Brown mustard (*Brassica juncea* Czern) processing by-products: Effects of mustard meal xenobiotics on the productive performance and antioxidant system of laying hens

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ABSTRACT

Brown mustard (*Brassica juncea Czern*) is a widely cultivated crop in Asia and throughout the world. Mustard meal, a by-product of mustard oil extraction, has potential as a feed ingredient for poultry. There are concerns, however, that it contains xenobiotic compounds such as mustard oils that may affect metabolism and productivity. This study evaluated the impact of including 5% mustard meal in layer diets on egg production, antioxidant status and vitamin content, identifying effective feed additives for metabolic normalization. A total of 500 Rhode Island White laying hens (150 days old) were divided randomly into five groups. The control group received a standard diet, while experimental groups received diets containing 5% mustard meal with different supplements: vermiculite sorbent, santoquin with vitamin E, or methionine with glucose. The incorporation of mustard meal retarded egg production during the first two weeks, but performance stabilized thereafter. Oxidative stress intensity was significantly higher in hens fed mustard meal compared to the control. All tested additives enhanced the antioxidant defense system, reflected in reduced malondialdehyde concentrations in blood serum. The antioxidant santoquin proved to be the most effective protector against mustard oil-induced oxidative stress. Supplementation with santoquin and vitamin E or methionine with glucose increased yolk carotenoid and tocopherol levels without affecting retinol or vitamin B_2 concentrations. In general, inclusion of mustard meal in the diet at a level of 5% may be feasible in combination with suitable protective additives, in particular santoquin+vitamin E, to ensure both productive stability and physiological resistance in laying hens.

Keywords: Mustard (*Brassica juncea*) seed oilmeal xenobiotics; Poultry nutrition and feeding of laying hens; Egg productivity; Lipid peroxidation; Antioxidant system

Contribution/Originality: This study contributes to knowledge of how mustard meal xenobiotics (mustard oils) influence the productivity and antioxidant system of laying hens. It documents the application of santoquin+vitamin E as the most effective dietary protector against oxidative stress caused by mustard meal inclusion in poultry diets.

1. INTRODUCTION

Mustard, such as *Sinapis alba* (white or yellow mustard), *Brassica juncea* (brown mustard) and *Rhamphospermum nigrum* (black mustard), is widely cultivated across Asia and throughout the world, serving as a spice, medicinal raw material and a valuable source of edible oil (Budiasih et al., 2019; Ayadi et al., 2022; Sharma A. et al., 2024; Sharma R. et al., 2024). Its use in the food production industry has been gradually increasing because of the nutritional and functional properties of mustard seeds as they contain a wide range of biologically active components (Budiasih et al., 2019). These include isothiocyanates, which impart a characteristic flavor and pungency, but also have adverse physiological effects (Ayadi et al., 2022). In addition to beneficial bioactives, mustard seeds and derivatives thereof, which are meant for human/animal ingestion, may also contain potentially unwanted or even toxic substances, e.g., bisphenol F, erucic acid and/or allergenic proteins (Sharma et al., 2019; Ayadi et al., 2022; Grygier, 2023). Numerous studies (for example, Lietzow, 2021; Ayadi et al., 2022; Akhtar et al., 2024) provided a comprehensive overview of the hazardous compounds contained within mustard seeds and subsequently assessed the potential health risks that they present.

Given that they are the by-products of mustard oil extraction, the seed oilmeal and cake are good sources of plant protein for use in animal nutrition. This is especially the case for countries in Asia (Sarker et al., 2015). Mustard cake inclusion in poultry diets has been shown to enhance both growth and egg production performance and to improve product quality, being rich in protein, fatty acids, and fiber. In Bangladesh, protein contents in black (*R. nigrum*) and white (*S. alba*) mustard cakes were reported as 38% and 29%, with pepsin digestibility of 80% and 77%, respectively, as well as a favorable amino acid profile (Sarker et al., 2015). Mustard seed oilmeal has long been utilized in poultry feeding. For instance, in India, Panda and Pradhan (1966) compared peanut oilmeal and black mustard oilmeal in chick starter rations for White Leghorn layers up to 7 weeks of age. They found higher body weights in chicks fed mustard oilmeal. Although mustard contains antinutritional factors such as glucosinolates (Gilani et al., 2012), later studies demonstrated that low-glucosinolate meals from brown mustard (*B. juncea*) have nutritional values comparable or superior to canola meals derived from rapeseed (*B. napus*) or turnip rape (*B. rapa*) (Newkirk et al., 1997). Oryschak et al. (2020) showed that inclusion of canola or brown mustard coproducts (20%) in the diets of laying hens maintained egg quality and performance, with 75–85% ileal digestibility. In broilers, black mustard seeds also supported higher carcass growth and feed intake (Adegbeye et al., 2020). Furthermore, supplementation with brown

mustard seed extract in drinking water improved intestinal morphology, microbiota balance, blood biochemistry, growth and meat oxidative stability (Abdulameer et al., 2021; Abdulameer & Alwan, 2022).

