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In ovo study on the effect of natural antioxidants on NDV proliferation in chicken embryos

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Newcastle disease (NDV), caused by the highly virulent Newcastle disease virus affects both wild and domesticated bird species. This disease has spread over the years in all countries of the world, contributing to enormous economic losses in the poultry sector related to poultry death, farm liquidation costs and compensation, as well as suspending the trade and export of poultry products (Alexander, 2000).

The course and the image of the disease is varied and is determined by the virulence degree of the virus strain, route of the infection, the species and age of the bird, the immunological status of the organism, as well as concomitant infections or stress (Seal et al., 2000). Infected birds become apathetic and sleepy, loss of appetite, diarrhea and decrease in egg yield are also observed. Sometimes neurological symptoms also occur, including muscle twitching and paralysis of the limbs. NDV infection is characterized by high mortality, up to 100% of birds in the flock (Beard & Hanson, 1984). However, no effective drug against NDV has yet been developed (Orajak et al., 1999). Because of that, one of the most common ways to fight this disease at the moment is to take preventive measures in the form of routine preventive vaccination of breeding poultry (Seal et al., 2000, Boven et al., 2008). Vaccination of birds helps to reduce the incidence of Newcastle disease and alleviates clinical symptoms of infection, but it does not guarantee the absence of infection, and often results in increased susceptibility of immunized animals to other infections and inhibition of their growth (Alexander, 2003). Therefore, new methods of combating the NDV virus are still being sought. The use of antioxidants – compounds protecting the body against the harmful effects of free radicals and supporting its immunological status – seems to be promising solution. Available literature proves that antioxidants can be a barrier to protect against other pathogenic factors, including viruses (Beck, 2001; Berardi et al., 2009). Among the antioxidants with special interest as potential measures to fight the NDV virus, mention is made of e.g. vitamin E (tocopherol), resveratrol and coenzyme Q10 (Nutrition and Health, 2006).

Studies carried out so far included an analysis of the impact of individual antioxidants present only in the form of separate preparations on the development of viruses in bird embryos (Beck, 2001, Berardi et al., 2009). However, there are no reports of the simultaneous use of the foregoing antioxidants in the form of a mixture. It has been assumed, however, that the use of a preparation which is a mixture of various antioxidants may beneficially modify the development of pathogenic viruses in the body.

The aim of the study was to evaluate the effect of coenzyme Q10, resveratrol and RRR-d-alpha-tocopherol used as an antioxidant complex on the multiplication of NDV virus in chicken embryos and the oxidoreductive status of egg yolk of incubated eggs.

Materials and methods

The *in ovo* study was performed on 240 hen eggs incubated for 8 days at 37°C. The experimental agents included the NDV (Newcastle Disease Virus) and an antioxidant complex prepared from 98% coenzyme Q10, 98% RRR-d-alpha-tocopherol and 98% resveratrol in proportion of 1: 1: 1 (A1) and in proportion of 5: 3: 2 (A2). The initial concentration of the antioxidant complex was 30 µg/ml. The test was carried out according to the scheme shown in Table 1.

Table 1. Scheme of the experiment

Experimental factor	Healthy embryos (Z) (n=120)			Infected embryos (N) (n=120)		
	Z (n=40)	Z-A1 (n=40)	Z-A2 (n=40)	N (n=40)	N-A1 (n=40)	N-A2 (n=40)
NDV	-	-	-	+	+	+
A1	-	+	-	-	+	-
A2	-	-	+	-	-	+

