EFFECT OF AN ALOE PREPARATION AND 5-OXO-1,2,4-TRIAZINE ON THE REDOX PROFILE OF THE BLOOD OF TURKEY HENS SUBJECTED TO STRESS*

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Abstract

The aim of this study was to determine whether the introduction of stress affects corticosterone and malonyl dialdehyde levels and antioxidant indices of the blood of turkey hens, and whether additives used in conjunction with stress, such as aloe extract supplemented with trans-resveratrol and vitamin C or the amidrazone derivative 5-oxo-1,2,4-triazine, can mitigate adverse changes that may occur in these parameters. The experiment was carried out on 360 turkey hens allocated randomly to 6 groups of 60 birds each. Groups C and C(+)stress were the control groups and did not receive any additive. Birds from groups A and A(+)stress were administered aloe extract with the addition of trans-resveratrol and vitamin C in the amount of 0.70 ml/kg body weight (BW)/day. The turkey hens from groups T and T(+)stress received 5-oxo-1,2,4-triazine in the amount of 30 µg/kg BW/day. Blood tests included determination of the content of corticosterone and malonyl dialdehyde (MDA), total plasma antioxidant potential (FRAP) and vitamin C, as well as activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (PGx). The blood serum samples were also analysed for levels of zinc (Zn), copper (Cu) and iron (Fe) by atomic absorption spectrometry (AAS). The stress factors applied resulted in a significant increase in the level of corticosterone and MDA and in SOD and CAT activity in the blood plasma of the turkey hens. A significant decrease in the level of vitamin C and total plasma antioxidant potential were also recorded in the birds treated with stress. The aloe preparation supplemented with trans-resveratrol and vitamin C caused a significant increase in vitamin C, FRAP, Fe and Cu, and a decrease in the corticosterone and MDA levels in the blood plasma of the turkey hens, while in the blood plasma of birds treated with 5-oxo-1,2,4-triazine a significant increase was noted in superoxide dismutase activity and a decrease in the malonyl dialdehyde level. In order to alleviate the negative effect of stress, supplementation of diets with aloe extract supplemented with resveratrol and vitamin C may be considered.

Key words: turkey hens, aloe, 5-oxo-1,2,4-triazine, stress, blood, redox profile

During intensive breeding birds are exposed to various stress stimuli which may adversely affect their health status and cause significant economic losses. The stress

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response activates a series of mechanisms designed to adapt to the changed conditions and to restore the disturbed intrasystemic balance. Unfortunately, the adaptive changes result in less favourable production indicators, such as decreased body weight, less efficient use of feed (Quinteiro-Filho et al., 2012), reduced dressing percentage and deterioration of meat quality (Pijarska and Malec, 2005; Doktor, 2007; Rachwał, 2006). Stress can also impair antioxidant defence mechanisms, which is manifested as a decrease in the activity of antioxidant enzymes and in the concentration of low-molecular-weight antioxidants in the blood and tissues.

As several studies have demonstrated, an effective way to mitigate the potential effects of stress in poultry is to enrich the diet with exogenous antioxidants, especially vitamins (tocopherols, vitamin C, carotenoids), and some trace elements (Wójcik et al., 2000, 2001; Pandurang et al., 2011). In the last few years attention has been drawn to the possibility of exploiting the antioxidant and adaptogenic properties of herbs (Pandurang et al., 2011; Hashemi and Davoodi, 2012). Aloe Plus is a preparation based on the synergistic action of the active compounds of aloe, trans-resveratrol (an antioxidant isolated from Japanese knotweed) and vitamin C. Recent studies on turkeys have confirmed its ability to stimulate antioxidant defence mechanisms (Ognik and Czech, 2010). Aloe preparations are also known for their biostimulating properties and are used as a means of improving weight gain and health status in turkey hens (Ognik et al., 2004; Ognik and Sembratowicz, 2007, 2012). A stimulating effect on immune and antioxidative defence mechanisms in turkey hens was also demonstrated for the newly-synthesized amidrazone derivative 5-oxo-1,2,4-triazine (Ognik and Sembratowicz, 2011). This compound exhibits anti-inflammatory, antibacterial, antiviral and antifungal (*in vitro* analysis) properties (Modzelewska-Banachiewicz and Kamińska, 2000). Studies on turkeys have shown that 5-oxo-1,2,4-triazine had a stimulatory effect on weight gain and on the mineral composition of tissues (Ognik and Merska, 2011). Taking into account the biological properties of aloe extracts and the newly synthesized amidrazone derivative 5-oxo-1,2,4-triazine, an attempt was made to use these additives during artificially induced stress in turkey hens.