Compared to sunflower, olive, rapeseed and peanut oils, mustard oil contains the highest proportion of erucic acid (C22:1) at 11.38%, limiting its suitability for direct human consumption (Konuskan et al., 2019). Partial removal of glucosinolates has been shown to improve the bioavailability of mustard seed proteins across *R. nigrum*, *B. juncea*, and *S. alba*. The limited use of mustard meals in livestock and poultry feeding is mainly attributed to such antinutritional components. Recent studies (Garg et al., 2024) have, however, clarified these constraints, suggesting technological improvements for more efficient mustard meal utilization. The fermentation of oilseed meals is a promising approach to circumvent the negative actions of antinutritional factors and xenobiotics. To give an example, rapeseed meal when it is fermented leads to a reduction in glucosinolates and phytate phosphorus concentrations. It also increases turkey body weight, while not impairing metabolic nor immune parameters when administered at a 15% level. Moreover, at these concentrations, it improved the antioxidant (AO) status, as well as the histomorphology of the intestines (Drażbo et al., 2018).

Poultry productivity is influenced by a range of stressors that can arise in the food, or through technological intervention. The former are of particular importance and can, in conjunction with low-quality feed and/or vaccination stress, induce so called “free-radical pathology,” characterized by an imbalance between lipid peroxidation (LPO) activation processes and the generation of reactive oxygen species (ROS) (Shakhov et al., 2003; Klotz & Steinbrenner, 2017). The interaction between oxygen and phospholipids of the membrane in the presence of oxidative radicals initiates LPO activation (Onur Yaman & Ayhanci, 2021). Indeed, the stability during evolution of living systems under oxidative conditions is sustained by AO mechanisms that keep lipid peroxides low. The balance of LPO and AO activity is therefore fluid and changing, and may change when put under stress. This can lead to an increase in oxidative reactions (Abd El-Aal, 2012; El-Beltagi & Mohamed, 2013; Valgimigli, 2023).

The increased activity of free radicals takes place under both pathological and high-metabolic physiological conditions. These include broiler growth and egg production. Unphysiologically high LPO products inhibit cell division, destabilize membranes and inactivate enzymes, which can

impede poultry performance (Aw, 1999; Aydemir et al., 2000). The underlying risk is both from LPO activation of LPO, in the depletion of AO reserves, and in the disruption of oxidative balance. Perpetuating an optimal status for AO status is fundamental for sustaining health and productivity (Surai, 2020; Ponnampalam et al., 2022). Dietary fats increase feed energy but can become pro-oxidative if oxidized and this can initiate LPO activation (Wang et al., 1997; Zamora et al., 1997). Contemporary poultry diets contain 2–6% fat, which can rise to 10% in broiler feeds (Pesti et al., 2002). Too little fat reduces the utilization of proteins, whereas oxidized fat can lead to the accumulation of toxic aldehyde and peroxide (Vieira et al., 2017; Geng et al., 2023). It follows then that examining fats from different sources and containing different states of oxidation is crucial to understand their effects on LPO activity, AO system and thus overall productivity (Durand et al., 2005; Kujoana et al., 2024).

The AO system also reacts to xenobiotics and antinutritional substances contained within feeds. The vast majority of plant ingredients contain certain compounds with potential antinutritional or toxic effects. These include non-starch polysaccharides in cereals, trypsin inhibitors in legumes and glucosinolates in crucifers (Okolelova et al., 1999). The toxicity of these depends on dosage, exposure duration, and organism adaptability (Bratishko et al., 2008). Negative impacts and risks can be mitigated by technological processing, enzymatic treatment and/or supplementation with adaptogenic additives. The thermal processing of soybean inactivates antinutritional factors (Badjona et al., 2023), while enzyme preparations improve the digestion of polysaccharides (Choct, 2006), and the fortification methionine and iodine enhances the efficiency of rapeseed product utilization (Duborská et al., 2022; Mykytyn et al., 2023). By combining these strategies with the inclusion of bioactive substances that activate xenobiotic metabolism and strengthen AO defense, we can improve feeding efficiency considerably. Therefore, choosing feed additives with detoxifying and adaptogenic features represents a potentially useful approach that could enhance plant feed quality.

In poultry, vaccination is still the most effective strategy to prevent infectious diseases, although it can weaken the immune response and induce immunodeficiency (Slivka, 2003). The latter is typically associated with increased activity of LPO as well as the accumulation of malondialdehyde (MDA). This thereby reflects oxidative stress activation (Kichun et al., 2001; Tanir Basaranoglu et al., 2021). The impact of vaccines metabolism can vary according to type (live, inactivated, mono-, or polyvalent) and age of the animal. Food additives, particularly the vitamins A, E and C, are key to

improving the effectiveness of the immunization process (Kolb & Seehawer, 2001; McDowell, 2000; Aslam et al., 2017; Van Hieu et al., 2022; Shastak & Pelletier, 2023, 2024). It is crucial, however, to balance their dosage because too high levels can cause antagonistic effects and/or immunosuppression (Muir et al., 2002; Kurtyak & Yanovich, 2004; Schoendorfer & Davies, 2012; Fernández-Villa et al., 2019). Other promising additives include plant and microbial preparations as well as selected amino acids and microelements (Dietert & Golemboski, 1998; Nys, 2001; Ghadban, 2002; Lee et al., 2002).