Evaluation of NDV multiplication in chicken embryos

Evaluation of the NDV multiplication in chicken embryos was carried out according to the method proposed by Kandefer-Szerszeń (1997). The eggs selected for the test (n = 240) were divided into three equal groups, and then they were subjected to X-ray scan, with the air chamber boundary and the position of the embryo being determined. After disinfection of the shell with 70% ethyl alcohol, there were two holes drilled in it – one above the air chamber, the other on the opposite side of the embryo. After repeated disinfection, 100 µl of the anti-oxidation complex A1 or A2 was introduced via injection into the allantoic fluid of the prepared embryos (N-A1, N-A2), through the hole on the side of the egg. The control group (N) did not receive any experimental supplementation. In addition, 100 µl of NDV (Z, Z-A1, Z-A2) was injected into the allantoic fluid in half of the embryos from each experimental group. After introducing the experimental agents, the opening was closed with liquid paraffin. All eggs were then incubated at 37°C for 24 h, 48 h or 72 h. After a certain time, the eggs were disinfected with 70% ethanol and the shells were removed just above the observed air chamber line. The chorioallantoic membrane was disrupted using sterile tweezers. Allantoic fluid sample was taken for microscopic analysis. The NDV titer in the allantoic fluid was also determined using the Reed and Muench method (1938). For this purpose, a continuous L 929 fibroblast cell line suspended in MEM growth fluid (50 µl) was inserted into a 96-well plate. In order to reduce the error of the result, four experimental objects were used for each allantoic fluid collected, i.e. each dilution was performed in four replications. In addition, a series of dilutions from 10^{-1} to 10^{-7} were performed for allantoic fluid (50 µl taken in each case) through serial dilution method. Dilutions were performed for allantoic fluid of the tested groups of eggs (Z-A1, Z-A2, N, N-A1, N-A2). The embryos were then transferred to Petri dishes, and macroscopic changes were observed resulting from NDV infections of chicken embryos, comparing to control (healthy) embryos.

Evaluating the oxidoreductive status of egg yolks

After 120 h of incubation, 6 yolk samples were taken from each egg Z, Z-A1, Z-A2, N, N-A1, N-A2, using them next to prepare homogenates for the analysis of oxidoreductive status indicators.

The contents of lipid peroxides (LOOH) and malondialdehyde in yolk homogenates were determined according to Gay & Gębicki (2000), and Botsoglou et al. methods (1994), respectively. Moreover, the activity of peroxide dismutase (SOD) in the prepared samples was determined using the adrenaline method proposed by Misra in: Greenwald (1985) at 320 nm

wavelength. The method was modified to achieve greater selectivity of transient reaction products at this light length. The analyzes also included the determination of catalase activity (CAT) according to Bartosz (2004).

Statistical analysis

The test results were analyzed statistically using the Statistica Ver 5.1.G software (Stat, Inc. 1997). An analysis of ANOVA variance in the double-agent plan was conducted for all analyzed dependent variables (oxidoreduction status).

Results and discussion

Based on the results of the initial tests, aimed at determining the effect of the antioxidant complex on cells of the allantoic fluid (Tab. 2 and 3) it was found that this complex does not adversely affect the cells. There was no lysis of cells under the influence of the preparation being used, on the contrary – it was noted that it increases the cell density after 48 hours of incubation. The obtained results suggest that the analyzed experimental complex stimulates cell proliferation.

Literature data confirm that natural antioxidants do not have a negative effect on cells (Beck, 2001; Berardi et al., 2009). Furthermore, thanks to specific properties they support the the immune system actions and provide effective protection when fighting against viruses.

Data determining the effect of NDV virus and antioxidant complexes A1 and A2 on allantoic fluid cells are presented in Tables 4–6.

Table 2. Results of the effect of antioxidant complex A1 and A2 on allantoic fluid cells after 24 h incubation

Dilution rate	A1	A1	A1	A1	A2	A2	A2	A2
10^{-1}	+	+	+	+	-	-	-	-
10^{-2}	-	-	-	-	-	-	-	-
10^{-3}	-	-	-	-	-	-	-	-

A1 – antioxidant complex of coenzyme Q10, 98% RRR-d-alpha-tocopherol and 98% resveratrol in a 1: 1: 1 ratio.
A2 – antioxidant complex of coenzyme Q10, 98% RRR-d-alpha-tocopherol and 98% resveratrol in a 5: 3: 2 ratio.

Table 3. Results of the effect of antioxidant complex A1 and A2 on allantoic fluid cells after 48 h incubation

Dilution rate	A1	A1	A1	A1	A2	A2	A2	A2
10^{-1}	+	+	+	+	-*	-*	-*	-*
10^{-2}	-	-	-	-	-	-	-	-
10^{-3}	-	-	-	-	-	-	-	-

* *Increased cell density.*
A1 – antioxidant complex of coenzyme Q10, 98% RRR-d-alpha-tocopherol and 98% resveratrol in a 1: 1: 1 ratio.
A2 – antioxidant complex of coenzyme Q10, 98% RRR-d-alpha-tocopherol and 98% resveratrol in a 5: 3: 2 ratio.

