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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.

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 plus vitamin E as the most effective dietary protector against oxidative stress caused by mustard meal inclusion in poultry diets.

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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 (Ayadi, Debouba, Rahmani, & Bouajila, 2022; Sharma, Garg, Sharma, & Rai, 2024; Sharma et al., 2024). Its use in the food production industry has been gradually increasing because of the nutritional and functional properties of mustard seeds, which contain a wide range of biologically active components. 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 (Ayadi et al., 2022; Grygier, 2023; Sharma, Verma, Gupta, Neelabh, & Dwivedi, 2019). Numerous studies (for example, Akhtar & Khan, 2024; Ayadi et al., 2022; Lietzow, 2021) provided a comprehensive overview of the hazardous compounds contained within mustard seeds and subsequently assessed the potential health risks they present.

Given that they are by-products of mustard oil extraction, seed oilmeal and cake are valuable sources of plant protein for use in animal nutrition. This is particularly relevant for countries in Asia (Sarker, Saha, Begum, Zaman, & Rahman, 2015). Mustard cake inclusion in poultry diets has been shown to enhance growth, egg production performance, and 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, Xiao, & Cockell, 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, Classen, & Tyler, 1997). Oryschak, Smit, and Beltranena (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 & Alwan, 2022; Abdulameer, Hamzah Ajeel, & Bakir Al-Hilli, 2021).

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, Arslan, & Oksuz, 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, Gairola, Punetha, & Gangola, 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 fermented, leads to a reduction in glucosinolates and phytate phosphorus concentrations. It also increases turkey body weight, while not impairing metabolic or 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, Ognik, Zaworska, Ferenc, & Jankowski, 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) (Klotz & Steinbrenner, 2017; Shakhov, Argunov, & Buzlama, 2003). The interaction between oxygen and phospholipids of the membrane in the presence of oxidative radicals initiates lipid peroxidation (LPO) activation (Onur & Ayhanci, 2021). Indeed, the stability during the evolution of living systems under oxidative conditions is maintained by antioxidant (AO) mechanisms that keep lipid peroxides low. The balance between LPO and AO activity is therefore dynamic and can change, especially when subjected to stress. This shift can lead to an increase in oxidative reactions, potentially resulting in cellular damage and dysfunction (Abd El-Aal, 2012; El-Beltagi & Mohamed, 2013; Valgimigli, 2023).

The increased activity of free radicals occurs under both pathological and high-metabolism 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, Öztürk, Bozkaya, & Tarhan, 2000). The underlying risk involves both LPO activation leading to depletion of antioxidant reserves and disruption of oxidative balance. Maintaining optimal antioxidant status is fundamental for sustaining health and productivity (Ponnampalam et al., 2022; Surai, 2020). Dietary fats increase feed energy but can become pro-oxidative if oxidized, and this can initiate LPO activation (Wang, Bottje, Maynard, Dibner, & Shermer, 1997; Zamora, Alaiz, & Hidalgo, 1997). Contemporary poultry diets contain 2–6% fat, which can rise to 10% in broiler feeds (Pesti, Bakalli, Qiao, & Sterling, 2002). Too little fat reduces the utilization of proteins, whereas oxidized fat can lead to the accumulation of toxic aldehydes and peroxides (Geng, Liu, & Zhang, 2023; Vieira, Zhang, & Decker, 2017). It follows that examining fats from different sources and containing different states of oxidation is crucial to understanding their effects on lipid peroxidation activity, antioxidant system, and overall productivity (Durand, Scislowski, Gruffat, Chilliard, & Bauchart, 2005; Kujoana, Mabelebele, & Sebola, 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, Rumyantsev, Kulakov, Morozov, & Ievlev, 1999). The toxicity of these depends on dosage, exposure duration, and organism adaptability (Bratishko, Gaviley, Pritulenko, & Tereshchenko, 2008). Negative impacts and risks can be mitigated by technological processing, enzymatic treatment, and/or supplementation with adaptogenic additives. The thermal processing of soybeans inactivates antinutritional factors (Badjona, Bradshaw, Millman, Howarth, & Dubey, 2023) while enzyme preparations improve the digestion of polysaccharides (Choct, 2006), and the fortification of methionine and iodine enhances the efficiency of rapeseed product utilization (Duborská, Šebesta, Matulová, Zvěřina, & Urík, 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 remains 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 reflects oxidative stress activation (Kichun, Vishchur, Skorokhid, & Kvachov, 2001; Tanir, Cekic, Kirhan, Dirican, & Kilic, 2021). The impact of vaccine metabolism can vary according to type (live, inactivated, mono-, or polyvalent) and age of the animal. Food additives, particularly vitamins A, E, and C, are key to improving the effectiveness of the immunization process (Aslam, Muhammad, Aslam, & Irfan, 2017; Kolb & Seehawer, 2001; McDowell, 2000; Shastak & Pelletier, 2023, 2024; Van Hieu, Guntoro, Qui, Quyen, & Al Hafiz, 2022). It is crucial, however, to balance their dosage because too high levels can cause antagonistic effects and/or immunosuppression (Fernández-Villa, Aguilar, & Rojo, 2019; Kurtyak & Yanovich, 2004; Muir, Husband, & Bryden, 2002; Schoendorfer & Davies, 2012). Other promising additives include plant and microbial preparations as well as selected amino acids and microelements (Dietert & Golemboski, 1998; Ghadban, 2002; Lee, Austic, Naqi, Golemboski, & Dietert, 2002; Nys, 2001).