Feed and technological stressors primarily disrupt the AO defense system. Identifying vulnerable links and implementing corrective strategies allows for the stabilization of metabolic processes, prevention of pathological changes, as well as improvement of productivity and immune responses. Preprocessing of feed combined with compounds that facilitate xenobiotic metabolism can enhance adaptation and detoxification further. Vermiculite has been established to be an effective mineral supplement and sorbent in poultry nutrition, reducing mortality, improving growth rate and enhancing feed digestibility. Its ion-exchange properties also facilitate the removal of radionuclides such as cesium and strontium (Bomko et al., 2023). The inclusion of vermiculite up to 5% in quail diets improved mineral and amino acid composition of meat and increased overall productivity (Apdraim et al., 2023).

Given the growing evidence supporting the hypothesis that LPO activation represents an initial link in the stress response mechanism (Bozhkov et al., 2022), it can be assumed that strengthening the AO system enhances the adaptability of birds to xenobiotic stress. Our previous studies (Svjezhentsov et al., 2005) evaluated mustard oilcake as a protein component in young chicken diets, confirming its suitability in poultry feeding. We also conducted preliminary experiments on the presence of xenobiotics in mustard oilcake and their influence on the AO system of adult hens. We proposed its potential efficiency as a feed ingredient (Bratyshko et al., 2003). Therefore, the present study further investigated, in a broader experiment and larger flock, the effects of mustard xenobiotics and various feed additives on the performance and AO system of laying hens. As feed additives, we tested sorbent (vermiculite), santoquin + vitamin E, and methionine + glucose and examined their impact on poultry egg productivity, as well as oxidative processes and vitamin content in tissues.

2. MATERIALS AND METHODS

In this study, untreated and treated, mustard-fed (without and with feed additives) laying hens were compared in terms of their performance, oxidative processes and vitamin content in tissues, which enabled to conclude with regard to treatment effects and applicability of the tested treatment regimes in poultry nutrition.

2.1. Description of the Mustard Plant Materials Used

The experiments used seeds of brown (or Sarepta) mustard (*B. juncea* Czern; Figure 1) of the "Korona" variety, from which mustard meal was produced.

(A) (B) (C)

Figure 1. Brown (or Sarepta) mustard (*Brassica juncea* Czern). (A) Mustard field in India. (B) Flowers. (C) Seeds. Credit: (A) https://commons.wikimedia.org/wiki/File:Mustard_Field-1.jpg (by Trikutdas, CC-BY-SA-4.0); (B) https://commons.wikimedia.org/wiki/File:Yellow_mustard_flower.jpg (by Indiaphotoblog/Xabier Armendaritz, CC-BY-3.0); (C) https://commons.wikimedia.org/wiki/File:Brown_Mustard_Seed_(Close).jpg (by Dsaikia2015, CC-BY-SA-4.0).

The spring mustard variety "Korona" (Belvet, 2025; Agronom.Info, 2025) has a vegetation period of 85–95 days and seed oil content of 40–45%. It is a newly registered (2020) technological, early-maturing, low-erucic acid Sarepta mustard variety suitable for mechanized cultivation. The variety grows rapidly within 85–90 days after sowing and is intended for producing edible oil and mustard powder. The plant has light-yellow corolla flowers (Figure 1C), is well adapted to different soil types and growing conditions and requires minimal care. Due to its high adaptability and disease resistance, it is recommended for cultivation in steppe and forest-steppe zones. The variety has high resistance to lodging, with a potential yield of up to 2.8 t/ha. The 1000-seed mass is 3.8–4.0 g, which facilitates seed cleaning. "Korona" seeds can be used for feeding purposes, as well as a green manure that can improve the fertility of the soil. Sticking to optimal sowing dates and control weeds in a timely manner are essential for successful cultivation. Treatment of the seeds before planting should improve germination. Because of its pest and disease resistance properties, "Korona" can help ensure stable yields keeping losses to a minimum.

2.2. Place, Time of Research, Birds and Egg Performance

All experiments were undertaken at the State Poultry Research Station, an institution of the National Academy of Agrarian Sciences of Ukraine. The animals were kept in BKN-3 cage batteries certified for compliance with the usual technological parameters. The study involved Rhode Island White laying hens (100 birds per group) aged 150 days (a commercial layer line described elsewhere, e.g., Semerdzhiev et al, 2005; Calik et al., 2017, 2018; Garamvölgyi & Sütő, 2021; Szász et al., 2023). The experimental period lasted from May to August.

Egg-laying performance was assessed by daily recording of egg production per group. The laying intensity (in %) was calculated based on the daily egg count, and egg weight (in g) was determined by weighing eggs collected over a 5-day period from each group at the beginning of each month during the experiment.

2.3. Research on Laying Hens and Experimental Design

Rhode Island White laying hens were randomly divided into five groups (100 birds each). The experimental design is presented in Table 1. Group 1 served as the control and received a standard diet without additives. In the diets of Groups 2–5, 5% of the feed was replaced with mustard meal and various supplements.

Table 1. Experimental design.