The aim of the study was to determine whether the introduction of stress affects corticosterone and malonyl dialdehyde levels (treated as stress factors) and antioxidant blood indices, and whether additives used in conjunction with stress, such as aloe extract supplemented with trans-resveratrol and vitamin C or 5-oxo-1,2,4-triazine, mitigates adverse changes in these parameters.

Material and methods

Animals and experimental design

The experiment was carried out on 360 turkey hens of the BUT-9 line, aged from 6 to 16 weeks. The birds were randomly assigned to 6 experimental groups of 60 birds. Each group was further subdivided into 6 replicates, each with 10 birds. The birds were reared under standard conditions following recommendations by Faruga and Jankowski (1996). The experimental procedure was approved by the

Second Local Ethics Commission for Experiments with Animals in Lublin (approval no. 11/2009). During the experimental period, the birds from all groups had access to drinking water and were fed *ad libitum* with complete feed mixtures (Table 1) balanced according to nutrient requirements for poultry (NRP, 2005).

Table 1. Ingredients (g kg⁻¹) and calculated nutrient content of standard diets

	Feeding period (in weeks of age)				
	starter (1–2 wk)	grower I (3–5 wk)	grower II (6–9 wk)	grower III (10–12 wk)	finisher I (13–15 wk)
Ingredient:					
maize meal	256	274	238	352	474
wheat	200	250	300	250	250
rape cake	-	-	-	-	-
wheat bran	30	-	-	-	-
soybean meal 46% protein	410	417	388	327	204
soybean meal 45% protein	20	-	-	-	-
fish meal 60%	35	-	-	-	-
fodder chalk	12	17	17	14	15
soybean oil	5	10	25	30	30
Cytromix Plus ¹	2	2	2	2	2
Farmix ²	30	30	30	25	25
Calculated nutrient composition					
crude protein (g kg ⁻¹)	271.0	255.0	245.0	220.0	175.0
ME (MJ kg ⁻¹)	11.45	11.73	12.19	12.58	13.09
crude fibre (g kg ⁻¹)	28.6	27.7	27.2	27.1	27.0
Lysine (g kg ⁻¹)	18.1	17.1	15.7	13.8	11.7
Meth.+ cyst. (g kg ⁻¹)	9.8	9.0	8.8	7.9	7.0
Tryptophan (g kg ⁻¹)	3.4	2.8	2.7	2.3	1.9
Arginine (g kg ⁻¹)	17.7	15.7	15.0	13.2	9.8
Calcium (g kg ⁻¹)	13.9	12.3	11.7	10.6	9.4
Avail. P (g kg ⁻¹)	7.7	6.7	5.9	5.7	4.7
Sodium (g kg ⁻¹)	1.5	1.6	1.5	1.5	1.5

¹Cytromix Plus: citric acid, fumaric acid, phosphoric acid (62%).

²Farmix mineral and vitamin premix provided the following per kilogram of diet: 3,000,000 IU vitamin A; 900,000 IU vitamin D₃; 10,000 mg vitamin E; 500 mg vitamin K₃; 700 mg vitamin B₁; 2,000 mg riboflavin; 1,200 mg vitamin B₆; 6 mg vitamin B₁₂; 400 mg folic acid; 72 mg biotin; 15,000 mg niacin; 120,000 mg choline; 4,200 mg calcium pantothenic; 30,000 mg Mn; 18,000 mg Zn; 12,000 mg Fe; 3,000 mg Cu; 200 mg I; 60 mg Se; 40 mg Co; 15 g Ca; 15.5 g P.

