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ANTIOXIDANT ACTIVITY OF COMBINED FEED INGREDIENTS AND ITS IMPACT ON THE ANTIOXIDANT STATUS, METABOLIC INDICATORS, AND PRODUCTIVITY OF LAYING HENS

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Aim. To study how variations in the antioxidant activity of diets formed exclusively from basic ingredients with different natural antioxidant potential affect the antioxidant status, oxidative stress indicators, biochemical and hematological blood indices, as well as the main productivity indicators of laying hens. **Methods.** The experiment, conducted from December 2023 to June 2024 at the experimental farm of the State Poultry Research Station of the Institute of Animal Husbandry of the NAAS (Kharkiv region), involved the use of laying hens of the Birkivska barvysta breed (2 similar groups of 40 birds each), which were kept in group cages. The birds were fed isonutrient diets with the same levels of crude protein (17%) and metabolizable energy (270 kcal/100 g), but contrasting in terms of total antioxidant activity: 12.55 and 23.34 mg/g as recalculated per ascorbic acid. The antioxidant activity of the feeds was modeled based on the actual values of the antioxidant activity of the ingredients (corn, wheat, bran, soybean, and sunflower meal, yeast), which were previously determined by the authors' titrimetric method (Kotyk et al., 2023), expressed as recalculated per ascorbic acid (mg/g). The duration of feeding was 30 weeks. At the end of the experiment, the following indicators of antioxidant status and oxidative stress were determined in hens using common methods: the antioxidant activity of serum, egg white and yolk, the activity of superoxide dismutase and catalase, the level of malondialdehyde in serum; metabolic and hematological indices — total protein, total lipids, cholesterol, alanine aminotransferase, aspartate aminotransferase, hemoglobin, erythrocytes, leukocytes; the markers of non-specific resistance — phagocytic activity of neutrophils and bactericidal activity of serum and egg white regarding Escherichia coli. The antioxidant activity of excrements was additionally analyzed. The productivity was assessed by egg laying intensity, average egg weight, and survival of the hens; the incubation properties were evaluated by egg hatchability. **Results.** Higher antioxidant activity of the diet was accompanied by a significant improvement in antioxidant indicators: the antioxidant activity of serum increased by 12.1%, that of egg white by 19.3%, of yolk by 32.0%, superoxide dismutase and catalase activity by 7.4% and 5.5%, respectively, while the level of malondialdehyde in serum decreased by 11.0%. At the metabolic level, an increase in total blood serum protein (by 12.0%) was detected with a simultaneous decrease in total lipids (by 17.4%) and cholesterol (by 14.5%) without changes in the activity of liver enzymes; hematological indices (hemoglobin, erythrocytes, leukocytes) remained within the physiological norm and did not differ between groups. Among the indicators of non-specific resistance, an increase in the bactericidal activity of egg white (by 3.3%) was noted with unchanged phagocytic activity of neutrophils and bactericidal activity of serum. The antioxidant activity of excrements was higher for the birds that consumed a diet with higher antioxidant activity. The productivity indicators did not differ significantly (egg-laying intensity — 52.8% and 52.6%; average egg weight — 58.8 and 59.0 g; survival — 95.6% and 100.0%), and egg hatchability was 90.7% and 91.8% in groups with lower and higher antioxidant activity of the diet, respectively.

Conclusions. The optimal combination of ingredients containing natural antioxidants provides an increase in the antioxidant status of laying hens and egg components, a decrease in the intensity of lipid peroxidation, and an improvement in individual metabolic indices without any deterioration in the productivity and incubation indicators. This approach is an effective strategy for increasing the antioxidant protection of both hens and products obtained from them, which is especially important during periods of higher oxidative stress (heat stress, vaccinations, technological stressors).

Key words: antioxidant activity of feed, natural antioxidants, hens, antioxidant protection, oxidative stress, non-specific resistance, egg-laying productivity.

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INTRODUCTION

In modern poultry breeding, significant attention is paid to reducing the use of synthetic additives, and instead, the main efforts are aimed at the natural functionalization of feed, in particular, by increasing its antioxidant potential (Mahfuz et al., 2021). Synthetic antioxidants, traditionally added to feed to prevent fat rancidity, are under increasing regulatory and consumer pressure due to safety issues (Xiao et al., 2024). Therefore, a current trend is a search for natural alternatives — using the antioxidant properties of the feed ingredients instead of artificial additives. In this context, feed is considered not only as a source of nutrients, but also as a carrier of biologically active compounds that contribute to the antioxidant protection of animals. This approach is reflected in the assessment of the antioxidant activity (AOA) of raw materials: the quality of grain and its processed products is now characterized not only by nutritional indicators, but also by the level of AOA, which determines the ability of the feed matrix to neutralize prooxidants and stabilize biomolecules in feed and livestock products.