Group	Diet composition
1 (control)	Standard complete feed (PC)
2	95% PC + 5% mustard meal
3	95% PC + 5% mustard meal + 0.4 g/bird/day vermiculite sorbent
4	95% PC + 5% mustard meal + 200 g/t santoquin + 40 mg/kg vitamin E
5	95% PC + 5% mustard meal + methionine (10% above the normative level) + 0.1% glucose

The following supplements were tested to normalize metabolic processes in laying hens:

- Vermiculite sorbent Sorbover, characterized by its ion-exchange capacity, facilitates the removal of radionuclides (cesium, strontium, etc.) and LPO products from the organism. Sorbover is highly efficacious (Bomko et al., 2023) and can improve chemical, mineral and amino acid composition of quail meat (Apdraim et al., 2023). Vermiculite is often added to poultry feed to enhance nutrient absorption, immunity and resistance to stress.
- Santoquin with vitamin E was applied in order to strengthen the AO defense system (Bozhkov et al., 2022).
- Methionine in combination with glucose was applied to inactivate adverse factors via the mechanism of of sulfur-containing conjugate synthesis.

Mustard meal was administered for a period of 60–62 days, after which a similarly long observation period followed.

2.4. Research Methods

2.4.1. Sample Collection: Blood/Serum, Liver and Eggs

Samples of both breast muscle and blood were collected on days 3, 7, 14, 28 and 60 after the supplementation of experimental additives. At each time point, a total of five birds from each group were sacrificed for sampling purposes. Following decapitation under anesthesia, blood was collected, allowed to stand at 26 °C for 30 minutes, and centrifuged at 1500 g for 10 min at room temperature to obtain serum, which was transferred into sterile tubes.

At the end of the feeding period, five birds per group were slaughtered to obtain liver samples. Concentrations of general biochemical parameters in serum and liver were controlled by standard methods (e.g., Maurer, 2011; Hoekstra et al., 2013; Bora et al., 2017; Kadhum & Hadwan, 2021; Hadwan et al., 2024).

Egg samples (30 per group) were collected monthly to assess egg quality parameters, including shell strength and the content of carotenoids and vitamins A, E, and B_2 (Egorov et al., 2007; Baydevlyatova et al., 2009; Surai et al., 2016).

2.4.2. Serum, Liver and Eggs Quality Analyses

At different ages, the concentration of MDA (in mmol/mL or mmol/g) in serum and muscles of five birds per group was determined by the thiobarbituric acid reaction (Konieczka et al., 2014). Serum

protein concentration (%) was determined by the Lowry method (Niamke et al., 2005; Waterborg, 2009; Shen et al., 2013).

In liver samples obtained at the end of the feeding period, concentrations of vitamins C, E, and A were determined monthly. Vitamin C (μg/g) was measured as described previously (e.g., Du et al., 2022). Vitamins E and A were quantified as described elsewhere (e.g., Zhang et al. 2021; Liu et al. 2021). Specifically, vitamin A concentration (μg/g) in the liver was measured by the reaction with boron trifluoride (Bozhkov et al., 2022, 2024), while vitamin E concentration (μg/g) was determined by thin-layer chromatography (Polak & Pajurek, 2021; Kröpfl et al., 2022).

Egg quality was assessed by measuring carotenoid concentration after extraction from egg yolks with acetone at 450 nm (Strati et al., 2012; Islam & Schweigert, 2015; Wang et al., 2016). Vitamin B_2 content in yolk and albumen was determined by the fluorometric method based on the fluorescence of riboflavin after ethanol extraction (Pinto & Rivlin, 2013).

2.5. Statistical Analyses

Data were analyzed using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA) and STATISTICA 8 (StatSoft, Inc./TIBCO, Palo Alto, CA, USA) for repeated-measures analysis of variance (rANOVA). Data visualization was performed with Microsoft Excel 2013. Results were expressed as means plus or minus the standard error (M ± SE). Then statistical significance was established using both non-parametric Mann–Whitney U test and Student's *t*-test (GraphPad Software, 2024). As is convention, differences considered significant at $p < 0.05$. The changes of biochemical parameters and vitamin content were assessed using rANOVA. Liver regenerative potential indicators were evaluated using both Mann–Whitney U test (area index) and Fisher's exact test (degree of adhesion). We used the Phantasus web platform (version 1.27.1) to perform principal component analysis (PCA) and hierarchical clustering (Zenkova et al., 2018; Kleverov et al., 2024); index values were normalized by $\log_2$ transformation implemented in Phantasus.

3. RESULTS AND DISCUSSION

3.1. Poultry Egg Productivity

The inclusion of mustard oilmeal containing mustard-derived xenobiotics initially reduced egg production by approximately 7% (48% vs. 55%) in the first 14 days compared to the control. By the fourth week, however, the intensity of egg-laying recovered to the level of controls. In the final

phase of the experiment, it exceeded the level of the controls by 1–2%, perhaps due to the higher protein and energy content (1.5% increase) in the supplemented diet.

Egg weight stayed stable across all groups throughout the trial, showing no statistically significant differences between control and treatment chickens (Table 2). Some transient deviations were, however, observed in Groups 4 and 5, particularly in weeks, where the mean egg weight had decreased slightly. This did not impair overall productivity.

Table 2. The effect of mustard meal on egg weight in hens of Groups 1–5 (M ± m; *n* = 30).