Tabela 4. Odczyt wyników wpływu kompleksu antyoksydacyjnego i wirusa NDV na komórki płynu omoczniowego po 48-godzinnej inkubacji
Table 4. Results of the effect of antioxidant complex and NDV virus on allantoic fluid cells after 48 h incubation

Rozcieńczenie *Dilution rate*	N	N	N	N	N-A1	N-A1	N-A1	N-A1	N-A2	N-A2	G.N-2	N-A2	
10^{-1}	+	+	+	+	+	+/-	+	+/-	+	+	+	+	kontrola 1 bez zmian *control 1 no change*
10^{-2}	+	+	+/-	+/-	-	-	-	-	-	-	-	-	kontrola N-A1 *control N-A1*
10^{-3}	+/-	+/-	-	-	-	-	-	-	-	-	-	-	kontrola N-A1 *control N-A1*
10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	kontrola N-A2 *control N-A2*
10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-	kontrola N-A2 *control N-A2*
10^{-6}	-	-	-	-	-	-	-	-	-	-	-	-	MEM kom. bez zmian *MEM cells no change*
10^{-7}	-	-	-	-	-	-	-	-	-	-	-	-	MEM kom. bez zmian *MEM cells no change*
Wynik miareczkowania *Titration result*	$4420000=2*10^{3,00+0,45}CCID_{50}/ml$				$4000000=2*10^{1,50+0,32}CCID_{50}/ml$				$4000000=2*10^{1,50+0,32}CCID_{50}/ml$				

N – zarodki zakażone wirusem NDV – *embryos infected with NDV virus.*
N-A1 – zarodki zakażone wirusem NDV otrzymujące dodatek kompleksu antyoksydacyjnego A1 – *embryos infected with NDV virus and supplemented with A1 antioxidant complex.*
N-A2 – zarodki zakażone wirusem NDV otrzymujące dodatek kompleksu antyoksydacyjnego A2 – *embryos infected with NDV virus and supplemented with A2 antioxidant complex.*

Tabela 5. Odczyt wyników wpływu kompleksu antyoksydacyjnego i wirusa NDV na komórki płynu omoczniowego po 72-godzinnej inkubacji
Table 5. Results of the effect of antioxidant complex and NDV virus on allantoic fluid cells after 72 h incubation

Rozcieńczenie *Dilution rate*	N	N	N	N	N-A1	N-A1	N-A1	N-A1	N-A2	N-A2	N-A2	N-A2	
10^{-1}	+	+	+	+	+	+/-	+/-	+	+	+	+	+	kontrola 1 bez zmian *control 1 no change*
10^{-2}	+	+	+	+	-	-	-	-	-	-	-	-	kontrola N-A1 *control N-A1*
10^{-3}	+	+	+/-	+	-	-	-	-	-	-	-	-	kontrola N-A1 *control N-A1*
10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	kontrola N-A2 *control N-A2*
10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-	kontrola N-A2 *control N-A2*
10^{-6}	-	-	-	-	-	-	-	-	-	-	-	-	MEM kom. bez zmian *MEM cells no change*
10^{-7}	-	-	-	-	-	-	-	-	-	-	-	-	MEM kom. bez zmian *MEM cells no change*
Wynik miareczkowania *Titration result*	$4440000=2*10^{3,50+0,32}CCID_{50}/ml$				$4000000=2*10^{1,50+0,32}CCID_{50}/ml$				$4000000=2*10^{1,50+0,32}CCID_{50}/ml$				

N – zarodki zakażone wirusem NDV – *embryos infected with NDV virus*
N-A1 – zarodki zakażone wirusem NDV otrzymujące dodatek kompleksu antyoksydacyjnego A1 – *embryos infected with NDV virus and supplemented with A1 antioxidant complex*
N-A2 – zarodki zakażone wirusem NDV otrzymujące dodatek kompleksu antyoksydacyjnego A2 – *embryos infected with NDV virus and supplemented with A2 antioxidant complex.*

Tabela 6. Odczyt wyników wpływu kompleksu antyoksydacyjnego i wirusa NDV na komórki płynu omoczniowego po 120 godzinach inkubacji
Table 6. Results of the effect of antioxidant complex and NDV virus on allantoic fluid cells after 120 h incubation