The AOA of grain components varies significantly depending on the species, variety, genotype, and cultivation conditions of crops; a key role in the antioxidant potential is played by free and bound phenolic compounds released in the digestive tract and collectively forming AOA (Fardet et al., 2008; Horvat et al., 2020; Suchowilska et al., 2020). Classical comparison demonstrates that corn generally has higher concentrations of polyphenols and a higher total AOA than wheat, oats, and rice (Adom and Liu RH. 2002). On the other hand, modern studies show that the profile of phenolic compounds and the value of AOA can vary significantly under the impact of agrotechnical factors and genetic specificities of the crop, for example, significant genotypic fluctuations in antioxidant potential were found in sorghum (Elsafy et al., 2024; Collins et al., 2024). High but variable AOA is also notable for grain processing by-products (meal, DDGS), which emphasizes the importance of origin and processing technology (Shin et al., 2018). Our previous studies confirm a wide range of AOA of basic feed ingredients: minimum values in ascorbic acid equivalent (AAE) were found in corn, wheat, rapeseed, respectively — 10.6; 10.2; 10.9 mg/g, improbably higher values for oats (12.0 mg/g AAE) and millet (12.6 mg/g AAE), while maximum values were recorded for sorghum (48 mg/g AAE) and especially for sunflower meal and cake — 57 mg/g AAE (Kotyk et al., 2023). Such variability creates the prerequisites for targeted selection of raw materials in order to form diets with different total levels of natural antioxidants.

For poultry, the relevance of the AOA diet is conditioned by the role of oxidative stress in the etiology of metabolic and productive disorders. Oxidative stress occurs when there is an imbalance between pro- and antioxidants in favor of the former, which leads to peroxidation of lipids, proteins, and nucleic acids and damage to cellular structures (Surai et al., 2019). In contrast, increasing the antioxidant potential of feed is an effective nutritional strategy to attenuate oxidative processes in the body: numerous studies demonstrated a decrease in the intensity of lipid peroxidation, improvement of antioxidant status, and product quality by feeding feed enriched with antioxidants (Shahidi and Ambigaipalan, 2015; Ouyang et al., 2016). In particular, the addition of certain natural antioxidants (vitamins, minerals, flavonoids, and herbal extracts) to the diet has a positive effect on the antioxidant protection and immunity of animals and poultry (Mahfuz et al., 2021). At the same time, more and more attention is paid to the search for ways to increase the intrinsic AOA of feed without the use of synthetic substances. Most available publications highlight either direct supplementation with isolated antioxidant micronutrients and plant extracts or the use of commercial synthetic antioxidants in

feed (Pitino et al., 2021). However, the potential of basic cereal components as a source of «built-in» antioxidants to formulate diets, aimed at targeting total dietary AOA, is yet to be studied.

Alternative approaches to enhancing antioxidant properties of feed envisage the use of biotechnological and technological solutions, such as fermentation, extrusion, or the use of enzyme combinations. In particular, the fermentation of feed ingredients, such as soybean products, can reduce the content of anti-nutritional factors and significantly increase the AOA of the raw material due to the accumulation of phenolic compounds and amino acids with antioxidant properties (Adebo and Medina-Meza, 2020). The extrusion technology changes the structure of the grain matrix and the availability of bioactive compounds, allowing, under certain conditions, to maintain or even enhance the anti-radical activity of the ingredients (Ramos-Enríquez et al., 2018). Additionally, the use of enzyme preparations (xylanases, cellulases, esterases) promotes the release of bound polyphenols, powerful natural antioxidants (Aliaga et al., 2018). The complex application of these approaches demonstrates the potential to increase the antioxidant value of feed without the use of chemical antioxidants (Kasote et al., 2021).

Our study is focused on a new approach to increasing the antioxidant status of poultry — the variation of the antioxidant activity of only basic diet ingredients without the addition of synthetic or specialized natural antioxidants. It was necessary to assess whether this difference in the natural potential of standard grain components can affect the physiological state of poultry.

The aim of the study is to determine how natural variation in AOA of the diets affects the antioxidant status, oxidative stress indicators of laying hens, biochemical and hematological blood parameters, and the productivity of hens.

MATERIALS AND METHODS

The study was conducted at the Department of quality and safety assessment of poultry feed and products of the State Poultry Research Station of the Institute of Animal Husbandry of the National Academy of Sciences of Ukraine (SPRS of IAH, the NAAS) and in the conditions of the experimental farm of the institution (Kharkiv region) from December 2023 to June 2024. The objects of the study were laying hens of the Birkivska barvysta breed. At the age of 18 weeks, 80 hens were randomly distributed into two similar groups of 40 birds each. The birds were kept in group cages, 9 birds per cage (8 hens and 1 rooster), under standard technological conditions of microclimate, illumination, and feeding. The testing lasted 30 weeks.