Experimental week	Egg weight, g				
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**
1	57.20±1.56	57.60±0.54	57.90±1.65	54.80±1.68	56.30±2.04
2	61.90±0.48	60.90±0.43	61.20±0.37	60.90±0.82	61.50±1.04
3	$62.50\pm0.60^{\uparrow *4}$	61.00±0.49	62.10±0.55	$60.90\pm0.29^{\downarrow *1}$	61.60±0.57
4	61.70±0.53	60.60±0.85	62.00±1.17	60.60±0.62	62.20±0.72
5	$61.40\pm0.44^{\uparrow ***4}$	60.70±0.35	60.70±1.4	$59.00\pm0.14^{\downarrow ***1}$	59.90±0.90
6	$62.50\pm0.99^{\uparrow **4}$	61.20±0.76	60.80±0.77	$59.50\pm0.40^{\downarrow **1}$	60.80±0.90
7	$62.00\pm0.72^{\uparrow *4}$	61.10±0.63	$62.60\pm0.62^{\uparrow **4}$	$60.10\pm0.41^{\downarrow *1,**3}$	$60.50\pm0.32^{\downarrow **3}$
8	$61.10\pm0.84^{\uparrow *4}$	60.30±0.78	60.20±0.68	$58.60\pm0.62^{\downarrow *1}$	59.40±0.75
9	$62.10\pm0.32^{\uparrow **4}$	61.10±0.45	61.90±1.25	$59.10\pm0.97^{\downarrow **1}$	$62.80\pm0.90^{\uparrow **4}$
Mean	$61.40\pm0.53^{\uparrow *4}$	60.50±0.77	$61.10\pm0.47^{\uparrow *4}$	$59.30\pm0.63^{\downarrow *1,3}$	60.60±0.64

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (↑ increase; ↓ decrease) relative to Groups 1 (control), 3, or 4.

3.2. Oxidative Processes and Vitamin Content in Tissues

Hens fed with mustard oilmeal experienced considerable changes in oxidative metabolism. In the first 72 hours, LPO process intensity declined; however, by the end of the first week, a notable increase was observed. In particular, under Fe^{2+}-induced stimulation, the concentration of thiobarbituric acid–reactive substances (TBARS) in serum exceeded the control value by 59% (Table 3). The difference between control and experimental groups gradually diminished, reaching 48% after one month and 27% after two months of feeding.

Table 3. The effect of mustard meal on blood serum protein and malondialdehyde (MDA) concentration in hens of Groups 1–5 after 3, 7, 14, 28 and 60 days (M ± m; *n* = 5).

Period	Group	MDA concentration, mmol/mL		Protein, %
		Spontaneous LPO	Fe^{2+} activation	
3 days	1	39.50±2.57$^{\uparrow *4}$	353.25±66.00$^{\uparrow *2,4}$	4.85±0.13
	2	37.00±1.60$^{\uparrow *4}$	143.00±6.56$^{\downarrow *1,***5}$	5.06±0.23
	3	41.25±2.60$^{\uparrow *4,5}$	232.40±64.56	5.50±0.31
	4	32.00±1.02$^{\downarrow *1,2,3}$	168.00±24.10$^{\downarrow *1,**5}$	4.77±0.31
	5	34.12±0.94$^{\downarrow *3}$	376.50±35.46$^{\uparrow ***2,**4}$	5.25±0.21
7 days	1	44.80±1.30$^{\downarrow **4,5}$	278.50±45.80$^{\downarrow *2,**3,5}$	6.11±0.37
	2	49.80±2.10$^{\downarrow *4,5}$	442.70±18.12$^{\uparrow *1,**4,\downarrow *3}$	6.23±0.37
	3	43.50±4.99$^{\downarrow *4,5}$	566.00±39.00$^{\uparrow **1,*2,5***4}$	6.53±0.29
	4	67.50±5.40$^{\uparrow **1,*2,3}$	268.00±35.70$^{\downarrow **2,5,***3}$	5.96±0.18
	5	66.60±5.55$^{\uparrow **1,*2,3}$	449.00±16.50$^{\uparrow **1,4\downarrow *3}$	5.81±0.26
14 days	1	51.20±2.94$^{\downarrow **3}$	364.50±40.15	4.74±0.34$^{\downarrow **5}$
	2	56.60±2.31$^{\downarrow *3}$	325.50±22.10	4.41±0.40$^{\downarrow **5}$
	3	64.25±1.28$^{\uparrow **1,*2}$	303.75±38.20$^{\downarrow *5}$	5.47±0.47
	4	48.00±8.70	361.88±51.30	4.94±0.52$^{\downarrow *5}$
	5	63.50±10.15	456.50±30.60$^{\uparrow *3}$	6.34±0.26$^{\uparrow **1,2,*4}$
28 days	1	37.15±4.15	204.10±54.10	6.24±0.30$^{\uparrow *4}$
	2	37.00±4.92	302.20±25.70$^{\uparrow **3,*4}$	6.15±0.23$^{\uparrow *4}$
	3	34.50±4.74	208.50±3.77$^{\downarrow **2,5}$	5.82±0.29
	4	40.50±4.50	211.40±17.19$^{\downarrow *2,**5}$	5.32±0.22$^{\downarrow *1.2}$
	5	32.00±6.75	334.50±30.30$^{\uparrow **3,4}$	5.42±0.46
60 days	1	86.20±5.07	327.20±25.10	6.35±0.23
	2	106.10±9.43	416.30±17.80	5.75±0.33
	3	101.70±6.70	290.50±32.70	5.45±0.31
	4	87.30±8.98	242.30±12.60	4.74±0.23
	5	86.50±8.51	375.40±41.20	6.08±0.43

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (↑ increase; ↓ decrease) relative to Groups 1 (control), 2, 3, or 4.

