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Effects of Grape Seed Proanthocyanidin Extract (Vinox) on Growth Performance, Immune Indices, Antioxidant Status, and Oxidative Stress in Broiler Chickens

Wahab Izadi[1*], Salahadin Khodamoradi[1]

1. Institute of Agriculture, Water, Food and Nutraceuticals, Mah.C., Islamic Azad University, Mahabad, Iran

*** Corresponding author email address:** wahabizadi@iau.ac.ir

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A B S T R A C T

Natural polyphenolic antioxidants are increasingly investigated as alternatives to synthetic antioxidants and antibiotic growth promoters in poultry nutrition. Objective: This study evaluated the effects of dietary grape seed proanthocyanidin extract (GSPE; Vinox) on growth performance, antioxidant status, oxidative stress, and selected immune-related indices in Ross 308 broiler chickens. Birds were allocated to four dietary treatments: basal diet, basal diet plus 0.05% Vinox, basal diet plus 0.10% Vinox, and basal diet plus 0.15% Vinox. The experimental allocation was four treatments with three replicate pens and 10 birds per pen. Growth performance was recorded through 42 days. Serum superoxide dismutase (SOD), glutathione peroxidase (GPx), ferric reducing antioxidant power (FRAP), and malondialdehyde (MDA) were assessed on days 28 and 42, together with relative bursa and spleen weights and peripheral lymphocyte percentage. Data were analyzed using a general linear model and Duncan's multiple-range test. Dietary 0.10% and 0.15% Vinox were associated with greater average daily gain and lower feed conversion ratio than the basal diet ($p < .05$), whereas feed intake did not differ significantly. Vinox supplementation increased SOD, GPx, and FRAP and decreased MDA. Relative bursa and spleen weights and lymphocyte percentage were also higher in supplemented groups. Under the reported experimental conditions, dietary GSPE was associated with improved growth efficiency, antioxidant status, and selected immune-related indices. These findings should be interpreted cautiously because the experimental protocol did not permit complete separation of dietary effects from the IBD vaccination procedure.

Keywords: *broiler chicken; grape seed proanthocyanidins; antioxidant enzymes; oxidative stress; immunity; growth performance*

1. Introduction

The poultry industry has traditionally used antimicrobial growth promoters to support feed efficiency and disease control. Concerns regarding antimicrobial resistance and restrictions on non-therapeutic antibiotic use have intensified the search for natural feed additives that can support productivity without contributing to antimicrobial selection pressure. Phytogenic compounds, particularly plant-derived polyphenols, are of interest because of their

antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties.

Rapid growth and high metabolic activity make modern broilers susceptible to oxidative imbalance. Heat stress, vaccination, infectious challenge, dietary oxidants, and intensive production can increase reactive oxygen species and lipid peroxidation. Excessive oxidative stress can damage cellular lipids, proteins, and nucleic acids and may impair nutrient utilization, immune function, and productive performance. Endogenous defense systems include enzymes such as superoxide dismutase and glutathione peroxidase, whereas malondialdehyde is commonly used as a marker of lipid peroxidation.

Grape seeds are rich in flavan-3-ols and oligomeric proanthocyanidins. These compounds can scavenge reactive species, chelate transition metals, reduce lipid oxidation, and influence redox-sensitive signaling pathways. Experimental work in poultry has shown that grape seed proanthocyanidin extract can improve antioxidant balance under infectious or toxicological stress, although effects on growth performance are heterogeneous and appear to depend on dose, product composition, bird genotype, and challenge conditions (1-3).

Recent evidence reinforces the need for dose-specific interpretation. Dietary grape proanthocyanidins at moderate concentrations have improved plasma antioxidant activity and intestinal morphology, whereas higher concentrations have not consistently produced additional benefits and may adversely affect selected biochemical indices (4). Studies using grape seed or fermented grape seed have also reported improvements in antioxidant capacity and selected performance outcomes (5). Conversely, a 2026 meta-analysis found no overall significant effect of grape products on growth, immunity, or oxidative balance, while identifying substantial heterogeneity across product forms and outcomes (6).

Vinox is a commercial grape seed proanthocyanidin preparation. The present study examined whether graded dietary levels of Vinox affected growth performance, serum antioxidant and oxidative indices, and selected immune indicators in broiler chickens. The hypothesis was that Vinox supplementation would improve redox status and immune indices and thereby support growth performance.