Given the known variability of AOA in different components of combined feed, the analytical assessment of the actual ingredients intended for use in the experiment (corn, wheat, bran, soybean and sunflower meal, yeast) was carried out in terms of antioxidant activity before forming the diets. To evaluate the total content of compounds with restoring properties, a titrimetric method, developed by the authors, based on the interaction of an aqueous sample extract with potassium permanganate in an acidic environment, was used (Kotyk et al., 2023). For the reproducibility of the titration, a titration solution of the following composition was used: 0.79 g $KMnO_4$ and 2.35 g H_2SO_4 per 100 ml of water. After preparing the aqueous extract of each sample, titration was carried out with a 0.05 H solution of potassium permanganate, $KMnO_4$, until discoloration; a standard solution of ascorbic acid was used as a reference. The antioxidant activity was expressed as recalculated per ascorbic acid (AAE, mg/g) according to the formula:

$$X = \frac{C_{AA} \cdot V_{AAT} \cdot V_{RS}}{V_{RST} \cdot m},$$

where X — the total content of compounds with AAE in the sample, mg/g; C_{AA} — the concentration of the ascorbic acid solution, mg/ml; V_{AAT} — the volume of the ascorbic acid solution, spent for the titration of 1 ml of 0.05 H $KMnO_4$, ml; V_{RS} — the volume of the studied solution, ml; V_{RST} — the volume of the studied solution, spent for the titration of 1 ml $KMnO_4$, ml; m — mass of the weighed quantity of the sample, g.

The obtained AAE values of the ingredients, reflecting the actual characteristics of specific batches, were used as a basis for formulations of two isonutrient diets with a distinct contrast in total AAE under fixed levels of crude protein and metabolizable energy, which corresponded to the norms for laying hens (crude protein content — 17%, metabolizable energy — 270 kcal/100 g). After mixing each combined feed, samples were taken, and the total AAE was re-determined using the method described above; these analyzed AAE levels were considered as an influencing factor in the experiment.

Thus, hens of the two groups were fed with feeds, identical in nutritional value and contrasting in antioxidant activity. The norms of hen feeding were defined according to recommendations for keeping the parent flock (Bratyshko, 2013), and water was provided without restrictions.

Blood for biochemical and hematological analyses was collected from the wing vein of 10 randomly selected hens from each group. Immediately after collection, the hemoglobin concentration (g/l) in whole blood was measured using the hemoglobin cyanide method, and the number of erythrocytes and leukocytes was measured using the melange method (Levchenko et al., 2010). Serum was obtained by centrifugation at 3,000 rpm for 15 minutes, then stored at +4°C and analyzed for 24 h. Serum was analyzed for total protein, total lipids, and cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activity on a BS-3000M semi-automatic analyzer (Sinnowa, People's Republic of China) using Bio-Systems reagents (Spain). The content of malondialdehyde (MDA) in serum was determined by the TBAR method with spectrophotometric detection at 532 nm (Ohkawa et al., 1979). Superoxide dismutase (SOD) and catalase (CAT) activity were assessed spectrophotometrically according to generally accepted methods (Stępniewska et al., 2016). The antioxidant activity of serum, excrements, egg white, and yolk was assessed by the titrimetric method and expressed in terms of AAE (mg/g), as described above. The bactericidal activity of blood serum and egg white against Escherichia coli was determined by bacteriological method with quantitative assessment of the percentage of inactivation relative to the control suspension. The phagocytic activity of neutrophils was assessed microscopically (the proportion of phagocytic cells, %). Eggs for the study were randomly selected from the daily collection — 10 eggs from each group. The samples of fresh excrements were taken for the investigation from the cage trays, combining samples from all cages within the group into composite samples; the samples were immediately cooled and stored at –20°C until analysis. Before the analysis, thawed samples were homogenized; a weighed portion (approximately 1 g) was extracted with distilled water (ratio 1:10) at 20–22°C with intensive shaking for 30 min, centrifuged for 10 min (3,000 rpm), and filtered.

During the experiment, the number of laid eggs and deaths was recorded daily, and the weight of eggs was measured separately for all eggs collected once a week. Based on the results of the experiment, the following productivity indicators were calculated: laying intensity, average egg weight per period, and stock survival. Laying intensity (%) was calculated as the total number of eggs collected during the observation period divided by the total number of live hens for each day of the period, expressed as a percentage. The average egg weight (g) was determined as the total weight of weighed eggs divided by the number of weighed eggs. Stock survival (%) was calculated as the proportion of stock that survived during the period multiplied by 100.

