USE OF WALNUT LEAVES (JUGLANS REGIA) AS AN ANTIOXIDANT IN THE STORAGE OF POULTRY FEED

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Objective. To investigate the effect of walnut leaves (Juglans regia) on the oxidative stability of prepared poultry feeds compared with a synthetic antioxidant and a control (without antioxidant). **Methods.** The object of the research was three samples of complete feed for the parent flock of adult egg-laying hens: BR – basic diet without added antioxidants; BRSA – basic diet with added synthetic antioxidant; BRLG – basic diet with added dry ground walnut leaves (2%). All samples were stored under the same conditions for 120 days. During storage, the indicators of oxidative status (acid and peroxide value of fat) and vitamin supply (content of vitamins A and E) were monitored. **Results.** During storage, feeds with added synthetic (BRSA) and natural (BRLG) antioxidants had better oxidative stability indicators compared to the sample free from preservatives. Both samples of feed with antioxidants after 120 days of storage were within the regulatory limits in terms of acid and peroxide values of fat, while the base sample (BR) exceeded the critical value of acid value already on the 90th day of storage (30.96 mg KOH/g), peroxide value – at the end of the experiment (0.35%J2), which made it unsuitable for feeding poultry. The introduction of walnut leaves and synthetic antioxidant into the feed also contributed to better preservation of vitamins A and E, maintaining their content within the regulatory limits. Losses of vitamin A after 120 days of feed storage were 12% for BRSA and 34.4% for BRLG, vitamin E – 8.2% and 15.5%, respectively. In contrast, in the basic compound feed, significantly worse preservation of vitamins and their reduction below the minimum required norm were noted after 90 days of storage for vitamin A and after 60 days for vitamin E. **Conclusions.** The antioxidant and preservative properties of walnut leaves were confirmed. Adding it to compound feed for chickens during long-term storage inhibits oxidative and hydrolytic processes of lipids and increases the preservation of vitamins, which makes walnut leaves an alternative to synthetic antioxidants.

Keywords: poultry, compound feed, oxidative stability, antioxidants, acid number, peroxide number, vitamins.

Introduction. Preservation of product quality is one of the most important tasks of food and compound feed industry manufacturers. In compound feeds for animals and poultry during storage, numerous chemical changes may occur with the formation of dangerous secondary products. This is because during the preparation of feeds, fats that are prone to oxidation upon contact with oxygen in the air are widely used to achieve the desired energy level. As a result, toxic products of unsaturated compounds (peroxide, aldehydes, ketones, etc.) are formed and accumulate. The greatest danger is the chain oxidation of polyunsaturated fatty acids, or lipid peroxidation (LPO). These processes lead to a deterioration in the quality of feeds, the destruction of many vitamins, as a result of which the feed value of the product is lost [1]. The consumption of such feeds negatively affects the productivity and viability of animals and poultry, there is a lag in growth and development, pathological changes occur in the blood, liver, kidneys, etc., which can cause diseases. It is believed that oxidative stress is associated with such diseases of birds as intestinal leakage, coccidiosis and ascites [2]. Oxidized dietary lipids have been reported to slow growth, impair antioxidant status, and impair some immune functions in dogs [3]. Various strategies can be used to improve the oxidative stability of feeds during storage, including: avoiding light, oxygen, and high temperatures, and adding antioxidant compounds. One of the most common ways to stabilize the composition of compound feeds and protect them from the effects of oxidative processes is to use antioxidants, which inhibit the activity of enzymes that cause oxidative reactions in feeds and extend their shelf life [4-5]. Synthetic antioxidant compounds are mostly used in feed production, the most widespread of which are phenolic oxidation inhibitors: butylhydroxytoluene, butylhydroxyanisole [6-7]. However, synthetic antioxidants are not safe for human and animal health, so consumers increasingly consider their presence in feeds undesirable. This stimulates the trend towards natural antioxidants, the role of which is played by phenolic compounds found in plants [8]. Plant extracts, such as thyme, curcumin, rosemary, oregano, are rich in polyphenols and other biologically active substances that exhibit antioxidant effects when added to food and animal feed [9-10]. In particular, the effectiveness of adding grape seed, curcumin, pomegranate and cranberry extracts to slow down lipid oxidation during storage of dog food has been established [11]. The possibility of replacing synthetic antio antioxidant butylhydroxytoluene in the preparation of extruded fish feeds with rosemary extract, which showed the greatest protection against induced oxidation during storage for 24 weeks [12]. The effectiveness of thymol, a component of oregano, as a natural alternative for increasing the oxidative stability of poultry feeds has been reported [13] and the potential usefulness of antioxidant properties of plant extracts for the physiological state and productive indicators of poultry [14]. According to many researchers, walnut leaves also have a significant content of natural phenolic compounds that exhibit antioxidant activity [15-16], and the presence of juglone in its composition determines the preservative effect and antibacterial potential [17-18]. The effectiveness of adding walnut leaves as an antioxidant to sunflower oil to increase its resistance to oxidation processes has been reported [19]. At the same time, to our knowledge, no studies have been conducted on the use of walnut leaves as a natural antioxidant for the preservation of lipids in poultry feed. Thus, the main objective of this study was to evaluate the effect of adding dry ground walnut leaves to chicken feed on its oxidative stability.