Table 2. Content of mineral elements, vitamin C and resveratrol in the aloe extract (Ognik and Sembratowicz, 2012)

Aloe extract	Macroelement (mg l ⁻¹)				Microelement (mg l ⁻¹)			Vit. C (mg ml ⁻¹)	Resveratrol (mg l ⁻¹)
	Na	K	Ca	Mg	Fe	Cu	Zn		
Aloes Plus	32.4	243.6	38.4	3.66	0.04	0.108	0.34	35.12	486.3

Groups C and C(+)*stress* were control groups that did not receive any additive (Table 3). Birds from groups A and A(+)*stress* were administered aloe extract with trans-resveratrol and vitamin C (Table 2) added in the amount of 0.70 ml/kg body weight (BW)/day (Table 3). The additive is a Polish aloe preparation with the commercial name Aloes Plus, produced by Herbapol Lublin, a producer of herbal products. Turkey hens from groups T and T(+)*stress* received the amidrazone derivative: 5-oxo-1,2,4-triazine in the amount of 30 µg/kg BW/day, dissolved in a small quantity of ethanol (ca. 2 ml). The 5-oxo-1,2,4-triazine was synthesized by the Department of Organic Chemistry of the Medical University of Lublin. The additives were administered to the birds in their drinking water for 28 days (Table 3) starting from the 36th day of life. The dose of the additives per kg BW/day was determined on the basis of average values for performance parameters (body weight and daily weight gain) in BUT-9 turkey hens. The experimental additives were administered every morning in drinking troughs with about 1–2 litres of water, which guaranteed that the additives would be consumed, and then clean drinking water was added as needed. A two-week break followed, during which the birds drank water without additives. After the break, the birds again received the additives for another 28 days. On days 26, 27 and 28 of administration of the additives an experimental factor in the form of stress stimuli was introduced in groups C(+)*stress*, A(+)*stress* and T(+)*stress* for 1 hour each day.

Table 3. Experimental design

Specification	Control		Experimental groups			
	C	C+ <i>stress</i>	A	A+ <i>stress</i>	T	T+ <i>stress</i>
36–63 days of life	-	-	aloe extract (0.70 ml/kg BW/day)	aloe extract (0.70 ml/kg BW/day)	5-oxo-1,2,4-triazine (30 µg/kg BW/day)	5-oxo-1,2,4-triazine (30 µg/kg BW/day)
61–63 days of life	-	+ <i>stress</i>	-	+ <i>stress</i>	-	+ <i>stress</i>
64–77 days of life	-	-	-	-	-	-
78–105 days of life	-	-	aloe extract (0.70 ml/kg BW/day)	aloe extract (0.70 ml/kg BW/day)	5-oxo-1,2,4-triazine (30 µg/kg BW/day)	5-oxo-1,2,4-triazine (30 µg/kg BW/day)
103–105 days of life	-	+ <i>stress</i>	-	+ <i>stress</i>	-	+ <i>stress</i>

The stress stimuli were crowding and changes in temperature and lighting, applied simultaneously. The birds were placed in 85×50×35 cm plastic containers (crowding) used to transport birds. A maximum of three birds were placed in one container. The containers had holes enabling access to cool fresh air when the birds were moved outdoors (to a place not shielded from wind) in order to induce a rapid change of lighting conditions from artificial light to daylight, as well as a decrease in

temperature of ca. 20–30°C, as during the study (November–January) the air temperature ranged from +5°C to –10°C. The treatments were conducted in the daytime, always at the same time of day. The experimental conditions were adjusted to resemble the conditions of the production process, especially during intensive rearing at large production farms. The same procedure for inducing the stress factor was applied on days 68, 69 and 70 of the life of the turkey hens (Table 3).