At the end of the experiment, eggs were incubated to assess their hatchability. The eggs from each group were collected for 5 consecutive days to complete a batch of at least 80 pieces per group. If the target volume was not reached in 5 days, the collection was continued to accumulate the minimum number. If there was a surplus, a random sampling was carried out to the target quantity, taking into account quality criteria (intact, clean shell without microcracks; weight of 55–62 g), ensuring an approximately proportional representation of eggs collected on different days. Before incubation, eggs were stored at 12–15°C and 70–80% relative humidity, blunt end up. The incubation was carried out in a laboratory incubator with forced ventilation with ILU-F-0.3, separate trays for each group, under the same standard conditions adopted for chicken eggs; quality control was performed by ovoscopy before incubation and before transfer to the hatching unit. Hatchability (%) was determined as the proportion of live day-old chicks and the number of fertilized eggs.

The statistical analysis was performed using STATISTICA software (version 8.0, StatSoft Inc., USA). The data were tested for normal distribution using the Shapiro-Wilk test. The paired Student's *t*-test was used to assess differences between experimental groups. If normality conditions were not met, the Mann-Whitney U-test was used. The results are presented as mean ± standard error of the mean value (SEM). The differences were considered statistically significant at $p \leq 0.05$. The exact significance levels ($p < 0.05$; $p < 0.01$; $p < 0.001$) are presented in the tables of results, depending on the magnitude of the detected effect (Woolson and Clarke, 2011).

RESULTS

The determination of antioxidant activity of individual feed ingredients demonstrated a significant

variability of this indicator (**Table 1**). The lowest values were observed in corn (7.0 mg AAE/g), the highest ones — in wheat (9.3 mg AAE/g) and bran (11.4 mg AAE/g). Relatively high antioxidant activity was notable for soybean meal (17.5 mg AAE/g) and yeast (16.4 mg AAE/g). The maximum values were found in sunflower meal (70.0 mg AAE/g).

Table 1. Antioxidant activity of some feed ingredients

Feed ingredients	Antioxidant activity (mg AAE/g)
Corn	7.0
Wheat	9.3
Bran	11.4
Yeast	16.4
Soybean meal	17.5
Sunflower meal	70.0

Note: AAE — ascorbic acid equivalent.

Based on these data, two feed formulations were made, which had the same level of crude protein (17%) and metabolizable energy (270 kcal/100 g), standard for laying hens of the parent flock of egg-laying hens, but differed in the level of antioxidant activity (**Table 2**). In the first diet, the total AAE was 12.55 mg AAE/g, in the second one — 23.34 mg AAE/g.

The studies of antioxidant status and non-specific resistance indicators are presented in **Table 3**. The birds of the second group had significantly higher values of antioxidant activity of blood serum (24.83 vs. 22.14 mg AAE/g, $p < 0.001$), egg white (33.10 vs. 27.75 mg AAE/g, $p < 0.001$), and yolk (8.45 vs. 6.40 mg AAE/g, $p < 0.001$). The AAE of the excrements of hens from groups 1 and 2 during the experiment was within 8.8 and 13.5 mg AAE/g, respectively, i.e., for both groups, a higher level of AAE in the feed was clearly associated with a higher level in the excrements.

At the same time, the level of malondialdehyde in the blood serum was significantly lower in the second group (152.6 versus 171.5 μmol/ml, $p < 0.05$). The activity of superoxide dismutase and catalase in hens of the second group was also significantly higher ($p < 0.05$). The indicators of non-specific resistance (phagocytic activity of neutrophils, bactericidal activity of blood serum) did not differ significantly between the groups ($p > 0.05$). At the same time, the bactericidal activity of egg white for hens of the second group was significantly higher (83.8 versus 81.1%, $p < 0.01$).

The comparison of hematological and biochemical blood parameters of chickens showed that both groups were characterized by values within the physiological norm (**Table 4**). Hemoglobin concentration, erythrocyte, and leukocyte counts did not differ significantly between groups ($p > 0.05$). In terms of biochemical

Table 2. Composition and antioxidant activity of the feed under investigation

Feed ingredients	Group 1		Group 2	
	%	AOA, mg AAE/g	%	AOA, mg AAE/g
Corn	10.00	0.70	40.48	2.83
Wheat	49.78	4.63	8.00	0.74
Bran	5.00	0.57	5.00	0.57
Soybean meal	18.00	3.15	5.00	0.88
Sunflower meal	5.00	3.50	25.00	17.50
Bone meal	1.40	—	0.80	—
Meat-and-bone meal	2.00	—	2.00	—
Limestone meal	8.00	—	8.00	—
Yeast	0.00	—	5.00	0.82
Mixture of vitamins	0.04	—	0.04	—
Mixture of microelements	0.06	—	0.06	—
Lysine	0.17	—	0.17	—
Methionine	0.25	—	0.15	—
Salt	0.30	—	0.30	—
Total	100.00	12.55	100.00	23.34

Note: AOA — antioxidant activity, AAE — ascorbic acid equivalent.