At the end of the feeding period, the concentration of MDA in breast muscle remained 26.7% higher in birds fed mustard oilmeal compared to the control (Table 4). These findings indicate the

activation of LPO and subsequent oxidative stress in the organism of the hens receiving mustard oilmeal.

Because of this oxidative activation, the reserves of endogenous AOs decreased. In the liver of hens from Group 2, the concentration of vitamin E was 30% lower than in the control, while vitamin A content tended to decline by approximately 11% (Table 4).

Table 4. Effect of mustard meal on selected antioxidant system parameters in hens (M ± m; *n* = 5).

Group	Vitamin concentration in liver, μg/g			MDA concentration in breast muscle, mmol/g	
	A	C	E	Spontaneous	Fe^{2+} activation
1	1190.00±133.80	$325.22±5.75^{\uparrow **4,5}$	$7.29±0.79^{\uparrow *2}$	144.00±8.48	$243.75±15.39^{\downarrow *2,5}$
2	$1060.00±64.81^{\downarrow *4}$	$332.40±6.51^{\uparrow **4,***5}$	$5.01±0.23^{\downarrow *1,3,4}$	200.40±23.2	$309.00±23.60^{\uparrow *1}$
3	1155.00±183.00	$315.12±3.94^{\uparrow *4,***5}$	$6.26±0.35^{\uparrow *2,\downarrow *4}$	163.30±5.42	276.56±14.63
4	$1530.00±98.15^{\uparrow **2,5}$	$250.08±20.78^{\downarrow **1,2,*3}$	$9.88±1.44^{\uparrow *2,3,5}$	163.70±23.20	259.25±25.88
5	$1070.00±100.20^{\downarrow *4}$	$229.68±10.76^{\downarrow ***1,2,3}$	$5.57±0.79^{\downarrow *4}$	159.90±13.40	$294.38±10.73^{\uparrow *1}$

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (↑ increase; ↓ decrease) relative to Groups 1 (control), 2, 3, or 4.

In parallel, the nutritional quality of eggs deteriorated. A progressive decline in egg vitamin E content was recorded—by 14.2% after four weeks and by 65.5% after six weeks. Toward the end of the experiment, however, the vitamin E concentration increased again, reducing the difference relative to the control to 16.5% (Table 5).

Table 5. Effect of mustard meal on vitamin concentrations in eggs of laying hens (M ± m; *n* = 5).

Period	Group	Vitamin concentration in eggs, μg/g				
		Carotenoids	A	E	B_2 (yolk)	B_2 (albumen)
21 days	1	13.80±2.13	6.90±0.91	$83.50±3.12^{\uparrow *2}$	5.07±0.91	3.21±0.11
	2	14.20±1.44	6.80±1.12	$71.60±2.98^{\downarrow *1}$	5.58±0.67	3.78±0.45
	3	17.80±1.09	7.10±1.01	–	4.47±1.12	3.59±0,70
	4	16.90±1.78	7.10±0.89	–	5.02±0.56	3.75±0.56
	5	13.10±2.15	7.10±0.79	–	5.12±1.08	3.49±0,61
42 days	1	15.85±3.11	8.60±1.78	$61.50±1.98^{\uparrow ***2,3,\downarrow ***4,5}$	4.88±1.44	2.90±0.24
	2	16.48±1.19	10.90±1.46	$21.20±4.12^{\downarrow ***1,4,5}$	4.93±1.98	3.41±0.31

	3	16.81±2.09	9.90±0.98	29.50±3.17$^{\downarrow***1,4,5}$	4.44±0.07	3.36±0.65
	4	17.02±1.40	10.90±2.01	123.50±3.21$^{\uparrow***1,2,3,5}$	5.13±0.92	2.95±1.01
	5	15.12±0.89	10.90±1.78	92.60±2.03$^{\uparrow***1,2,3,\downarrow***4}$	4.83±0.56	2.81±0.21
56 days	1	12.40±1.45$^{\downarrow*3,***4,**5}$	6.10±1.02	69.90±12.62$^{\downarrow**3,5,***4}$	4.17±0.44	3.11±0.12
	2	17.62±2.31$^{\downarrow*4}$	6.90±1.98	69.60±1.28$^{\downarrow***3,4,5}$	4.39±0.34	3.20±0.15
	3	22.44±2.98$^{\uparrow*1}$	9.00±2.45	129.10±2.95$^{\uparrow**1,***2,\downarrow***4}$	4.07±1.01	3.11±0.22
	4	24.97±1.11$^{\uparrow***1,*2}$	9.00±0.95	156.90±1.93$^{\uparrow***1,2,3,**5}$	4.82±0.69	3.13±0.45
	5	25.79±2.76$^{\uparrow**1}$	7.20±2.00	128.50±6.77$^{\uparrow**1,***2,\downarrow**4}$	4.03±0.84	3.17±0.26

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (↑ increase; ↓ decrease) relative to Groups 1 (control), 2, 3, or 4.

Supplementing mustard oilmeal with various feed additives affected metabolism and productivity differently. The use of a sorbent did not prevent the activation of LPO processes or the transient decline in egg-laying intensity during the early weeks. The level of MDA in blood serum increased by 108% ($p < 0.01$) during the first week, while the vitamin E concentration in eggs from Group 3 decreased by 52% after the first month. Nevertheless, hens in Group 3 exhibited better physiological adaptation by the end of the experiment, with their indicators approaching those of the control group.