Rozcieńczenie *Dilution rate*	N	N	N	N	N-A1	N-A1	N-A1	N-A1	N-A2	N-A2	N-A2	N-A2	
10^{-1}	+	+	+	+	+	+/-	+/-	+	+	+	+	+	kontrola 1 bez zmian *control 1 no change*
10^{-2}	+	+	+	+	-	-	-	-	-	-	-	-	kontrola N-A1 *control N-A1*
10^{-3}	+	+	+	+	-	-	-	-	-	-	-	-	kontrola N-A1 *control N-A1*
10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	kontrola N-A2 *control N-A2*
10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-	kontrola N-A2 *control N-A2*
10^{-6}	-	-	-	-	-	-	-	-	-	-	-	-	MEM kom. bez zmian *MEM cells no change*
10^{-7}	-	-	-	-	-	-	-	-	-	-	-	-	MEM kom. bez zmian *MEM cells no change*
Wynik miareczkowania *Titration result*	$4440000=2*10^{3,50+0,32}CCID_{50}/ml$				$4000000=2*10^{1,50+0,32}CCID_{50}/ml$				$4000000=2*10^{1,50+0,32}CCID_{50}/ml$				

N – zarodki zakażone wirusem NDV – *embryos infected with NDV virus.*
N-A1 – zarodki zakażone wirusem NDV otrzymujące dodatek kompleksu antyoksydacyjnego A1 – *embryos infected with NDV virus and supplemented with A1 antioxidant complex.*
N-A2 – zarodki zakażone wirusem NDV otrzymujące dodatek kompleksu antyoksydacyjnego A2 – *embryos infected with NDV virus and supplemented with A2 antioxidant complex.*

It was found that the addition of A1 and A2 antioxidant complexes to NDV infected 8-day-old chicken embryos inhibits the multiplication of the pathogen. The above effects were visible after 48 h of incubation (Tab. 4). In the embryos infected with the NDV virus (N), cell lysis was still observed at a dilution of 10^{-3}, and the lysis of cells infected with the NDV in the presence of antioxidant complexes (N-A1 and N-A2) was visible only at a dilution of 10^{-1}.

The effect of inhibiting the multiplication of NDV in chicken embryos by the antioxidant complex was also expressed in the form of a lower viral titer (Tab. 4). The virus titer of the infected embryos was 100,000 CCID50 in 1 ml, whereas the viral titer of infected eggs in the presence of antioxidant complexes was significantly lower – 31,620 CCID50 in 1 ml. Similar results were recorded after 72 h and 120 h of incubation. 72- and 120-hour incubation confirmed the capability of the tested antioxidant complexes to inhibit the multiplication of the NDV. The virust titer in the allantoic fluid after 72 and 120 h of incubating the NDV-infected embryos was 3,162,000 CCID50/ml, whereas the virus titer in the allantoic fluid of the NDV-infected embryos in the presence of antioxidant complexes was 31,620 CCID50/ml. Confrontation and comparison of the results obtained from authors' own research are difficult due to the lack of similar studies in available domestic and foreign literature. However, as reported by Glickman et al. (1988), antioxidants may demonstrate the ability to inhibit viral development.

The embryos (N) which die due to NDV infection were reddened, covered with petechiae on the back and the limbs. Skulls have been found to have fairly large haemorrhages. The allantoic fluid cells collected from the infected embryo were clearly destroyed by the virus. The absorbed allergic liquid was cloudy. On the other hand, embryos infected with NDV (N-A1 and N-A2) in the presence of polyphenol complexes A1 and A2 did not show such intensive symptoms of infection. Microscopy comparison of the appearance of healthy chicken embryos (Z-A1 and Z-A2) incubated in the presence of polyphenol complexes only, with reference to to embryos infected with NDV virus incubated in the presence of polyphenol complexes (N-A1 and N-A2) demonstrated that the cells of chicken embryos incubated in the presence of polyphenol complexes only were damaged to a much lower extent, and quire contrarily – they increased their density. This is probably the result of a positive effect of the polyphenolic complex on the process of health cell multiplication. On the other hand, embryo cells infected with NDV in the presence of polyphenol complex (N-A1 and N-A2) showed significantly less signs of destruction than NDV-infected embryo cells (N).

The results concerning the formation of oxidoreductive status indicators in fertilized chicken embryos infected with the NDV in the presence of polyphenol complexes are provided in Tables 7 and 8.

