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Dietary Supplementation of Grape (*Vitis vinifera*) Seed Powder in Broiler Chicken

Kharde S.R. [a], Kadam A.S. [a], Lonkar V.D. [a*], Mugalikar D.M. [b], Patodkar V.R. [c], Bhalerao S.M. [d] and Karande V.V. [e]

[a] Department of Poultry Science, Krantisinh Nana Patil College of Veterinary Sciences, Shirwal, Maharashtra Animal and Fishery Sciences University, Nagpur-440 006, Maharashtra, India.
[b] Department of Veterinary Microbiology, Krantisinh Nana Patil College of Veterinary Sciences, Shirwal, Maharashtra Animal and Fishery Sciences University, Nagpur-440 006, Maharashtra, India.
[c] Department of Veterinary Physiology, Krantisinh Nana Patil College of Veterinary Sciences, Shirwal, Maharashtra Animal and Fishery Sciences University, Nagpur-440 006, Maharashtra, India.
[d] Department of Animal Nutrition, Krantisinh Nana Patil College of Veterinary Sciences, Shirwal, Maharashtra Animal and Fishery Sciences University, Nagpur-440 006, Maharashtra, India.
[e] Department of Veterinary Pharmacology, Krantisinh Nana Patil College of Veterinary Sciences, Shirwal, Maharashtra Animal and Fishery Sciences University, Nagpur-440 006, Maharashtra, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**Corresponding author: Email: vijaysinhlonkar@mafsu.in;*

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ABSTRACT

The experiment was carried out to study the effect of dietary supplementation of grape seed powder (GSP) in broiler chicken. A total of 240 straight-run day-old commercial broiler chicks were randomly distributed to four groups *viz.* A, B, C and D and supplemented with GSP @ 0, 5, 10 and 15g/kg feed, respectively. The supplementation of grape seed powder @ 5, 10 and 15 g/kg feed did not affect body weight, weight gain and feed intake and carcass parameters. Grape seed powder @ 5g/kg diet was beneficial to improve feed efficiency in broilers. Graded levels of grape seed powder (5, 10, and 15g/kg feed) significantly ($P < .05$) increased serum total protein and albumin level in broilers without affecting serum globulins. Grape seed powder @ 10, and 15g/kg feed helps to maintain liver health and improve antioxidant capacity in terms of serum SOD activity. The GSP levels did not influence ileal *Escherichia coli* count but improved the count of beneficial *Lactobacillus spp*. The GSP @ 5g/kg diet was economically beneficial to increase the net profit in broilers. In conclusion, it is recommended to use grape seed powder @ 5g/kg of feed in broiler diet for improving feed efficiency, gut health and net profit without altering growth and carcass parameters.

Keywords: Broiler; growth performance; superoxide dismutase; polyphenols; flavonoids.

1. INTRODUCTION

Among all the inputs provided in poultry production, feed cost contributes near about 65-70%. This necessitates screening low prices with high-quality poultry feed ingredients by considering the growing demand for animal protein. However, the alternative feed ingredient should be locally and easily available with good qualities to include in the poultry diet. Therefore, it is very important to draw attention towards the use of some locally available by-products like winery by-products, which could be a promising feed ingredient for poultry production.

Grape (*Vitis vinifera*) is one of India's commercially important fruit crops. It is a temperate crop adapted to the sub-tropical climate of the Indian subcontinent. Maharashtra is the largest producer of grapes in India, contributing almost 78.30 % of the total Indian grape production. Nashik, Sangli, Satara, and Ahmednagar are the leading districts of Maharashtra State for grape production. Nashik district is the Wine Capital of India, which has a geographical indicator for grape wine. Grapes are popular for making wine, jam, juice and jelly. Various varieties of grapes raised in the Nashik Valley for the production of white wine are Chenin blanc, Sauvignon blanc, and Thompson Seedless while for red wine varieties grown are Merlot and Shiraz. There is huge production of winery by-products like grape pomace and grape seeds in these areas.

Grape flavonoids are quercetin, catechin and proanthocyanidin. The proanthocyanidin flavonoids of grapes have 20 times and 50 times greater antioxidant potential than vitamins E and C, respectively [1]. The grape wine, grape seeds and grape by-products possess antimicrobial activities [2]. Dietary supplementation of polyphenol-rich grape seeds in poultry has been shown to alter the intestinal microflora of chickens, which can potentially increase the growth of specific beneficial bacteria and decrease the harmful bacteria in chickens [3]. By considering the grape production in Maharashtra and its antioxidant and antimicrobial properties, winery products like grape seed can be a promising option for poultry feed as a feed additive. Recent advances suggested that the use of winery by-products in the broiler feed resulted in improved broiler performance [4,5]. The current study evaluated the effect of Dietary Supplementation of Grape Seed (*Vinis vinifera*) Powder in Broiler Chicken.

2. MATERIALS AND METHODS

An experiment was conducted on 240 straight-run day-old commercial broiler chicks for a period of six weeks. The broiler birds were randomly distributed into four groups A, B, C, and D with 60 birds in each. Each treatment was subdivided into three replicates containing 20 chicks in each replicate. The control basal diet of group A was prepared as per the nutrient requirement of the commercial broiler strain used in the experiment. The Grape Seed Powder (GSP) was supplemented in the diet @ 0, 5, 10 and 15 g/kg of feed in groups A, B, C and D, respectively. The broiler pre-starter, starter and finisher diets were made iso-caloric and iso-nitrogenous and were offered to the broilers from 0-9, 10-21, 22-

42 days of age, respectively. All the groups were provided with similar environmental and managemental conditions throughout the experimental period of six weeks. The broilers were vaccinated against Ranikhet disease (*LaSota* Strain) at 7th and 28th day of age and against Gumboro disease (Intermediate strain) at 14th and 21st day of age. The grape seeds (*Vitis vinifera*) were procured from Vintage Wines Private Limited, Nashik, Maharashtra. The grape seeds were separated from grape pomace. The seeds were finely ground using a grinder and stored in plastic bags for further use. The amount of total phenolic content (TPC) in the dried grape seed powder, estimated using Folin-Ciocalteu method using gallic acid as equivalent and absorbance was measured at 760nm using UV-VIS spectrophotometer. The total flavonoid content (TFC) quantification in the grape seeds was carried out using the aluminum nitrate colorimetric method and quercetin equivalent as the reference standard and absorbance was measured at 415nm using UV-VIS spectrophotometer.