2. Methods and Materials

2.1. Study Site, Birds, and Husbandry

The experiment was conducted in a commercial poultry facility in Mahabad, West Azerbaijan Province, Iran. One-day-old Ross 308 broiler chicks were obtained from a commercial hatchery and reared for 42 days. Birds had ad libitum access to feed and water, and lighting and ventilation were managed according to Ross 308 recommendations. The experimental allocation comprised four treatments, three replicate pens per treatment, and 10 birds per pen, corresponding to 120 experimental birds.

2.2. Experimental Diets

Birds were assigned to a completely randomized dietary design with four treatments: (1) basal diet without Vinox, (2) basal diet plus 0.05% Vinox, (3) basal diet plus 0.10% Vinox, and (4) basal diet plus 0.15% Vinox. The diets were formulated for starter (days 1-21) and grower (days 22-42) phases using NRC recommendations and UFFDA software. Vinox replaced an equivalent quantity of inert carrier.

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Table 1

Composition and calculated nutrient content of the experimental diets

Ingredient or nutrient	Starter, days 1-21	Grower, days 22-42
Corn	54.60	62.30
Soybean meal, 44% CP	38.90	31.00
Soybean oil	1.50	2.00
Dicalcium phosphate	1.65	1.70
Oyster shell powder	1.12	1.12
Salt	0.25	0.25
Vitamin premix	0.25	0.25
Mineral premix	0.25	0.25
DL-Methionine	0.26	0.37
L-Lysine	0.20	0.31
L-Threonine	0.07	0.15
Enzyme preparation	0.05	0.05
Vinox	0-0.15	0-0.15
Metabolizable energy, kcal/kg	3000	3150
Crude protein, %	22.0	19.0
Calcium, %	0.90	0.65
Available phosphorus, %	0.65	0.65
Methionine, %	0.78	0.78
Lysine, %	1.05	1.05

Note. Values are percentages unless otherwise indicated.

2.3. *Infectious Bursal Disease Procedure*

On day 18, birds in the Vinox-supplemented groups received a live intermediate-strain infectious bursal disease vaccine at 1.5 doses per bird by ocular administration, whereas the basal-diet control received physiological saline. Because challenge exposure was not identical across dietary groups, the resulting group differences cannot be attributed exclusively to Vinox supplementation. Accordingly, the vaccination procedure is described as a study limitation rather than as an independently tested challenge effect.

2.4. *Blood and Tissue Sampling*

Blood was collected from the wing vein after a 12-hour feed withdrawal on days 28 and 42. Serum was separated by centrifugation at 1500 rpm for 10 minutes and stored at -20°C until analysis. Relative weights of the bursa of Fabricius and spleen were calculated as percentages of live body weight, and peripheral blood lymphocyte percentage was determined from Giemsa-stained blood smears.

2.5. *Antioxidant and Oxidative Stress Assays*

Glutathione peroxidase and superoxide dismutase activities were measured spectrophotometrically using commercial RANDOX kits. Total antioxidant capacity was assessed using the ferric reducing antioxidant power assay at 593 nm (7), and serum malondialdehyde was measured using a thiobarbituric acid-based method at 532 nm (8).

2.6. *Growth Performance*

Body weight and feed consumption were recorded weekly. Average daily gain, average daily feed intake, and feed conversion ratio were calculated for the stated production periods. Mortality was recorded daily.

2.7. *Statistical Analysis*

Data were analyzed using the general linear model procedure in SPSS version 22 for a completely randomized design. Treatment means were compared using Duncan's multiple-range test at $p < .05$. The pen was considered the experimental unit for growth-performance outcomes. Antioxidant and immune-related outcomes were analyzed separately at each sampling time. Dose-response effects were interpreted as treatment-group differences because formal polynomial contrasts were not performed.