Materials and methods. Experimental studies were conducted in the testing laboratory of the Department of Quality and Safety Assessment of Poultry Feed and Products of the State Research Station of Poultry of the National Academy of Sciences of the Russian Academy of Sciences (DSSP NAAS) using available laboratory and analytical equipment. The material for the studies was a complete feed for the parent flock of adult chickens of the egg production direction. Three different feed samples were prepared: BR - basic diet without the addition of antioxidants; BRSA – basic diet with the addition of a synthetic antioxidant, which includes butylhydroxytoluene (BHT), propyl gallate (E310), citric acid (E330), sodium phosphate (E339) and colloidal silicic acid (E551a) (according to the instructions for use of the drug); BRLG – basic diet with the addition of dry ground walnut leaves in an amount of 20 g/kg. The basic diet was prepared in accordance with the standard for the parent flock of laying hens (crude protein content 17%, metabolizable energy 2650 kcal/kg), the recipe for the compound feed is given in Table 1. Table

1. Recipe for basic compound feed (BR)

Compound feed components	Component content, %
Corn	49,40
Wheat	10,00
Sunflower meal	12,30
Soybean meal	14,10
Meat and bone meal	5,00
Chalk	6,00
Turtle shell	1,50
Bone meal	1,15
Lysine	0,08
Methionine	0,11
Mixture (citric acid, vitamin mixture, microelement mixture, B4)	0,20

Table salt	0,16
100 g of compound feed contains	
Metabolic energy, kcal/kg	2648,3
Crude protein, %	16,6
Crude fat, %	4,8
Crude fiber, %	4,3

Walnut leaves were collected in early June in dry weather from adult plants growing on the territory of the National Academy of Sciences of Ukraine without the use of phytosanitary treatments. Plant raw materials were dried in natural conditions - at ambient temperature (20–24°C) in a dark (without access to direct sunlight), well-ventilated room. The samples were ground in an electric mill to a fraction that passes through a sieve with a hole diameter of 1 mm. Thus, three samples of compound feed weighing 1 kg each were used for research, which were stored under the same conditions at room temperature (18–22°C) for 4 months. During storage, the quality of the feed and its resistance to oxidation were monitored once a month according to the acid and peroxide values of fat and the content of vitamins A and E. These indicators were studied using generally accepted methods [20].

Research results and their discussion. According to the obtained indicators of acid and peroxide values of fat of the studied samples of compound feed, a rather active course of peroxide processes was established in the base sample prepared without the use of antioxidants (Fig. 1, 2).