A significant increase in the level of peroxidation products: LOOH (peroxides) and MDA (malloondialdehyde) was found in the yolk of NDV-infected eggs (N), compared to the yolks of healthy embryos (Z). This indicates the oxidative stress caused by viral infection. A significant increase in the activity of antioxidant enzymes: SOD (peroxide dismutase) and CAT (catalases), was also observed in egg yolks N. Significantly lower levels of oxidation products (LOOH and MDA) and lower enzyme (SOD and CAT) activity were observed in yolks infected with NDV in the presence of antioxidant complexes, compared to yolks N. Subbaiah et al. (2011), while conducting research on chickens infected with NDV virus, also noted the effect of increased oxidation (oxidative stress). The authors of the study observed a significant increase in the concentration of MDA in the brain and liver in chickens infected with the NDV virus in comparison to the control group. On the other hand, in the examined brain and liver tissues, the activity of enzymes: superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and S-glutathione transferase was significantly reduced in relation to the values of these enzymes in the control group. The same authors (Subbaiah et al., 2011), when administering vitamin E feed supplementation for the NDV-infected chickens noted decreased oxidation process intensity, which was expressed by a lower content of oxidation products (MDA), and a higher content of enzymatic antioxidants in the brain and liver tissues when compared to infected tissues. The histopathological examinations carried out by Subbaiah et al. (2011) have also shown that changes in hepatocytes, brain and

heart are observed as a result of NDV infection. However, such changes were not observed in chickens treated with vitamin E. The addition of vitamin stimulates the immune response of infected chickens by rapid growth of appropriate antibodies against the NDV virus and influences the immunological memory. This discovery forms the basis of the oxidative stress role in the pathogenesis of NDV and the therapeutic effect of antioxidants (Subbaiah et al., 2015).

Table 7. The level of oxidative parameters in fertilized yolk of eggs infected with NDV after 120 h incubation

Parameter	Z	Z-A1	Z-A2	N	N-A1	N-A2
LOOH nmol•mg^{-1}	4.23 b ± 0.33	4.13 b ± 0.37	4.01 b ± 0.15	7.59 a ± 1.98	3.43 c ± 0.57	3.47 c ± 0.17
MDA nmol•mg^{-1}	1.05 bc ± 0.33	0.72 c ± 0.08	0.99 c ± 0.22	2.40 a ± 0.15	1.59 b ± 0.06	1.62 b ± 0.03
SODIUM U•mg^{-1}	22.83 e ± 5.37	15.92 f ± 1.31	17.98 ef ± 8.85	626.7 a ± 65.4	138.4 d ± 28.9	226.8 c ± 28.5
CAT U•mg^{-1}	33.1 cd ± 6.82	22.4 e ± 7.37	27.85 d ± 6.85	69.47 a ± 5.79	53.29 b ± 1.89	48.82 c ± 7

Z – healthy embryos, Z-A1 – healthy embryos supplemented with A1 antioxidant complex, Z-A2 – healthy embryos supplemented with A2 antioxidant complex, N – embryos infected with NDV virus; N-A1 – embryos infected with NDV virus and supplemented with A1 antioxidant complex; N-A2 – embryos infected with NDV virus and supplemented with A2 antioxidant complex.
a, b, c… –P≤ 0,05 – means differ significantly at P≤ 0.05.

Table 8. Statistical analysis

Parameter	Effect of main factors			Interaction	
	A1	A2	N	A1xN	A2xN
LOOH	ns	ns	**	*	*
MDA	ns	ns	**	**	**
SODIUM	*	ns	*	*	*
CAT	*	**	*	**	*

A1 – antioxidant complex of coenzyme Q10, 98% RRR-d-alpha-tocopherol and 98% resveratrol in a 1: 1: 1 ratio.
A2 – antioxidant complex of coenzyme Q10, 98% RRR-d-alpha-tocopherol and 98% resveratrol in a 5: 3: 2 ratio.
N – infected with NDV virus; * significant at P≤0.01; ** significant at P≤0.05.