Feed and technological stressors primarily disrupt the AO defense system. Identifying vulnerable links and implementing corrective strategies allows for the stabilization of metabolic processes, the prevention of pathological changes, as well as the improvement of productivity and immune responses. Preprocessing of feed combined with compounds that facilitate xenobiotic metabolism can further enhance adaptation and detoxification. Vermiculite has been established as 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, Syvachenko, & Smetanina, 2023). The inclusion of vermiculite up to 5% in quail diets improved the mineral and amino acid composition of meat and increased overall productivity (Apdraim, Sarsembayeva, & Lozowicka, 2023).

Given the growing evidence supporting the hypothesis that LPO activation represents an initial link in the stress response mechanism (Chen, Weiskirchen, & Weiskirchen, 2023), it can be assumed that strengthening the AO system enhances the adaptability of birds to xenobiotic stress. Our previous studies (Svjezhentsov, Nedosek, Ionov, & Bratishko, 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, Ionov, & Gaviley, 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 us to conclude regarding treatment effects and the 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.

Note: 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" (Agronom.Info, 2025; Belvet, 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 green manure that can improve soil fertility. Sticking to optimal sowing dates and controlling weeds on time 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., Calik, 2017, 2018; Garamvölgyi & Sütő, 2021; Semerdzhiev, Sandev, Nikolova, Yarkov, & Tanchev, 2005; 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 the 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 stress resistance.
- Santoquin with vitamin E was applied to strengthen the AO defense system.
- Methionine in combination with glucose was applied to inactivate adverse factors via the mechanism 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, 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 minutes 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. The concentrations of general biochemical parameters in serum and liver were measured using standard methods (e.g., Bora, Gurram, & Sagi, 2017; Hadwan et al., 2024; Hoekstra et al., 2013; Kadhum & Hadwan, 2021; Maurer, 2011).