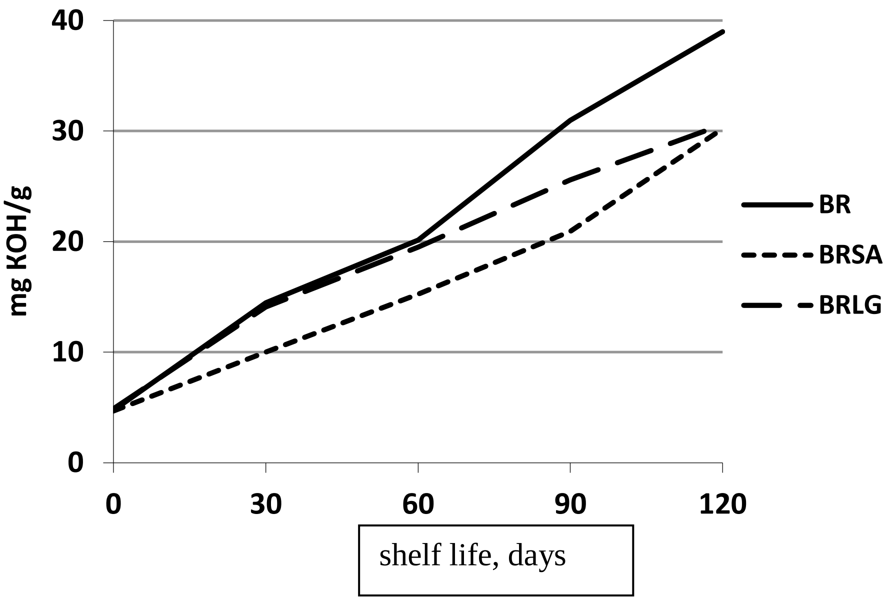

Figure 1. Dynamics of the level of acid number of fat in the studied compound feeds during their storage

Analysis of data on changes in the acid number of fat during compound feed storage shows that at the beginning of the experiment it was 4.69-4.75 mg KOH/g (Fig. 1). During storage, a gradual increase in this indicator was noted in all samples, by the end of the fourth month it was 30.21-38.98 mg KOH/g, which is 6.3-8.2 times higher than the initial level. At the same time, a positive effect of adding walnut leaves to the basic ration was noted. From the beginning of the experiment to 60 days of storage, the acid number of fat in compound feed containing walnut leaves (BRLG) was lower than in the basic ration (BR) by 2.7-3.2%. In subsequent observation periods, the difference in this indicator between BR and BRLG compound feeds reached 17.4-21.5%. At the same time, the best in terms of oxidative stability was the compound feed with the addition of a synthetic antioxidant (BRSA), since the acid number of fat in this sample was lower than in the other two throughout the entire storage period. The decrease in this indicator in the compound feed with a synthetic antioxidant relative to the basic compound feed was maximum, the difference was from 22.5% to 32.4% depending on the observation period. Compared to the compound feed with walnut leaves (BRLG), the differences in the acid number of fat were smaller (18.2-28.9%), and at the end of the experiment (120 days of storage) these two samples almost equalized in terms of the acid number of fat - the difference was only 1.3% in favor of the compound feed with the inclusion of a synthetic antioxidant (BRSA). Compound feeds with the addition of natural and synthetic antioxidants after 120 days of storage were at a critical level (30.2-30.6 mg KOH/g) relative to the maximum permissible value of the acid number of fat (30 mg KOH/g). For the sample without stabilizing additives (BR), the critical value was recorded already on the 90th day of storage (30.96 mg KOH/g), and at the end of the experiment, the acid number of fat exceeded the maximum permissible level by 1.26 times, which made it unsuitable for feeding poultry. The obtained data on the dynamics of changes in the peroxide numbers of fat in the studied compound feed samples show that the amount of peroxides in them during the experiment was at different levels (Fig. 2). After 120 days of storage, an increase of this indicator by 6-8 times relative to the initial value was noted. The BR compound feed, free from antioxidant additives, became unsuitable for consumption by poultry at the end of the experiment, as it had an increased fat peroxide value (1.17 times) relative to the maximum permissible level (0.3%J2).