Analysis of the chemical composition of the aloe preparation

Analysis of the chemical composition of the aloe preparation involved determination of the content of selected macro- and micronutrients, resveratrol and vitamin C. The concentration of minerals was determined by atomic absorption spectroscopy (AAS) using a Unicam 939 apparatus (AA Spectrometer Unicam). Vitamin C content in the Aloes Plus preparation was measured colorimetrically in a reaction with 2,6-dichlorophenylindophenol according to Omaye et al. (1979). Content of resveratrol was determined by HPLC chromatography using a C18 column (28 cm × 4 mm) and an acetonitrile/water (70:30) mobile phase.

Sample collection and analytical method

At the end of the 9th, 11th, and 15th weeks of life of the turkey hens, blood was collected (by a veterinarian) from the wing vein of 10 birds from each group. Blood samples were taken after 8-hour fasting with free access to drinking water. The blood serum level of the lipid peroxidation product malonyl dialdehyde (MDA), which is indicative of oxidative stress, was determined according to Salih et al. (1987). Corticosterone contents was determined in the blood plasma according to Beuving and Vonder (1977). Spectrophotometric assays were conducted to analyse antioxidant enzymes in the blood: superoxide dismutase (SOD) by the adrenaline method (according to Misra, cited by Greenwald, 1985) with a modification involving a wavelength of 320 nm to achieve better selectivity of transitory reaction products at the same wavelength (Bartosz, 2004); catalase (CAT) according to Bartosz (2004); and glutathione peroxidase (PGx) using the Ransel assay by Randox. Assays were performed for the following antioxidant system markers: total plasma antioxidant potential (FRAP) according to Benzie and Strain (1996) and vitamin C according to Omaye et al. (1979). Blood serum samples were analysed for levels of zinc (Zn), copper (Cu) and iron (Fe) by atomic absorption spectrometry (AAS).

Statistical analysis

Data were analysed using the STATISTICA software package, version 6.0 (StatSoft Corp., Kraków, Poland). Repeated measures two-way ANOVA was used to assess the effect of the main factors, stress (S) and additives (D), and their interactions (S×D). If the analysis revealed a significant interaction or that both factors had a significant influence, the differences between groups were then analysed using Tukey's multiple range post hoc test. Data were checked for normality before the statistical analysis was performed. Differences were considered to be significant at $P \leq 0.05$. The pooled SEM was calculated as the standard deviation from all measurements divided by their square root.

Results

When the turkey hens were assigned to groups (35th day of life) they had similar body weights (1.74–1.76 kg). The addition of the amidrazone derivative 5-oxo-1,2,4-triazine resulted in approximately 7.5% higher daily weight gain in the birds. The stress factor applied had no significant effect on the production results of the turkey hens (data in press). The data presented in Table 4 show that the stress factors resulted in a significant ($P \leq 0.05$) increase in corticosterone level in the blood of the turkey hens, which could be seen after the first application of stress (9th week of life). The concentration of malonyl dialdehyde (MDA) in the blood of turkey hens was found to decrease in groups A and T. A significant ($P \leq 0.05$) reduction in this compound in relation to group C was recorded, but only following long-term application. The stress factor alone increased the concentration of malonyl dialdehyde, especially at the beginning of the experiment, when the increase in MDA proved to be statistically ($P \leq 0.01$) significant. The results concerning the activity of enzymes involved in anti-oxidation reactions in the blood of the turkey hens are shown in Table 5. They indicate that the introduction of a stressor did not significantly influence the activity of superoxide dismutase (SOD) or glutathione peroxidase (PGx). The break in the application of stress (11th week of life) resulted in a significant increase in the activity of superoxide dismutase in the blood of the birds. Irrespective of the presence or absence of a stress factor, the use of the amidrazone derivative 5-oxo-1,2,4-triazine led to a visible ($P \leq 0.05$) increase in SOD activity. Analysis of catalase (CAT) activity in the groups of turkey hens found it to be higher in the stressed birds in the 9th week of rearing. Both additives analysed caused a significant decrease in CAT activity in the erythrocytes.