Table 3. The indices of antioxidant status and non-specific resistance of hens, fed with feed with different AOA levels

Indices	Group 1	Group 2	P-value
Antioxidant status			
AOA of blood serum, mg AAE/g	22.14 ± 0.46	24.83 ± 0.28***	<0.001
AOA of egg white, mg AAE/g	27.75 ± 0.51	33.10 ± 0.89***	<0.001
AOA of yolk, mg AAE/g	6.40 ± 0.34	8.45 ± 0.23***	<0.001
AOA of excrements, mg AAE/g	8.8	13.5	—
Indices of oxidative stress and enzymatic protection			
MDA in blood serum, mcmol/ml	171.5 ± 3.87*	152.6 ± 6.47	0.024
Superoxide dismutase, mmol/min/g of protein	4.7 ± 0.11	5.1 ± 0.06*	0.014
Catalase, mmol/min/g of protein	6.8 ± 0.11	7.2 ± 0.03**	0.008
Indices of non-specific resistance			
Phagocytic activity, %	31.6 ± 2.08	32.9 ± 1.16	0.594
Bactericide activity of blood serum, %	55.3 ± 1.47	56.9 ± 0.90	0.368
Bactericide activity of egg white, %	81.1 ± 0.67	83.8 ± 0.23**	0.003

Notes: The data are presented as the mean values ± SEM ($n = 10$). * — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$. AOA — antioxidant activity, AAE — ascorbic acid equivalent.

Table 4. The state of metabolism and the organism functioning of hens, fed with feed with different AOA levels

Indices	Group 1	Group 2	P-value
Hematological indices			
Hemoglobin, g/l	96.03 ± 0.27	95.97 ± 0.37	0.897
Erythrocytes, 109/ml	2.77 ± 0.51	2.85 ± 0.38	0.901
Leukocytes, 106/ml	24.2 ± 0.80	24.6 ± 0.54	0.684
Biochemical indices			
Total protein, g/l	45.20 ± 0.5	50.63 ± 0.75***	<0.001
Total lipids, g/l	5.56 ± 0.24**	4.59 ± 0.10	0.003
Cholesterol, mmol/l	2.14 ± 0.06***	1.83 ± 0.02	<0.001
ALT, units/l	9.45 ± 0.06	9.70 ± 0.23	0.317
AST, units/l	121.22 ± 2.04	121.03 ± 0.88	0.933

Notes: The data are presented as the mean values ± SEM ($n = 10$). ** — $p < 0.01$; *** — $p < 0.001$.

parameters, a significantly higher level of total protein was noted in hens of the second group (50.63±0.75 g/l versus 45.20 ± 0.5 g/l, $p < 0.001$). The concentration of total lipids and cholesterol in the second group was significantly lower ($p < 0.01$), while the activity of ALT and AST remained at the same level compared to the first group ($p > 0.05$).

The main productivity indicators of both groups were practically the same (**Table 5**). The intensity of egg laying during the observation period was 52.8% and 52.6%, respectively, for the group with low and high antioxidant activity of the feed, the average egg weight was 58.8 g and 59.0 g. The survival rate of the birds in the groups was high — 95.6% and 100%, with an advantage in the second group. According to the results of egg incubation, the hatchability in the group that consumed the feed with higher AAE was 91.8% versus 90.7% in the first group.

The formation of combined feed with different levels of antioxidant activity made it possible to identify certain differences in the metabolic state and organism functioning of laying hens. An increased AOA level in the diet was accompanied by an increase in the protein concentration in the blood serum, a decrease in the level of lipids and cholesterol, an increase in the activity of antioxidant enzymes, and a decrease in the MDA content. Higher values of the antioxidant activity of blood serum, egg white,

Table 5. The productivity of hens, fed with feed with different AOA levels

Indices	Group 1	Group 2
Egg production intensity, %	52.8	52.6
Average egg weight, g	58.8 ± 0.32	59.0 ± 0.40
Livability, %	95.6	100.0
Hatchability of eggs, %	90.7	91.8

and yolk, and an increase in the bactericidal activity of egg white were also noted. At the same time, the productivity and reproductive indicators of hens remained stable, which demonstrated the absence of a negative impact of different levels of antioxidant activity of feed.

DISCUSSION

The increase in the antioxidant activity of the diet at constant levels of crude protein and metabolizable energy was accompanied by an improvement in the indicators of the antioxidant status of hens (an increase in AOA of blood serum, egg white, and yolk), a decrease in the lipid peroxidation marker (MDA) and an increase in the activity of antioxidant enzymes (SOD, catalase). At the same time, productivity indicators (egg laying intensity, egg weight, survival, hatchability) remained stable between groups. Taken together, this indicates that the modification of the formulation in terms of AOA can improve the physiological and biochemical state of the poultry without negative consequences for productivity.