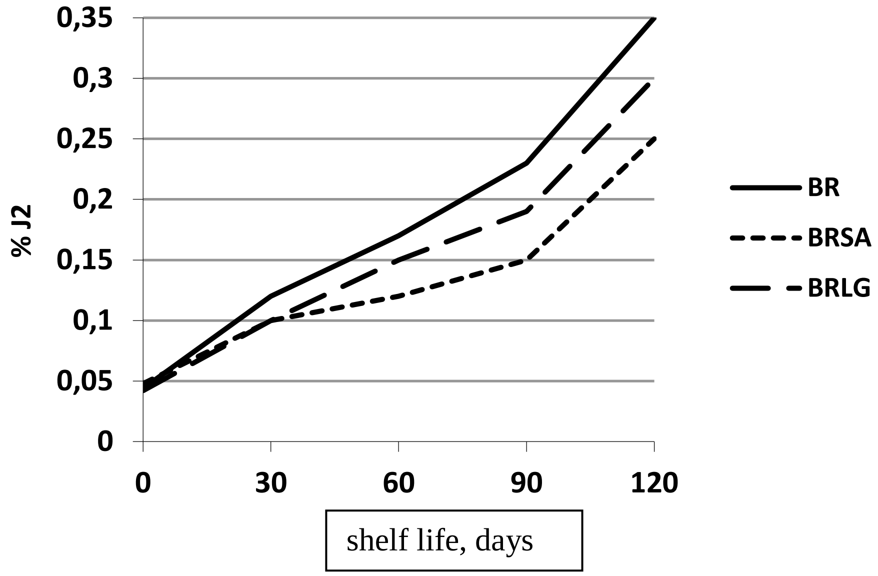

Figure 2. Dynamics of the level of peroxide value of fat in the studied compound feeds during their storage

At the same time, the addition of natural and synthetic antioxidants to compound feeds caused a significant decrease in the values of peroxide values of fat compared to the control (BR). After 30 days of storage, the peroxide value of fat in these two samples was at the same level - 0.1%J2, which is 16.7% lower than the indicator of the basic compound feed. Subsequently, the compound feed with walnut leaves occupied an intermediate position, and the sample with the addition of a synthetic antioxidant showed the highest stability with respect to peroxide processes. After 120 days of storage, the peroxide value of fat in the compound feed containing the addition of walnut leaves did not exceed the maximum permissible value and was 0.3%J2, which is 14.3% lower than the BR and 16.7% higher than the sample with a synthetic antioxidant (BRSA). Thus, the synthetic antioxidant showed the maximum stabilizing properties for long-term preservation of feed quality. The introduction of walnut leaves, due to the content of a number of polyphenolic compounds and juglone, contributed to a significant slowdown in oxidative processes relative to the sample free from antioxidant inclusions. The addition of walnut leaves to the feed as a natural antioxidant made it possible to extend its storage period by at least 30 days relative to the base sample. The stabilizing effect of natural and synthetic antioxidants was assessed based on the dynamics of the content of vitamins A and E in feed with their addition during storage (Fig. 3-4). Given the usually insufficient content of vitamins in feed and its significant decrease during storage, the regulatory value is considered to be their guaranteed introduction into feed (without taking into account their content in the components). In compound feeds for parent flocks of laying hens, the minimum required content of vitamin A is considered to be 10 IU/g, and vitamin E is 30 mcg/g.