3. Findings and Results

3.1. *Growth Performance*

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Table 2

Effects of dietary Vinox on broiler growth performance

Outcome	Control	0.05% Vinox	0.10% Vinox	0.15% Vinox
Average daily gain, days 1-28, g	54.4 ± 1.8^{b}	61.8 ± 0.9^{ab}	63.7 ± 1.2^{a}	64.1 ± 1.5^{a}
Average daily gain, days 29-42, g	58.4 ± 1.6^{b}	63.8 ± 1.4^{ab}	65.2 ± 1.8^{a}	66.3 ± 2.1^{a}
Average daily feed intake, days 1-42, g	112.4 ± 2.1	115.8 ± 1.8	116.5 ± 2.3	117.2 ± 1.9
Feed conversion ratio, days 1-42	2.10 ± 0.04^{a}	2.01 ± 0.03^{ab}	1.97 ± 0.02^{b}	1.95 ± 0.03^{b}

Note. Values are reported as mean ± standard error. Within a row, values with different superscripts differ at $p < .05$.

Broilers receiving 0.10% or 0.15% Vinox had higher reported average daily gain and lower feed conversion ratio than control birds. Average daily feed intake did not differ significantly among treatments.

3.2. Antioxidant Status and Lipid Peroxidation

Table 3

Effects of dietary Vinox on serum antioxidant and oxidative indices

Outcome	Control	0.05% Vinox	0.10% Vinox	0.15% Vinox
SOD, day 28	12.4 ± 0.6^{c}	15.8 ± 0.8^{b}	18.2 ± 0.9^{a}	19.1 ± 1.0^{a}
GPx, day 28	8.2 ± 0.5^{c}	10.5 ± 0.6^{b}	12.3 ± 0.7^{a}	13.1 ± 0.8^{a}
FRAP, day 28	0.62 ± 0.04^{b}	0.75 ± 0.05^{ab}	0.88 ± 0.06^{a}	0.92 ± 0.07^{a}
MDA, day 28	3.8 ± 0.3^{a}	2.9 ± 0.2^{b}	2.1 ± 0.2^{c}	1.9 ± 0.2^{c}
SOD, day 42	8.8 ± 0.5^{c}	12.1 ± 0.7^{b}	15.0 ± 0.9^{a}	16.4 ± 0.8^{a}
GPx, day 42	5.9 ± 0.4^{c}	8.8 ± 0.6^{b}	10.7 ± 0.7^{a}	11.8 ± 0.6^{a}
FRAP, day 42	0.42 ± 0.03^{b}	0.58 ± 0.04^{ab}	0.72 ± 0.05^{a}	0.79 ± 0.06^{a}
MDA, day 42	5.2 ± 0.4^{a}	4.1 ± 0.3^{b}	3.2 ± 0.3^{c}	2.8 ± 0.2^{c}

Note. SOD, superoxide dismutase; GPx, glutathione peroxidase; FRAP, ferric reducing antioxidant power; MDA, malondialdehyde. Values are mean ± standard error. Within a row, values with different superscripts differ at $p < .05$.

Vinox supplementation was associated with higher SOD, GPx, and FRAP values and lower MDA values at both reported sampling times. The largest differences were generally observed in the 0.10% and 0.15% groups. Because no formal dose-trend analysis was reported, these results are presented as treatment-group differences rather than as a confirmed linear response.

3.3. Immune-Related Indices

Table 4

Effects of dietary Vinox on selected immune-related indices

Outcome	Control	0.05% Vinox	0.10% Vinox	0.15% Vinox
Bursa weight, % body weight, day 28	0.58 ± 0.04^{b}	0.60 ± 0.05^{ab}	0.61 ± 0.04^{ab}	0.65 ± 0.05^{a}
Spleen weight, % body weight, day 28	0.15 ± 0.02^{b}	0.18 ± 0.02^{ab}	0.20 ± 0.02^{ab}	0.22 ± 0.03^{a}
Lymphocytes, %, day 28	45.2 ± 2.1^{b}	49.8 ± 2.3^{ab}	52.5 ± 2.5^{ab}	55.1 ± 2.8^{a}
Bursa weight, % body weight, day 42	0.38 ± 0.03^{c}	0.43 ± 0.04^{b}	0.48 ± 0.04^{ab}	0.51 ± 0.05^{a}
Spleen weight, % body weight, day 42	0.09 ± 0.01^{c}	0.11 ± 0.01^{b}	0.14 ± 0.02^{ab}	0.16 ± 0.02^{a}
Lymphocytes, %, day 42	35.2 ± 2.5^{c}	40.1 ± 2.8^{b}	44.8 ± 2.9^{ab}	47.2 ± 3.0^{a}

Note. Values are mean ± standard error. Within a row, values with different superscripts differ at $p < .05$.