The data, obtained in our experiments, confirmed the significant variability of AOA among basic feed ingredients (cereals, meal, bran, yeast), which is in agreement with the idea about the leading role of bound and free polyphenols and associated phytonutrients in the formation of the antioxidant potential of grain and its processed products (Adom and Liu, 2002; Masisi et al., 2016). The literature data indicate differences between grain species (in particular, high values in corn compared to wheat, oats, and rice), as well as the influence of genotype and environmental factors on the content of phenolic compounds and total AOA, which is well demonstrated for sorghum (Pontieri et al., 2021; Xu et al., 2021; Collins et al., 2024). At the same time, the significant variability of AOA in grain processing products and meal in different studies emphasizes the role of origin and production technology (Shin et al., 2018; Rezaee et al., 2021). Due to differences in determination methods (DPPH/ABTS/FRAP), extraction agents, and units of expression (the equivalents of gallic, ascorbic acids, or vitamin C), a direct numerical comparison between studies is not always accurate, but most importantly, the same «rank» of ingredients according to AOA is maintained. This is a valid basis for «adjustment» of formulations to the target AOA level of the diet by selecting the proportions of basic ingredients within nutritional restrictions.

We obtained two isonutrient diets with contrasting AOA (12.55 and 23.34 mg/g AAE) due to a successful combination of ingredients and enrichment of feed with natural antioxidants, inherent in grain and other raw materials. This strategy differs from approaches, based on synthetic antioxidants or micronutrients, and is consistent with the «clean label» trend. Most commercial feeds contain synthetic antioxidants, such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT), to reduce oxidation. However, these substances are known for their safety and toxicity issues, and their use is limited by legislation (Nimalaratne and Wu, 2015). In their turn, Surai and Kochish (2019) highlighted the modulation of antioxidant capacity mainly via the addition of specific antioxidants to the poultry diet — isolated micronutrients such as vitamin E, selenium, and carotenoids. In our study, we showed that a similar result can be achieved by forming a high antioxidant potential of the feed «inside» the basic formulation, not changing its nutritional profile and not introducing synthetic preservatives. This is important both from the standpoint of regulatory restrictions on synthetic agents and from the point of view of consumer perception.

The resulting diets with contrasting AOA were naturally reflected in systemic markers of antioxidant protection in poultry. The increase in AOA of the feed was accompanied by a significant ($p < 0.05$) increase in AOA of blood serum (by 12.1%) and egg components (protein by 19.3%, yolk by 32%). At the same time, there was an increase in the enzymatic link — the activity of superoxide dismutase increased by 7.4%, that of catalase — by 5.5% (both $p < 0.05$) in the blood serum of birds of the second group,

against the background of a decrease in the level of MDA by 11% ($p < 0.05$). The sequence of processes is interpreted as an increased intake of exogenous antioxidants from the diet, support and activation of the enzymatic link of antioxidant defense, and, as a result, inhibition of lipid peroxidation (LPO). This mechanistic chain corresponds to modern ideas about the integral effect of natural antioxidants in poultry feed and the data of studies where natural antioxidants and individual micronutrients increased the antioxidant potential of the egg and reduced the intensity of LPO, in particular during storage (Al-Khalaifah et al., 2024; Amevor et al., 2021; Liu et al., 2023; Vlaicu et al., 2021; Vlaicu and Untea, 2024).

It is likely that higher AOA values in egg white and yolk in the group with high AOA of the diet may indicate an improved intake or deposition of antioxidant compounds in the egg. Additionally, it is significant that the AOA of the excrements, as an indicator of the transportation of antioxidants through the gastrointestinal tract, was higher in the group with high AOA of the feed, reflecting both the input level of the diet and the excretion of some ingredient antioxidants and/or their metabolites. At the same time, the observed AOA levels of the yolks (6.4–8.4 mg/g) in the ascorbic acid equivalent considerably exceed the values given by Muhammad A.I. et al. (2021) — 0.82 mg/g by the phosphomolybdenum method and 1.73 mg/g by FRAP (Ferric Reducing Antioxidant Power Assay) in the gallic acid equivalent (GAE). Such discrepancies are most likely due to different methods of determination and equivalents of expression, but the direction and interrelation of the effects remain constant.

Non-specific resistance indicators in our study (neutrophil phagocytic activity and serum bactericidal activity) generally remained similar between groups, but the bactericidal activity of egg white against *Escherichia coli* was 3.3% higher ($p < 0.05$) in the group with the higher AOA of the diet. Given the increase in AOA of the white and yolk, this seems logical and is consistent with the idea of the deposition of some dietary antioxidants and associated protective proteins in the egg (Vlaicu et al., 2021). Given that the bactericidal activity of egg white is considered an integral indicator of non-specific resistance and an important factor in embryo protection during incubation, this result may have practical significance for reducing the microbial load during incubation and early embryogenesis. Since the intestinal microbiota and metabolites of phenolic compounds were not profiled, their contribution to the change in the bactericidal properties of the protein, as well as the causal relationship between egg AOA and bactericidal activity, requires a targeted study.