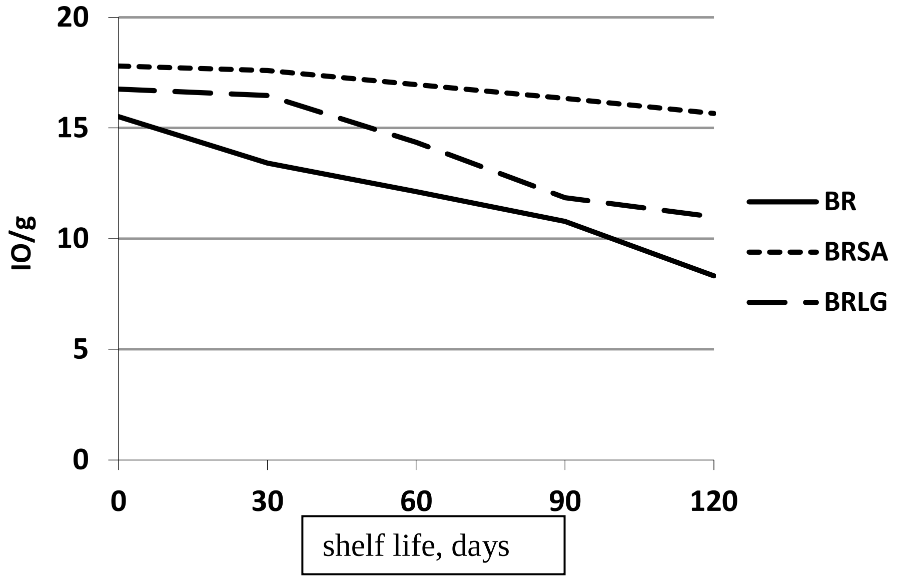

Figure 3. Dynamics of vitamin A content in the studied compound feeds during their storage

A slight difference was noted between the compound feed samples in terms of vitamin A content already at the beginning of the experiment, when, presumably, due to its presence in additives to the basic diet, the initial content of this vitamin in the BRSA and BRLG compound feeds was somewhat higher. The same applies to vitamin E, however, if the difference between the basic compound feed and the sample with the addition of a synthetic antioxidant at the beginning of the experiment was insignificant, then the compound feed with the addition of walnut leaves had a much higher vitamin E content compared to them - by 41.7-49.7%. This is obviously due to the rather high concentration of vitamin E in walnut leaves, which, according to our data, is at the level of 128.8 μg/g, and according to Untea et al. [21] - at the level of 157.54 μg/g. According to the results of the experiment, it was found that the introduction of walnut leaves and a synthetic antioxidant into the feed inhibits the oxidative and hydrolytic processes of lipids and thereby increases the preservation of vitamins A and E. As can be seen from Figure 3, the loss of vitamin A during the storage of the basic feed sample for 120 days was 46.4%, while in the experimental samples using the antioxidant properties of walnut leaves and a synthetic agent - 34.4% and 12%, respectively. As we can see, the vitamin A content in the basic feed exceeded the maximum required norm after 90 days of storage, while the experimental samples stabilized with synthetic and natural antioxidants remained within the regulatory limits for up to 120 days. At the same time, in the study by Dudarev et al. [22] noted a worse preservation of vitamin A in chicken feed, since after 60 days of storage without a preservative, the loss of vitamin A was 57% (in our case – 21.9%), and with the addition of a synthetic antioxidant – 25% (in our experiment – 4.7%). Less noticeable were the losses of vitamin E in all samples (Fig. 4). In the basic feed at the beginning of the experiment, its content corresponded to the norm (31.8 μg/g), and at the end – it decreased by 30%. However, a decrease in the concentration of vitamin E in the control sample below the regulatory limit was observed after 60 days. At the same time, changes in the content of vitamin E during storage of feed with walnut leaves and a synthetic antioxidant were significantly smaller. The decrease in its content by the end of the experiment was 15.5% for the sample with the addition of walnut leaves (BRLG) and 8.2% for the sample stabilized with a synthetic antioxidant (BRSA). At the same time, both experimental samples of compound feed in terms of their vitamin E content remained within the regulatory limits throughout the experiment (120 days). It is obvious that in compound feed with the addition of walnut leaves this occurred due to the presence of BAS (phenolic compounds, including juglone), which have antioxidant properties, and a higher initial content of vitamin E. In the experiment of Dudarev et al. [22], a more rapid decrease in the content of vitamin E in compound feed for chickens after 60 days of storage was observed - by 23.2% without the addition of a preservative (according to our data - 2.9%) and by 12.2% with the addition of a synthetic antioxidant (in our case - 2.3%). In our opinion, such discrepancies are due to the fact that in the above experiments, at the beginning of the experiment, the compound feed contained an insufficient amount of vitamins A and E, which caused their faster loss during storage.