Relative bursa and spleen weights and lymphocyte percentage were higher in Vinox-supplemented birds, with

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the largest values generally observed in the 0.15% group. These variables were interpreted as immune-related indices rather than direct evidence of enhanced protective immunity.

4. Discussion

The findings indicate that dietary Vinox was associated with differences in redox balance, selected immune-related indices, and growth performance in broiler chickens. Supplementation at 0.10% and 0.15% was associated with greater average daily gain, lower feed conversion ratio, increased antioxidant enzyme activity, greater ferric reducing capacity, and lower malondialdehyde. However, causal interpretation should remain cautious because the vaccination procedure was not identical across all dietary groups.

Proanthocyanidins can donate hydrogen atoms or electrons to reactive species, reduce metal-catalyzed oxidation, and limit propagation of lipid peroxidation. The increased SOD and GPx activities and reduced MDA observed in the supplemented groups are consistent with earlier work showing that grape seed proanthocyanidin extract improved antioxidant balance in broilers exposed to Eimeria tenella or aflatoxin B1 (3, 9). Dietary grape proanthocyanidins have also been associated with improved plasma antioxidant activity and intestinal morphology at moderate doses (4).

The improvement in feed conversion without a significant increase in feed intake suggests a possible improvement in nutrient-use efficiency. Comparable findings have been reported for dietary grape seed or fermented grape seed in broilers, although the literature is not uniform (1, 5). A recent meta-analysis concluded that grape and grape by-products did not significantly improve pooled growth performance, immune response, or antioxidant outcomes overall, indicating that individual positive trials should be interpreted in the context of marked between-study heterogeneity (6).

The higher relative bursa and spleen weights and lymphocyte percentages may reflect altered lymphoid development or immune activation. However, organ weight and differential leukocyte counts are indirect indices and do not alone demonstrate enhanced protective immunity. Antibody titers, cytokine concentrations, lymphocyte proliferation, histopathology, and pathogen-specific outcomes would provide stronger confirmation. Previous studies have reported favorable immune and antioxidant responses to grape seed products, but these effects vary with dose, product composition, and challenge model (2, 9).

Several limitations should be considered. The study used 120 experimental birds according to the stated pen allocation, although the initial flock size was described differently in an earlier version of the protocol. The control group did not receive the same IBD vaccination procedure as the supplemented groups, which limits separation of dietary and vaccination effects. The number of birds sampled per pen, mortality outcomes, exact assay units, and detailed product composition were unavailable. Laboratory outcomes measured at two time points were analyzed separately rather than with a repeated-measures or mixed-effects model. These limitations reduce the precision and generalizability of the findings.

Future studies should use a factorial design that separates dietary treatment from infectious or vaccine challenge, include an identically challenged unsupplemented control, report the standardized proanthocyanidin content and batch information of the commercial product, use the pen as the experimental unit for performance outcomes, and apply mixed-effects models for repeated observations. Orthogonal polynomial contrasts should be used when testing dose-response trends.

5. Conclusion

Dietary Vinox at 0.10% and 0.15% was associated with improved growth efficiency, antioxidant enzyme activity, ferric reducing capacity, and selected immune-related indices, together with reduced lipid peroxidation. The results support further investigation of grape seed proanthocyanidins as natural poultry feed additives. The study does not establish product safety or equivalence to antibiotic growth promoters, and the conclusions should remain limited to the measured outcomes under the reported conditions.

Authors' Contributions

Wahab Izadi: Conceptualization, methodology, investigation, data curation, and writing-original draft. Salahadin Khodamoradi: Methodology, validation,

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interpretation, and writing-review and editing. Both authors reviewed and approved the final manuscript.

Declaration

Generative artificial intelligence was used for English translation, academic editing, structural organization, reference verification, and document formatting. It was not used to generate experimental data or statistical findings. The authors remain responsible for the accuracy and integrity of the manuscript.

Transparency Statement

The data supporting the findings may be available from the corresponding author upon reasonable request, subject to institutional requirements.

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Declaration of Interest

The authors report no conflict of interest.

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Ethics Considerations

The study was conducted in accordance with institutional and educational research requirements. Participation was voluntary, confidentiality was maintained, no identifying information was collected, and students were informed of their right to withdraw without penalty.

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