When hens received mustard oilmeal together with elevated doses of the AO santoquin and vitamin E, significant metabolic disturbances were prevented. The concentrations of LPO products were comparable to the control throughout the study duration; however, carotenoid, vitamin A, and vitamin E contents in eggs and liver were higher. In addition, the hens of Group 4 showed no initial decline in egg production. By the end of the trial, the laying intensity exceeded the control by around 4–5%, albeit with a mean egg weight about 1 g lower.

The addition of methionine and glucose with mustard oilmeal exerted a distinct protective effect, with egg production staying stable and not decreasing compared to the control. Vitamin storage levels in eggs of Group 5 hens were comparable to those of the control. However, the intensity of LPO processes increased significantly, with MDA concentrations in serum and muscles remaining similar to Group 2. The hepatic concentrations of vitamins A and E were below control values. Therefore, although productivity losses were prevented, the metabolic profile revealed impaired

AO protection and elevated oxidative sensitivity. Methionine and glucose likely contributed to the formation of glucuronic conjugates with mustard oils, facilitating their elimination from the body, but were insufficient to completely neutralize their toxic effects.

3.3. Integrated Analysis of Diet Treatment Effects

To assess the overall impact of diet treatments on productivity and AO response, a multivariate analysis combining principal component analysis (PCA) and hierarchical clustering was conducted (Figure 2).

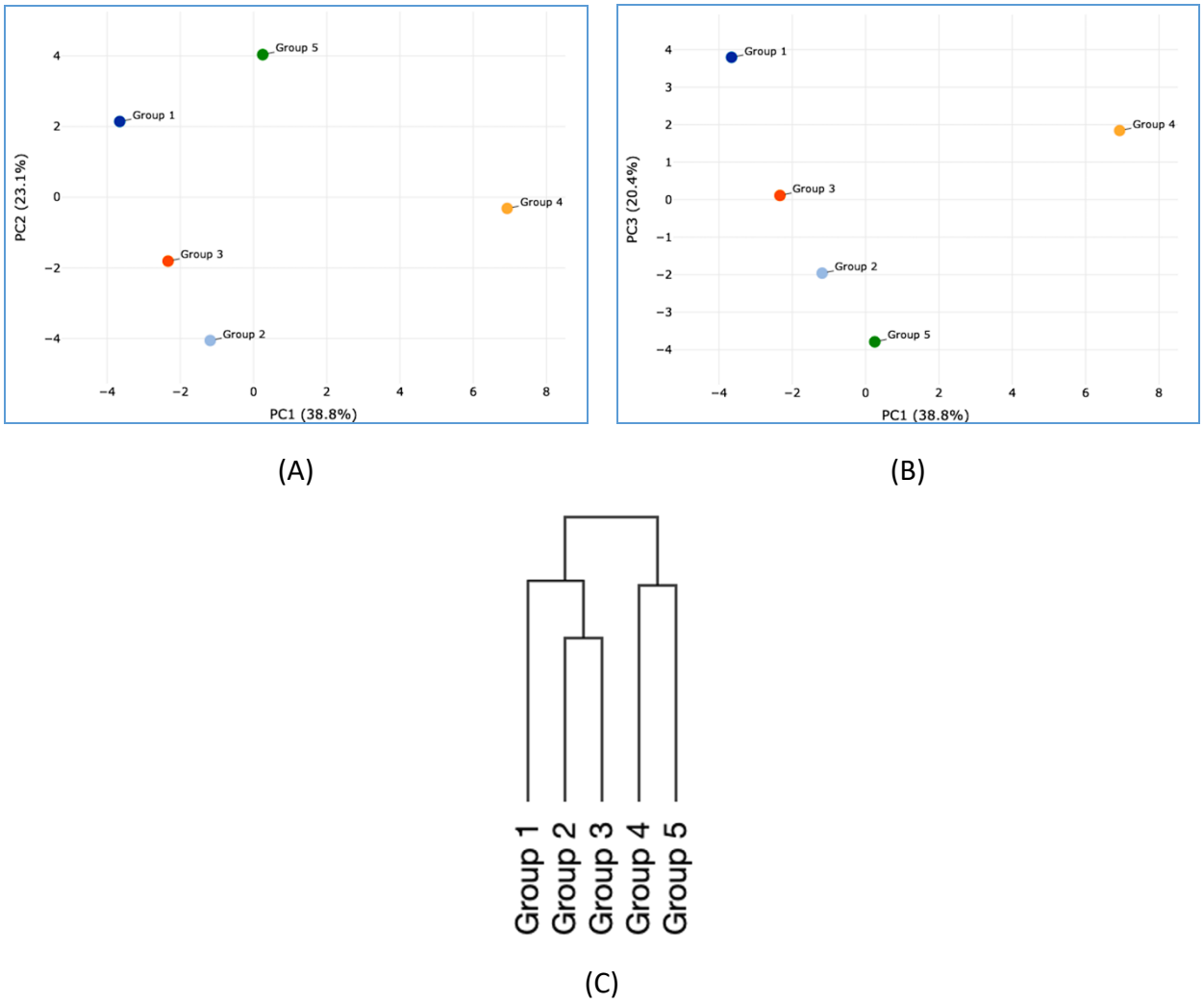

Figure 2. Principal component analysis (PCA) and hierarchical clustering tree showing the combined effects of dietary treatments on productivity and antioxidant response in control (Group 1) and experimental (Groups 2–5) laying hens. (A, B) PCA plots; the *X*- and *Y*-axes represent principal components 1 (PC1) and 2 (PC2) or 3 (PC3), which explain 38.8% and 23.1% or 20.4% of the total variance, respectively. (C) Hierarchical clustering tree constructed using the Euclidean distance metric and the average linkage method.