Table 4. Level of corticosterone and malonyl dialdehyde in the blood plasma of the turkey hens

Experimental factors	Corticosterone, nmol l ⁻¹				MDA, μ mol l ⁻¹			
	9	11	15	$\bar{x}$	9	11	15	$\bar{x}$
C (-)stress	9.26 b	10.02	10.11 b	9.79 b	1.18 b	1.16	1.21 ab	1.18 b
A	10.12 b	10.32	12.29 b	10.91 b	1.13 b	1.03	1.08 b	1.08 b
T	13.81 ab	12.08	14.01 ab	13.30 ab	1.06 b	1.02	1.10 b	1.06 b
C (+)stress	16.25 a	12.52	16.95 a	15.24a	1.40 a	1.29	1.54 a	1.41 a
A	10.67 b	11.48	13.17 ab	11.77 b	1.34 ab	1.12	1.09 b	1.18 b
T	14.01 ab	12.31	14.33 ab	13.55 ab	1.23 ab	1.09	1.11 b	1.14 b
SEM	0.021	0.019	0.025	0.022	0.017	0.021	0.018	0.016
Additive C	12.75 ab	11.27	13.53	12.51	1.29	1.22	1.37 a	1.29
effect (D) A	10.39 b	10.9	12.73	11.34	1.23	1.07	1.08 b	1.13 b
T	13.91 a	12.19	14.17	13.42	1.14	1.05	1.10 b	1.10 b
Stress (-)stress	11.06 b	10.81	12.1b	11.3b	1.12 b	1.07	1.13	1.11 b
effect (S) (+)stress	13.64 a	12.10	14.8a	13.5a	1.32 a	1.17	1.25	1.24 a
D effect	**	ns	ns	ns	ns	ns	**	**
S effect	**	ns	**	**	*	ns	ns	ns
DxS interaction	ns	ns	ns	ns	**	ns	ns	ns

a, b – means in the same column without common superscripts differ significantly at * $P \leq 0.01$; ** $P \leq 0.05$; ns $P > 0.05$.

(-) stress – without stress.

(+) stress – with stress.

Table 5. The activity of antioxidant enzymes in the blood of turkey hens

Experimental factors	SOD, U/gHb				PGx, U/gHb				CAT, U/gHb			
	9	11	15	$\bar{x}$	9	11	15	$\bar{x}$	9	11	15	$\bar{x}$
C	484.1 b	420.2 b	489.0 ab	464.4 b	5.06	5.44	6.39	5.63	1268.8 ab	1399.3 a	1195.7 b	1287.9
A	553.9 ab	449.8 b	493.7 ab	499.1 ab	5.10	4.96	5.07	5.04	1161.9 b	1296.4 ab	1207.8 b	1222.0
T	579.8 a	479.5 ab	507.2 a	522.2 a	5.80	5.44	5.49	5.57	1209.5 b	1334.6 a	1205.6 b	1249.9
C	496.6 b	531.4 a	458.4 b	495.5 ab	5.60	5.33	6.00	5.64	1462.2 a	1211.9 b	1323.1 a	1265.8
A	444.8 b	555.6 a	481.6 ab	494.0 ab	5.60	4.83	5.99	5.47	1234.0 b	1375.0 a	1248.5 ab	1285.8
T	525.1 ab	553.0 a	504.9 a	527.6 a	5.16	5.63	5.84	5.54	1308.1 ab	1325.1 a	1241.9 ab	1291.7
SEM	0.486	0.452	0.308	0.158	0.286	0.145	0.108	0.114	1.124	1.085	0.896	0.854
Additive	C	490.3 b	475.8	473.7	479.9 b	5.33	5.39	5.64	1365.5 a	1305.6	1259.4	1310.2
effect (D)	A	499.3 b	502.7	487.6	496.5 ab	5.35	4.90	5.26	1198.0 b	1335.7	1228.2	1253.9
T	552.4 a	516.2	506.0	524.9 a	5.48	5.54	5.67	5.56	1258.8 b	1329.9	1223.8	1270.8
Stress	(-)stress	539.2	449.8 b	496.6	495.2	5.32	5.28	5.41	1213.4 b	1343.4	1203.0	1253.3
effect (S)	(+)stress	488.8	546.6 a	481.6	505.7	5.45	5.26	5.55	1334.8 a	1304.0	1271.2	1278.3
D effect	*	ns	ns	**	ns	ns	ns	ns	*	ns	ns	ns
S effect	ns	*	ns	ns	ns	ns	ns	ns	**	ns	ns	ns
D×S interaction	**	**	ns	ns	ns	ns	ns	ns	**	ns	ns	ns

