

^{a, b, c}Statistically significant differences at $p \leq 0.05$

SEM – standard error for mean values

The achieved numerical results were subjected to statistical analysis calculating the arithmetic means. The difference in the significance between experimental groups and control was estimated by means of single-variable variance analysis (ANOVA) at significance level of 0,05 ($p \leq 0.05$) applying Statistica ver. 5 software.

RESULTS AND DISCUSSION

The data regarding the effects of an addition of 1,2,4-triazole derivative on indices of blood are recorded in Tables 2, 3 and 4. In the present study a significant ($p \leq 0.05$) increase of RBC was recorded (Table 2). However, they still remained within normal physiological limits [Koncicki and Krasnodębska-Depta 2005]. The present study did not observe the influence of 1,2,4-triazole derivative on the level of Ht and concentration of Hb. There were no significant differences among the groups in relation to WBC and leukogram. Immunological tests showed that 1,2,4-triazole addition caused an increase ($p \leq 0.05$) of NBT-positive cells percentage among the groups. The highest level of this indicator was affirmed especially in the group II and III as compared to control. The obtained results of NBT-test can suggest a larger leukocytes' capability to phagocytosis in the groups with 1,2,4-triazole addition, than control. Moreover, biochemical tests affirmed a statistical decrease ($p \leq 0.05$) of the content of glucose in groups II, III and IV as compared to control. The main source for phagocytosing cells is glucose. The percentage of phagocytosing cells maximum value was recorded in the group with 1,2,4-triazole derivative addition. Maybe, it provided strong phagocytosing cells' activity in phagocytosis. The results of immunological tests did not reveal significant differences among the groups in the level of phagocytosis index and lisozyme. The increase of phagocytosis index and lisozyme level testify to the lasting inflammable condition in the organism. In the groups with 1,2,4-triazole derivative a statistical decrease of the content of total protein was observed. It may suggest a better feed conversion ratio. The turkey-hens from groups II, III and IV achieved the higher slaughter weight and lower value of feed conversion ratio than in the control. Slightly better results of rearing achieved in groups with 1,2,4-triazole derivative addition might result from bacteriostatic and antiviral properties of this substance [Modzelewska-Banachiewicz and Kamińska 2001]. A statistical increase of AST and ALT activity was found out. However, they still remained within normal physiological limits [Krasnodębska-Depta and Koncicki 1999]. A stronger effort of liver after intake of synthetic 1,2,4-triazole derivative may be suggested.

CONCLUSIONS

On the basis of the obtained results affirmed the possibility of using 1,2,4-triazole derivative on turkey-hens' rearing was suggested. An essentially lower content of total protein, glucose and increased AST and ALT activity, percentage of phagocytosing cells and NBT noticed in the blood reveal the possibility in turkey-hens which received 1,2,4-triazole additive. The present study did not observe hematological indicators changes except RBC number. The body weight of the group with 1,2,4-triazole derivative was higher to the weight of the control. The feed conversion ratio was higher in this group and amounted to the control group. The survivability of the experimental groups was better than control.

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Streszczenie. Doświadczenie przeprowadzono na 300 jednodniowych indyczkach Big-6, którym przez 16 tygodni podawano pochodną 1,2,4-triazolu [kwas 3-(2-pirydylo)-4-fenylo-1,2,4-triazolo-5-karboxylowy] jako dodatek do wody pitnej. Celem podjętych badań była ocena wpływu testowanej substancji na niektóre wskaźniki krwi oraz efektywność odchowu indyczek rzeźnych. Po 5, 9, 13 i 16 tygodniach od 60 indyczek z każdej grupy pobrano krew w celu oznaczenia wskaźników hematologicznych, immunologicznych oraz biochemicznych. U indyczek, którym podawano pochodną 1,2,4-triazolu odnotowano znaczące obniżenie poziomu białka całkowitego i glukozy oraz podwyższenie aktywności AST i ALT, odsetka komórek fagocytujących oraz NBT. Masa ciała indyczek otrzymujących pochodną 1,2,4-triazolu była wyższa aniżeli w kontroli. Wykorzystanie paszy przez indyczki tych grup było lepsze niż w kontroli. W grupach eksperymentalnych stwierdzono również lepszą przeżywalność zwierząt.

Key words: pochodna 1,2,4-triazolu, wskaźniki krwi, indyczki rzeźne Big-6