Favorable changes in antioxidant indicators were consistently reflected in the metabolic profile of the blood: a higher concentration of total protein was combined with a decrease in total lipids and cholesterol in the absence of changes in transaminase activity and stable hematological parameters. In the higher AOA diet group, total protein increased by 12.0% ($p < 0.001$), while total lipids and cholesterol decreased by 17.4% and 14.5%, respectively ($p \leq 0.01$). These protein, lipid, and cholesterol profiles indicate a shift in liver metabolism under conditions of the diet with higher AOA and the absence of cytolytic events, which is consistent with better resistance to oxidative stress (Shahidi and Ambigaipalan, 2015; Surai and Kochish, 2019). Serum protein content in both groups was within the reference values for laying hens, which is supported by modern sources with reference intervals (Board et al., 2018; Sauer et al., 2019), and further demonstrated the absence of systemic stress or inflammatory response. The significant decrease in the content of lipids and cholesterol in the blood serum of laying hens of the second group compared to the first one ($p < 0.05$) may be explained by the fact that with a higher AOA diet, the liver uses cholesterol for the synthesis of bile acids more actively and better regulates lipid metabolism against the background of a lower oxidative load, thus, its concentration in the blood decreases (Ding et al., 2023).

The hematological parameters (mean hemoglobin, erythrocyte, and leukocyte counts) remained within normal limits and did not differ between groups, which is evidence of normal erythropoiesis and indirect confirmation of the absence of systemic stress or inflammatory response and is in good agreement with the biochemical profile (Sauer et al., 2019).

Importantly, the evident antioxidant and metabolic changes were not accompanied by a deterioration in production indicators. Egg laying intensity and egg weight practically did not change, the survival of the population was high in both groups, and egg hatchability tended to be higher in case of the diet with higher AOA. This is consistent with the communications that «natural» strengthening of the antioxidant background of diets via the selection of plant components can improve the antioxidant stability of

products without compromising productivity (Vlaicu et al., 2021; Surai and Kochish, 2019). The data for pigs also suggest that the high natural antioxidant potential of certain feed ingredients (e.g., treated corn grain with increased sulfur content) may even offset the need for increased doses of vitamin E under oxidative challenges without loss of productivity (Song et al., 2013; Shurson, 2018), which is consistent with our concept of increasing the AOA of the diet via natural properties of the ingredients; this is further supported by comparisons of the antioxidant capacity of DDGS and corn grain (Shin et al., 2018).

Considering the observed variability in AOA of ingredients, it is advisable to include the operational assessment of AOA of raw material batches in routine quality control and use it to «fine-tune» formulations to target AOA values of the diet. This approach makes it possible to maintain the antioxidant status of laying birds without changing basic nutritional parameters and without the risk of productivity deterioration, which is important both under standard conditions and during periods of increased oxidative stress (heat stress, vaccinations, technological stressors).

CONCLUSIONS

The formation of isonutrient diets, contrasting in terms of the natural antioxidant activity of the basic ingredients, was accompanied in the studies by an increase in the antioxidant activity of blood serum, egg white and yolk in hens against the background of a decrease in the intensity of lipid peroxidation (MDA) and an increase in the activity of key enzymes of antioxidant protection (SOD, catalase). At the metabolic level, this was accompanied by a higher concentration of total protein with a simultaneous decrease in total lipids and cholesterol in serum without any signs of cytolytic changes (ALT, AST) and without deviations of hematological indices from the physiological norm.

The optimal combination of ingredients containing natural antioxidants provides an increase in the antioxidant status of laying hens and egg components, a decrease in the intensity of lipid peroxidation, and an improvement in individual metabolic indices without any deterioration in the productivity and incubation indicators. This approach is an effective strategy for increasing antioxidant protection of both animals and products derived from them, which is especially important during periods of increased oxidative stress (heat stress, vaccinations, technological stressors).

ADDITIONAL INFORMATION

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Conflict of interests. The authors declare the absence of any conflicts of interests.

Adherence to ethical principles. All the manipulations with animals were carried out in accordance with the current requirements for humane treatment (provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 2005), Council of Europe Directive No. 2010/63/EU, Law of Ukraine «On Protection of Animals from Cruelty» No. 3447-IV (as amended), as well as the general ethical principles of experiments involving animals, adopted by the VII National Congress on Bioethics (Kyiv, 2019). The ethical approval of the experiment was confirmed by the Bioethics Commission of the SPRS, the IAH of the National Academy of Sciences of Ukraine (protocol No. 1 dated February 8, 2023).