a, b – means in the same column without common superscripts differ significantly at * $P \leq 0.01$; ** $P \leq 0.05$; ns $P > 0.05$.

(-) stress – without stress.

(+) stress – with stress.

Statistical analysis of the values for total plasma antioxidant potential (FRAP) in the turkey hens showed that the stress factor significantly ($P \leq 0.05$) decreased this parameter (Table 6). Application of the aloe preparation, however, resulted in a significant increase in FRAP. In the birds treated with the aloe preparation there was also a significant increase in vitamin C, which is one of the components of FRAP. The stress factor decreased the level of ascorbic acid for the entire experimental period.

Table 6. Level of total antioxidant potential (FRAP) and vitamin C in the blood plasma of the turkey hens

Experimental factors		FRAP, $\mu\text{mol l}^{-1}$				Vit. C, mg l^{-1}			
		9	11	15	$\bar{x}$	9	11	15	$\bar{x}$
C	(-)stress	243.1 ab	223.8 ab	205.0 b	223.9 ab	0.111 b	0.112 b	0.114 b	0.112 b
A		272.7 a	268.0 a	237.2 a	259.3 a	0.256 a	0.222 a	0.258 a	0.245 a
T		187.1 b	212.1 ab	200.6 b	199.9 b	0.106 b	0.101 b	0.104 b	0.103 b
C	(+)stress	185.2 b	215.8 ab	190.4 b	197.1 b	0.105 b	0.105 b	0.109 b	0.106 b
A		254.9 ab	221.6 ab	232.6 a	236.3 a	0.172 ab	0.186 ab	0.256 a	0.204 a
T		174.4 b	196.6 b	190.0 b	187.0 b	0.104 b	0.109 b	0.106 b	0.106 b
SEM		0.89	0.67	0.72	0.68	0.53	0.48	0.46	0.39
Additive	C	214.1 ab	219.8 ab	197.7 b	210.5 ab	0.108 b	0.108 b	0.111 b	0.109 b
effect (D)	A	263.8 a	244.8 a	234.9 a	247.8 a	0.214 a	0.204 a	0.257 a	0.224 a
	T	180.7 b	204.3 b	195.3 b	193.4 b	0.105 b	0.105 b	0.105 b	0.104 b
Stress	(-)stress	234.3 a	234.6 a	214.3	227.7 a	0.158 a	0.145	0.159	0.153 a
effect (S)	(+)stress	204.8 b	211.3 b	204.3	206.8 b	0.127 b	0.133	0.157	0.139 b
D effect		*	*	*	*	*	*	*	*
S effect		*	*	ns	**	**	ns	ns	**
D×S interaction		**	ns	**	ns	**	ns	ns	**

a, b – means in the same column without common superscripts differ significantly at * $P \leq 0.01$; ** $P \leq 0.05$; ns $P > 0.05$.

(-) stress – without stress.

(+) stress – with stress.