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 (Baydevlyatova, Ogurtsova, Shomina, & Tereshchenko, 2009; Egorov, Chesnokova, Ivachnick, Papazyan, & Surai, 2007; Surai, Fisinin, & Karadas, 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, Rozbicka-Wieczorek, Więsyk, Smulikowska, & Czauderna, 2014). Serum protein concentration (%) was determined by the Lowry method (Niamke et al., 2006; Shen, Xiao, Liang, Ma, & Huang, 2013; Waterborg, 2009). 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., Liu et al., 2021; Zhang et al., 2021). Specifically, vitamin A concentration (μg/g) in the liver was measured by the reaction with boron trifluoride (Bozhkov et al., 2024), while vitamin E concentration (μg/g) was determined by thin-layer chromatography (Kröpfl, Schweizer, & Vetter, 2022; Polak, 2021). Egg quality was assessed by measuring carotenoid concentration after extraction from egg yolks with acetone at 450 nm (Islam & Schweigert, 2015; Strati, Sinanoglou, Kora, Miniadis-Meimaroglou, & Oreopoulou, 2012; Wang, Wu, & Yang, 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 the non-parametric Mann–Whitney U test and Student's *t*-test (GraphPad Software, 2024). As is conventional, differences are considered significant at $p < 0.05$. The changes in biochemical parameters and vitamin content were assessed using rANOVA. Liver regenerative potential indicators were evaluated using both the 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 (Kleverov et al., 2024; Zenkova, Kamenev, Sablina, Artyomov, & Sergushichev, 2018); 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.

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.

Egg weight remained stable across all groups throughout the trial, showing no statistically significant differences between control and treatment chickens (Table 2). However, some transient deviations were observed in Groups 4 and 5, particularly during weeks when the mean egg weight decreased slightly. These deviations did not impair overall productivity.

3.2. Oxidative Processes and Vitamin Content in Tissues

Hens fed with mustard oilmeal experienced significant changes in oxidative metabolism. In the first 72 hours, the intensity of the lipid peroxidation (LPO) process 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	$39.50\pm2.57^{\uparrow *4}$	$353.25\pm66.00^{\uparrow *2,4}$	4.85±0.13
	2	$37.00\pm1.60^{\uparrow *4}$	$143.00\pm6.56^{\downarrow *1,***5}$	5.06±0.23
	3	$41.25\pm2.60^{\uparrow *4,5}$	232.40±64.56	5.50±0.31
	4	$32.00\pm1.02^{\downarrow *1,2,3}$	$168.00\pm24.10^{\downarrow *1,**5}$	4.77±0.31
	5	$34.12\pm0.94^{\downarrow *3}$	$376.50\pm35.46^{\uparrow ***2,**4}$	5.25±0.21
7 days	1	$44.80\pm1.30^{\downarrow **4,5}$	$278.50\pm45.80^{\downarrow *2,**3,5}$	6.11±0.37
	2	$49.80\pm2.10^{\downarrow *4,5}$	$442.70\pm18.12^{\uparrow *1,**4,\downarrow *3}$	6.23±0.37
	3	$43.50\pm4.99^{\downarrow *4,5}$	$566.00\pm39.00^{\uparrow **1,*2,5***4}$	6.53±0.29
	4	$67.50\pm5.40^{\uparrow **1,*2,3}$	$268.00\pm35.70^{\downarrow **2,5,***3}$	5.96±0.18
	5	$66.60\pm5.55^{\uparrow **1,*2,3}$	$449.00\pm16.50^{\uparrow **1,4\downarrow *3}$	5.81±0.26
14 days	1	$51.20\pm2.94^{\downarrow **3}$	364.50±40.15	$4.74\pm0.34^{\downarrow **5}$
	2	$56.60\pm2.31^{\downarrow *3}$	325.50±22.10	$4.41\pm0.40^{\downarrow **5}$
	3	$64.25\pm1.28^{\uparrow **1,*2}$	$303.75\pm38.20^{\downarrow *5}$	5.47±0.47
	4	48.00±8.70	361.88±51.30	$4.94\pm0.52^{\downarrow *5}$
	5	63.50±10.15	$456.50\pm30.60^{\uparrow *3}$	$6.34\pm0.26^{\uparrow **1,2,*4}$
28 days	1	37.15±4.15	204.10±54.10	$6.24\pm0.30^{\uparrow *4}$
	2	37.00±4.92	$302.20\pm25.70^{\uparrow **3,*4}$	$6.15\pm0.23^{\uparrow *4}$
	3	34.50±4.74	$208.50\pm3.77^{\downarrow **2,5}$	5.82±0.29
	4	40.50±4.50	$211.40\pm17.19^{\downarrow *2,**5}$	$5.32\pm0.22^{\downarrow *1,2}$
	5	32.00±6.75	$334.50\pm30.30^{\uparrow **3,4}$	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 antioxidants 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	$325.22\pm5.75^{\uparrow **4,5}$	$7.29\pm0.79^{\uparrow *2}$	144.00±8.48	$243.75\pm15.39^{\downarrow *2,5}$
2	$1060.00\pm64.81^{\downarrow *4}$	$332.40\pm6.51^{\uparrow ***4,***5}$	$5.01\pm0.23^{\downarrow *1,3,4}$	200.40±23.2	$309.00\pm23.60^{\uparrow *1}$
3	1155.00±183.00	$315.12\pm3.94^{\uparrow *4,***5}$	$6.26\pm0.35^{\uparrow *2,\downarrow *4}$	163.30±5.42	276.56±14.63
4	$1530.00\pm98.15^{\uparrow **2,5}$	$250.08\pm20.78^{\downarrow ***1,2,*3}$	$9.88\pm1.44^{\uparrow *2,3,5}$	163.70±23.20	259.25±25.88
5	$1070.00\pm100.20^{\downarrow *4}$	$229.68\pm10.76^{\downarrow ***1,2,3}$	$5.57\pm0.79^{\downarrow *4}$	159.90±13.40	$294.38\pm10.73^{\uparrow *1}$