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АНТИОКСИДАНТНА АКТИВНІСТЬ ІНГРЕДІЄНТІВ КОМБІКОРМУ ТА ЇЇ ВПЛИВ НА АНТИОКСИДАНТНИЙ СТАТУС, МЕТАБОЛІЧНІ ПОКАЗНИКИ Й ПРОДУКТИВНІСТЬ ЯЄЧНИХ КУРЕЙ

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Мета. Дослідити як варіювання антиоксидантної активності раціонів, сформованих виключно з базових інгредієнтів із різним природним антиоксидантним потенціалом, впливає на антиоксидантний статус, показники оксидативного стресу, біохімічні та гематологічні індикатори крові, а також на основні показники продуктивності яєчних курей. **Методи.** У досліді, проведеному з грудня 2023 по червень 2024 року в умовах експериментальної ферми Державної дослідної станції птахівництва Інституту тваринництва НААН (Харківська область), використано яєчних курей породи Бірківська барвиста (2 групи-аналоги по 40 голів), яких утримували в групових кліткових батареях. Птахам згодовували ізонутрієнтні раціони з однаковими рівнями сирого протеїну (17%) й обмінної енергії (270 ккал/100 г), але контрастні за сумарною антиоксидантною активністю: 12,55 та 23,34 мг/г у перерахунку на аскорбінову кислоту. Антиоксидантну активність кормів моделювали на підставі фактичних значень антиоксидантної активності інгредієнтів (кукурудза, пшениця, висівки, соєва та соняшникова макухи, дріжджі), яку попередньо визначали авторським титрометричним методом (Kotyk et al., 2023) з вираженням у перерахунку на аскорбінову кислоту (мг/г). Тривалість згодовування — 30 тижнів. Наприкінці досліду у курей загальновизнаними методами визначали показники антиоксидантного статусу та оксидативного стресу: антиоксидантну активність сироватки, яєчного білка та жовтка, активність супероксиддисмутази і каталази, рівень малонового діальдегіду у сироватці; метаболічні та гематологічні індикатори — загальний білок, загальні ліпіди, холестерин, аланінамінотрансферазу,

аспартатамінотрансферазу, гемоглобін, еритроцити, лейкоцити; маркери неспецифічної резистентності — фагоцитарну активність нейтрофілів і бактерицидну активність сироватки та яєчного білка щодо *Escherichia coli*. Додатково аналізували антиоксидантну активність посліду. Продуктивність оцінювали за інтенсивністю яйцекладки, середньою масою яйця, збереженістю поголів'я курей; інкубаційні властивості — за виводимістю яєць. **Результати.** Вища антиоксидантна активність раціону супроводжувалася суттєвим поліпшенням антиоксидантних показників: антиоксидантна активність сироватки зросла на 12,1%, яєчного білка — на 19,3%, жовтка — на 32,0%, активність супероксиддисмутази та каталази — на 7,4% і 5,5% відповідно, тоді як рівень малонового діальдегіду в сироватці знизився на 11,0%. На метаболічному рівні виявлено підвищення загального білка у сироватці крові (на 12,0%) за одночасного зниження загальних ліпідів (на 17,4%) і холестерину (на 14,5%) без змін активності печінкових ферментів; гематологічні показники (гемоглобін, еритроцити, лейкоцити) залишалися в межах фізіологічної норми і не різнилися між групами. Серед індикаторів неспецифічної резистентності відмічено підвищення бактерицидної активності яєчного білка (на 3,3%) за незмінної фагоцитарної активності нейтрофілів і бактерицидної активності сироватки. Антиоксидантна активність посліду була вищою у птиці, яка споживала раціон з більшою антиоксидантною активністю. Показники продуктивності суттєво не відрізнялися (інтенсивність яйцекладки 52,8% і 52,6%; середня маса яйця 58,8 і 59,0 г; збереженість 95,6% і 100,0%), а виводимість яєць становила 90,7% та 91,8% у групах з нижчою та вищою антиоксидантною активністю раціону відповідно. **Висновки.** Оптимальне поєднання інгредієнтів, що містять природні антиоксиданти, забезпечує підвищення антиоксидантного статусу яєчних курей та компонентів яйця, зменшення інтенсивності пероксидного окиснення ліпідів і поліпшення окремих метаболічних індикаторів без погіршення продуктивності й інкубаційних показників. Такий підхід є ефективною стратегією для підвищення антиоксидантного захисту як курей, так і продуктів, отриманих від них, що є особливо важливим у періоди підвищеного оксидативного навантаження (тепловий стрес, вакцинації, технологічні стресори).

Ключові слова: антиоксидантна активність корму, природні антиоксиданти, кури, антиоксидантний захист, оксидативний стрес, неспецифічна резистентність, яєчна продуктивність.