The results presented in Table 7 show that the stress applied did not result in major changes in the concentration of the trace elements tested in the blood of the birds. Use of the amidrazone derivative 5-oxo-1,2,4-triazine also had no effect on their content in the blood. In contrast, the addition of the aloe preparation caused a significant increase in the iron and copper concentration in comparison with the control group.

Table 7. Level of microelements in the blood plasma of turkey hens

Experimental factors	Fe, $\mu\text{mol l}^{-1}$				Zn, $\mu\text{mol l}^{-1}$				Cu, $\mu\text{mol l}^{-1}$			
	9	11	15	$\bar{x}$	9	11	15	$\bar{x}$	9	11	15	$\bar{x}$
C	28.1 b	35.4 a	26.7 b	30.0 ab	29.0 a	29.2	28.4	28.8	18.9 b	17.5	17.1	17.8 b
A	34.0 a	30.9 ab	33.5 a	32.8 a	24.7 ab	32.1	30.9	29.2	24.5 a	20.8	19.8	21.7 a
T	27.4 b	24.8 b	30.9 ab	27.7 b	23.2 b	29.3	29.2	27.2	15.2 b	16.8	14.4	15.4 b
C	27.6 b	26.8 b	27.0 b	27.1 b	21.1 b	28.7	26.1	25.3	16.4 b	19.3	17.2	17.6 b
A	29.5 ab	29.0 ab	32.9 a	30.5 ab	22.9 b	31.5	27.9	27.4	23.7 a	17.7	17.4	19.6 ab
T	27.0 b	30.8 ab	28.6 ab	28.8 b	22.8 b	28.9	29.8	27.1	14.3 b	17.5	14.6	15.4 b
SEM	0.53	0.89	0.89	0.77	0.76	0.76	0.91	0.81	1.78	0.47	0.47	0.91
Additive	27.85 b	31.1	26.85 b	28.55	25.05	28.95	27.25	27.05	17.65 b	18.4	17.15	17.7 b
effect (D)	31.75 a	29.95	33.2a	31.65	23.8	31.8	29.4	28.3	24.1 a	19.25	18.6	20.65 a
T	27.2 b	27.8	29.75 ab	28.25	23.0	29.1	29.5	27.15	14.75 b	17.15	14.5	15.4 b
(-)stress	29.83	30.37	30.37	30.17	25.63	30.20	29.50	28.40	19.53	18.37	17.10	18.30
effect (S)	28.03	28.87	29.50	28.80	22.27	29.70	27.93	26.60	18.13	18.17	16.40	17.53
D effect	**	ns	**	ns	ns	ns	ns	ns	*	ns	ns	**
S effect	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
D×S interaction	**	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	**

a, b – means in the same column without common superscripts differ significantly at * $P \leq 0.01$; ** $P \leq 0.05$; ns $P > 0.05$.

(-) stress – without stress.

(+) stress – with stress.