A significant increase in the level of peroxidation products: LOOH (peroxides) and MDA (malloondialdehyde) was found in the yolk of NDV-infected eggs (N), compared to the yolks of healthy embryos (Z). This indicates the oxidative stress caused by viral infection. In egg yolk N, there was also a significant increase in the activity of antioxidant enzymes: SOD (peroxide dismutase) and CAT (catalase). Significantly lower levels of oxidation products (LOOH and MDA) and lower enzyme activity (SOD and CAT) than in yolks N were recorded in yolks infected with NDV in the presence of antioxidant complexes. Subbaiah et al. (2011), when conducting research involving chickens infected with NDV, also noted the effect of increased oxidation (oxidative stress). The authors of the study observed a significant increase in the concentration of MDA in the brain and liver in chickens infected with the NDV virus in comparison to the control group. On the other hand, enzyme activity in the subject of the brain

and liver tissues: peroxide dysmutase, catalases, glutathione peroxidase, glutathione reductase and S-glutathione transferase has been reduced to the value of these enzymes in the control group. On the other hand, the same authors (Subbaiah et al., 2011), when administering vitamin E feed supplementation for the NDV-infected chickens noted decreased oxidation process intensity, which was expressed by a lower content of oxidation products (MDA), and a higher content of enzymatic antioxidants in the brain and liver tissues when compared to infected tissues. The histopathological examinations carried out by Subbaiah et al. (2011) have also shown that changes in hepatocytes, brain and heart are observed as a result of NDV infection. Such changes were not observed in chickens provided with vitamin E. Vitamin supplementation stimulates the immune response of infected chickens through the rapid growth of appropriate antibodies against the NDV virus and affects immune memory. This discovery forms the basis of the oxidative stress role in the pathogenesis of NDV and the therapeutic effect of antioxidants (Subbaiah et al., 2015).

Studies were also carried out on NDV-infected chickens provided with either Q or resveratrol. The results of these studies have shown that these compounds significantly improve the function of the immune system and reduce the symptoms of NDV infection (Mezes & Bulogh, 2011). Due to the fact that the studied topic has not been the subject of many authors' researches, comparing the results of own studies is difficult.

Conclusions

The studies have shown that the tested antioxidant complex has the ability to inhibit the proliferation of NDV virus in chicken embryos. Moreover, the pathology of NDV infection in chicken embryos is accompanied by strong oxidative stress, which results in an increase in the final peroxidation product (MDA) content, and the activity of antioxidant enzymes (SOD and CAT). However, it was found that this stress can be reduced by the addition of an antioxidant complex (coenzyme Q10, + RRR-d-alpha-tocopherol + resveratrol).

The use of an antioxidant complex (coenzyme Q10 + RRR-d-alpha-tocopherol + resveratrol), composed in a ratio of 1: 1: 1 and 5: 3: 2 therefore seems promising as a supplement used in prevention or supporting the treatment of chicken embryos infected with NDV. Nevertheless, the studies conducted in *in vitro* settings only required continuation and confiming the results in *in vivo* studies.

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IN OVO STUDY ON THE EFFECT OF NATURAL ANTIOXIDANTS ON NDV PROLIFERATION IN CHICKEN EMBRYOS

Summary

The aim of the study was to evaluate the effects of coenzyme Q10, resveratrol and RRR-d-alpha-tocopherol used as an antioxidant complex on NDV proliferation in chicken embryos and the antioxidant status of yolk in incubated eggs.

The *in vitro* study was conducted on 240 fertilized eggs which were divided into 3 equals groups. The first group did not receive any experimental supplement. Groups 2 and 3 received 100 µl antioxidant complex conta- ining coenzyme Q10, RRR-d-alpha-tocopherol and resveratrol respectively in a 1: 1: 1 (A1) or 5: 3: 2 (A2) ratio. In addition to the allantoic fluid, half of the embryos from each group were injected with 100 µl NDV. After 24, 48 or 72 h incubation, the NDV proliferation rate in chicken embryos was evaluated and the virus profile in the allantoic fluid was determined. The yolks, which were incubated for 120 h, contained lipid hyperoxides, malondialdehyde, superoxide dismutase and catalase.

The results indicate that the tested antioxidant complex has the ability to inhibit NDV proliferation in chicken embryos. The pathology of NDV infection is accompanied by oxidative stress, resulting in increased con- tent of MDA and SOD and CAT activity. However, it has been found that stress may be reduced by the addition of the antioxidant complex.

Key words: NDV, chickens, embryo, antioxidants