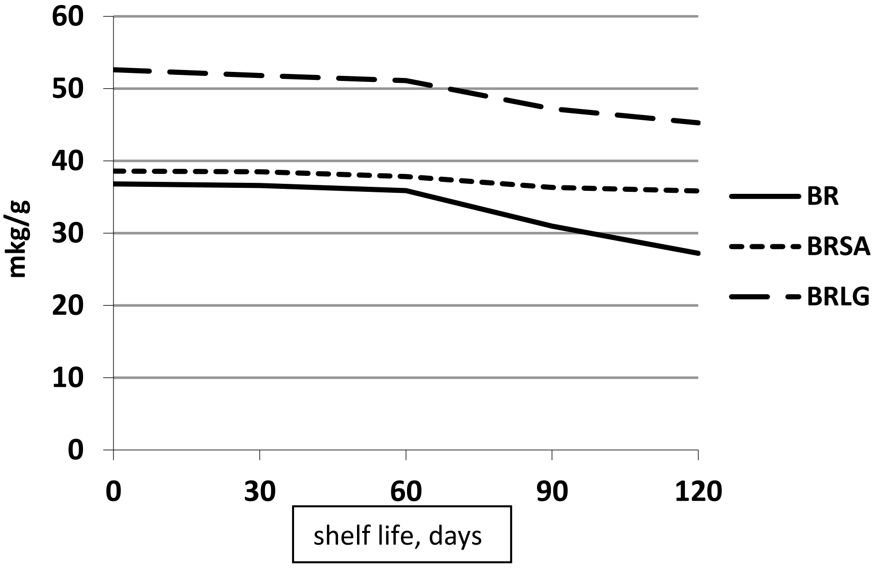

Figure 3. Dynamics of the level of vitamin E content in the studied compound feeds during their storage

The obtained data on the change in the content of vitamins A and E in compound feeds during their storage indicate their better preservation in samples stabilized with natural (BRLG) and synthetic antioxidants (BRSA). This allowed maintaining the content of these vitamins in the experimental feeds within the regulatory limits for at least 120 days, in contrast to which in the basic feed (BR) their decrease to a critical level occurred after 60 days of storage. Therefore, given the antioxidant and preservative properties of walnut leaves, during long-term storage of compound feeds, it is possible to use this raw material as an alternative to a synthetic antioxidant, which allows stabilizing the values of the acid and peroxide numbers and increasing the preservation of vitamins A and E for 4 months.

Conclusions. According to the results of the study of the oxidative status and vitamin supply of compound feeds during their long-term storage, a positive effect of adding synthetic and natural antioxidants was established, which ensured oxidative protection of feeds for 120 days of storage. The lowest resistance to oxidation and critical loss of vitamins A and E was shown by the sample free from antioxidants after 60 days of storage. The compound feed with the addition of a synthetic antioxidant turned out to be the least oxidized with minimal vitamin losses. At the same time, a significant decrease in the values of the acid and peroxide values of fat and vitamin losses was noted in compound feed with the addition of walnut leaves at a concentration of 2% relative to the basic recipe without an antioxidant. This confirms the effectiveness of using this plant raw material to improve oxidative stability, vitamin preservation and extend the shelf life of feeds. Thus, walnut leaves can be recommended as a powerful source of natural antioxidants and an alternative to synthetic feed stabilizers to protect them from oxidation.

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