Discussion

Corticosterone is a hormonal mediator of the stress reaction in birds. It can be used both as an indicator that a stress reaction is taking place and as a factor inducing it. The significant increase in corticosterone in the turkey hens treated with stress stimuli in the form of concurrent crowding and changes of temperature and light confirms that they are stressogenic. The pronounced increase in the level of the lipid peroxidation end product malonyl dialdehyde (MDA) in the blood of the turkey hens treated with stress indicates the severity of oxidation processes and the occurrence of what is known as oxygen shock. According to studies by other authors, this phenomenon is often accompanied in animals by stress, particularly thermal stress (Sahin et al., 2001, 2003; Wernicki et al., 2003). An increase in corticosterone and MDA in the blood plasma was also reported in another study, in which identical turkey hens were treated with stressors continuously (for 28 consecutive days) or intermittently (every two days) (Truchliński et al., 2006). In the present study analysing the effect of additives – an aloe preparation and the amidrazone derivative 5-oxo-1,2,4-triazine – on these blood parameters in turkey hens treated with stress, the reaction to the stress factors in the birds which received the aloe preparation was found to be weaker. This can be seen in the smaller increase in the concentration of the stress hormone and of malonyl dialdehyde than in the stressed birds that were not treated with additives. This could be due to various components of the preparation, such as trans-resveratrol, high content of vitamin C, trace elements and other active compounds with antioxidant properties. The positive impact of various preparations made from aloe on the antioxidant defence mechanisms has also been demonstrated in experiments by Ognik and Sembratowicz (2007) and by Ognik and Czech (2010). Results obtained by Skoromucha and Sosnówka-Czajka (2013) show that herbal mixtures with sedative effects have a beneficial effect during stress in poultry, because they reduce the level of corticosterone and other stress hormones, adrenaline and noradrenaline. Administration of vitamin C also causes a beneficial reduction in stress in birds. While this vitamin is synthesized by birds, such synthesis does not meet the increased demand under stress conditions (Abidin and Khatoon, 2013). A decrease in the secretion of the stress hormone (corticosterone) and a decrease in lipid oxidation as a result of administration of vitamin C during heat stress in chickens was observed by Abidin and Khatoon (2013). Ascorbate has the ability to destroy lipid peroxides, resulting in the termination of lipid peroxidation (Weidtmann et al., 1995). The effects observed in the turkey hens treated with stress and receiving the aloe preparation could therefore also be the result of an additional supply of vitamin C, which was a major component of the preparation.

Analysis of the influence of the amidrazone derivative 5-oxo-1,2,4-triazine on levels of corticosterone and malonyl dialdehyde treated as indicators of stress in the turkey hens indicates that its impact was associated with the inhibition of antioxidant reactions rather than its effect on the secretion of corticosterone. The reduction in oxidative processes may have been associated with an increase in the activity of one of the key antioxidant enzymes, superoxide dismutase. An increase in SOD activity in the erythrocytes of turkey hens induced by the application of the amidrazone de-

rivative 5-oxo-1,2,4-triazine was also observed in a previous study (Ognik and Sembratowicz, 2011). A similar effect on SOD has been exhibited by other amidrazones, such as the derivative 1,2,4-triazole (Ognik et al., 2004), most likely due to stimulation of the transcriptional activity of genes encoding the synthesis of this enzyme.

The stressor alone did not significantly affect the activity of SOD or glutathione peroxidase, but did cause a significant increase in catalase activity. Truchliński et al. (2007), however, used similar stress factors and noted a decrease in the activity of this enzyme in the erythrocytes. Catalase is an enzyme containing four porphyrin heme (iron) groups, whose main task is the decomposition of hydrogen peroxide. It has been suggested that it is responsible for the removal of half of the hydrogen peroxide produced in the red blood cells under normal conditions (Gaetani et al., 1994). The increased activity of this enzyme under the influence of the stress applied may therefore indicate intensive synthesis of its substrate, whose level was not determined, and to be an adaptive response, especially when we consider that when the stress was stopped CAT activity in the erythrocytes decreased in the stressed birds. The decrease in catalase activity in the turkey hens given additives during stress indicates a reduction in peroxidation processes, which is also evidenced by the decreased levels of MDA in these birds. Results obtained by other authors have shown (Blahova et al., 2007; Truchliński et al., 2007) that stress factors may result in the loss of many valuable macro- and micronutrients, first in the blood serum, and then in tissues and muscles. The use of the aloe preparation increased the concentrations of iron and copper in the blood, which may have resulted from their additional supply in the preparation or from the beneficial effect of the active ingredients of aloe on their digestibility and retention.

There are reports indicating that polyphenolic compounds having one or more active sites in the form of hydroxyl groups may form complexes (chelates) of various stability with trace elements (Roy et al., 2012), which can increase their stability in the body and consequently their content in the blood. According to Gülçin (2010) resveratrol has strong chelating properties.

To sum up, a sudden change in temperature and light together with overcrowding triggers a strong stress response in birds. In order to alleviate the negative effect of stress, supplementation of diets with aloe extract to which resveratrol and vitamin C have been added may be considered.

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