On the PCA plots (Figure 2A, B) and hierarchical clustering dendrogram (Figure 2C), all five layer groups were clearly separated, confirming distinct physiological responses to the different dietary

treatments. Principal components 1 and 2 (PC1, PC2) explained 38.8% and 23.1% of the total variance, respectively, while PC3 accounted for 20.4%. The spatial separation of groups on the PCA map and clustering tree confirmed pronounced, systematic differences between the control group (Group 1) and the experimental groups (2–5).

These results provide compelling evidence that the supplementation of mustard oilmeal and associated dietary treatments significantly affects productive and biochemical parameters in laying hens. The observed PCA and hierarchical clustering patterns (Figure 2) of the effects of these dietary treatments on the performance of laying hens and on the AO response also reflected the degree of physiological adaptation. That is, hens receiving and AO fortification (Group 4) were more similar to the control, while groups exposed to the unmitigated (Group 2) and the methionine + glucose-mitigated (Group 5) mustard oilmeal toxicity had the greatest deviation. This appears to confirm the protective manner of AO supplementation (Group 4) and methionine + glucose detoxification (Group 5).

Our observations are in line with other studies that tested mustard oilmeal in poultry nutrition. For example, inclusion of 10–20% of *B. juncea* meal in chicken diets did not affect egg production, egg quality characteristics, feed consumption, feed efficiency and mortality of Lohmann LSL-Lite laying hens (Savary et al., 2017, 2019). On the other hand, our data on the selective mitigating effect of certain feed additives may differ from the studies by Savary et al. (2017, 2019) who used a supplemental enzymatic (phytase + multicarbohydrase) treatment of the meal. As shown in Japanese quail (Malik & Lone, 2011; Malik et al., 2012), 5–25 % mustard seed meal included in the diets had no toxic/detrimental effects on the growth, liver weight and hepato-somatic index of birds. Thus, the previous and our research suggests the efficiency/safety of mustard meal-based ration usage in poultry nutrition.

4. CONCLUSIONS

This study investigated the effects of mustard meal xenobiotics (mustard oils) both on the productivity and on the AO system of laying hens. It identified additives to the feed that are capable of normalizing metabolic processes. Ingested mustard meal xenobiotics led to notable metabolic disturbances, firstly by intensifying the activation of LPO and secondly by altering AO defense mechanisms. These xenobiotics had a role as stress factors that led to a temporary

reduction in productive traits and vitamin concentrations. The hens demonstrated an adaptive capacity to some degree to mustard meal exposure. This was further enhanced by feed additives that promoted metabolism activation, detoxification and/or AO protection. The addition of AOs santoquin + vitamin E demonstrated the highest protective efficacy, and this helped maintain metabolic balance while minimizing oxidative damage. The sorbent Sorbover and the methionine + glucose supplement acted to maintain the levels of egg production that were comparable to the control. However, their effects on AO normalization were less pronounced. In total, the dietary inclusion of mustard meal at 5% level may be feasible when in combination with suitable protective additives (especially santoquin + vitamin E) to ensure both productive stability and physiological resilience in laying hens.

In terms of our study limitations, these may include sample size, region-specific mustard meal, and the fact that we did not tested treatment variants with >5% oilmeal inclusion. Therefore, future research directions should be focused on expanding experimental conditions/treatments and validating the conclusions made in this research.

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Institutional Review Board Statement: Experiments on laboratory animals using mustard meal were conducted by permission of the Animal Ethics Committee of the H.S. Skovoroda Kharkiv National Pedagogical University. This is guided by the provisions of the “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” (Strasbourg, March 18, 1986).

Transparency: The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics. All content was reviewed and verified by the authors.

Data Availability Statement: Upon a reasonable request, the supporting data of this study can be provided by the corresponding author.

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Authors' Contributions: **Igor A. Ionov**, **Olena V. Gaviley** and **Tetiana I. Fotina** designed the research and carried out the experiments. **Igor A. Ionov** and **Olena V. Gaviley** conducted laboratory analysis. **Igor A. Ionov**, **Tetiana I. Fotina** and **Darren K. Griffin** supervised the research project. **Igor A. Ionov**, **Tetiana I. Fotina** and **Michael N. Romanov** conducted data interpretation. **Darren K. Griffin** and **Michael N. Romanov** provided data analysis support. **Tetiana I. Fotina** and **Michael N. Romanov** were involved in the review of the literature. **Igor A. Ionov** and **Michael N. Romanov** wrote the initial draft. **Darren K. Griffin** and **Michael N. Romanov** made the manuscript revision. **Tetiana I. Fotina**, **Darren K. Griffin** and **Michael N. Romanov** conducted proofreading. All authors contributed to writing and revising the manuscript, have read and agreed to the published version of the manuscript.

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