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 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 remaining stable and not decreasing compared to the control. Vitamin storage levels in the eggs of Group 5 hens were comparable to those of the control. However, the intensity of lipid peroxidation (LPO) processes increased significantly, with malondialdehyde (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 antioxidant (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).

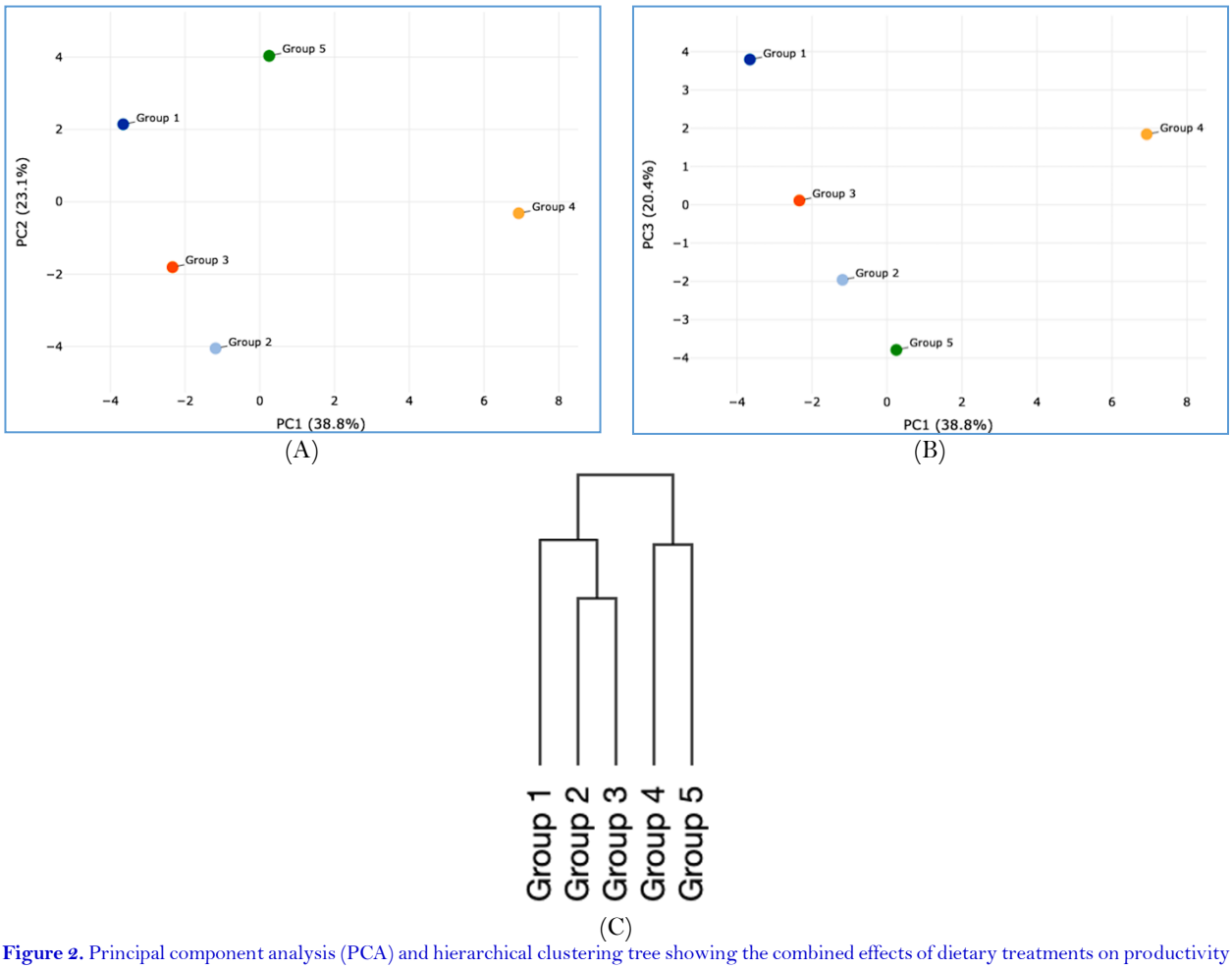

Figure 2. Principal component analysis (PCA) and hierarchical clustering tree showing the combined effects of dietary treatments on productivity and antioxidant response in control (Group 1) and experimental (Groups 2–5) laying hens. (A, B) PCA plots; the *X*- and *Y*-axes represent principal components 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 affect 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 reflect the degree of physiological adaptation. That is, hens receiving AO fortification (Group 4) were more similar to the control, while groups exposed to unmitigated (Group 2) and methionine + glucose-mitigated (Group 5) mustard oilmeal toxicity had the greatest deviation. This appears to confirm the protective effect 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, MacIsaac, Rathgeber, McLean, & Anderson, 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. (2019), who used a supplemental enzymatic (phytase + multicarbohydrase) treatment of the meal. As shown in Japanese quail (Malik & Lone, 2011), 5–25% mustard seed meal included in the diets had no toxic or detrimental effects on the growth, liver weight, and hepato-somatic index of birds. Thus, our previous research suggests the efficiency and safety of mustard meal-based ration usage in poultry nutrition.

4. CONCLUSIONS

This study investigated the effects of mustard meal xenobiotics (mustard oils) on both the productivity and the antioxidant (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, first by intensifying the activation of lipid peroxidation (LPO) and second by altering antioxidant defense mechanisms. These xenobiotics acted as stress factors, resulting in a temporary reduction in productive traits and vitamin concentrations. The hens demonstrated an adaptive capacity to some degree to mustard meal exposure. This adaptation 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, helping to maintain metabolic balance while minimizing oxidative damage. The sorbent Sorbover and the methionine + glucose supplement helped maintain egg production levels comparable to the control. However, their effects on AO normalization were less pronounced. Overall, the dietary inclusion of mustard meal at a 5% level may be feasible when combined 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 test treatment variants with >5% oilmeal inclusion. Therefore, future research directions should focus on expanding experimental conditions and treatments and validating the conclusions made in this research.

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Institutional Review Board Statement: Experiments involving laboratory animals using mustard meal were conducted with the permission of the Animal Ethics Committee of the H.S. Skovoroda Kharkiv National Pedagogical University. These experiments adhere to 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.
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