The performance parameters like body weight, weight gain, feed consumption and feed conversion ration were recorded at weekly intervals. One male and one female bird were randomly selected from each replicate and were sacrificed at the end of the experiment to study the carcass parameters. The blood samples (six samples per group) were collected at the end of experiment to study serum biochemicals. The Serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), serum total protein, albumen, globulin levels were estimated at the end of the experiment. Superoxide dismutase (SOD) activity of serum samples was estimated using Cu/Zn Superoxide Dismutase ELISA kit (Calbiochem). The *Escherichia coli* was counted on plates of MacConkey agar after incubation at 37 °C for 24 hours. The *Lactobacillus* spp. were enumerated on plates of Man–Rogosa–Sharpe agar 37 °C for 48 hours. The total bacterial population was expressed as colony-forming units per gram. The net profit per kg of live broiler birds was calculated for different groups by considering live body weight and recurring expenditure.

The data generated in a completely randomized design was analyzed by one-way ANOVA with the help of IBM SPSS Software-20. The Duncan Multiple Range Test (DMRT) post-hoc analysis was done to test the significant mean differences between the groups with significance levels defined at $P < .05$ [6].

3. RESULTS AND DISCUSSION

3.1 Total Phenolic and Flavonoid Content

Total phenolic content in the dried grape seed powder 7.07mg/g and total flavonoid content was 6.92mg/g. The total phenolic content and total flavonoid content of the grape seed seem to vary with the variety of the grape cultivars and the present results depict that the total phenolic content and total flavonoid content are within the range as reported by earlier workers [7,8]

3.2 Growth Performance

The results on growth performance parameters are depicted in Table 1. The overall body weight, weight gain and feed intake of the birds fed with GSP did not differ significantly. The overall FCR of broiler birds fed with GSP @ 5 and 10g/kg diet recorded significantly ($P < .05$) better FCR than the inclusion of 15g/kg feed and control. The birds supplemented with GSP @ 5g/kg diet recorded the best FCR among all groups. The results were in accordance with the observations of El-Kelawy et al [5], who reported significantly ($P < .05$) better FCR of broilers fed with GSP @ 0.5 and 1.0g/kg diet. Similarly, [4,9,10] reported that feed efficiency is significantly ($P < .05$) improved by dietary supplementation of grape seeds in broiler diet. Meanwhile, the supplementation of 5g/kg dietary grape seed [11] did not alter the FCR of broiler chickens. Feeding broilers with a diet containing grape seed @ 15 and 30g/kg [12], @ 7.5 and 10g/kg [13] did not have any influence on FCR of the birds, which was similar to the present finding. Moreover, observed deterioration of FCR in Cobb broilers [3] when fed with a diet containing grape seed extract @ 7.2g/kg while there was a deterioration in FCR at 40g grape seed supplementation per kg feed [4] in broiler chickens. This confirms that a higher level of inclusion of grape seed powder more than 5g/kg feed did not help to improve FCR. High levels of polyphenols could interact with proteins and form tannin-protein complexes that decrease the efficiency of nutrient utilization and depress growth performance [14,15], which may be the reason for getting better FCR at the lowest level of GSP.

Table 1. Effect of dietary supplementation of GSP on overall body weight, weight gain, feed intake and carcass parameter

Parameters	Group				P Value	CD
	A	B	C	D		
Body weight, g	2584.96 ± 59.37	2693.66 ± 49.17	2753.77 ± 28.54	2585.13 ± 23.33	0.05	-
Weight gain, g	2537.96 ± 17.4	2646.93 ± 74.51	2706.9 ± 43.08	2537.96 ± 22.73	0.14	-
Feed intake, g	4474.31 ± 31.85	4487.48 ± 11.51	4687.87± 91.77	4669.86± 20.57	0.15	-
Feed conversion ratio	1.76^{a} ± 0.003	1.69^{c} ± 0.01	1.73^{b} ± 0.009	1.76^{a} ± 0.009	0.001	0.029
% Eviscerated yield	64.39 ± 1.22	66.22 ± 0.37	66.25 ± 0.97	63.01 ± 2.82	0.44	-
% Giblet yield	4.56 ± 0.26	4.04 ± 0.13	4.39 ± 0.15	4.18 ± 0.18	0.24	-
% Ready-to-Cook yield	68.95 ± 1.15	70.26 ± 0.35	70.64 ± 0.86	67.19 ± 2.93	0.46	-
% Breast meat yield	25.12 ± 0.80	24.25 ± 3.62	26.50 ± 0.38	25.99 ± 1.45	0.86	-
% Abdominal fat	1.63 ± 0.13	1.56 ± 0.19	1.50 ± 0.14	1.81 ± 0.17	0.57	-

Means bearing different superscripts within the row differed significantly (P < .05)

3.3 Carcass Parameters

Feeding broilers with GSP @ 5, 10, and 15g/kg feed did not influence carcass parameters *viz.* abdominal fat, eviscerated yield, giblet yield, R-to-C yield, and breast meat yield of the broiler chicken (Table 1). The result of the experiment corroborated with the observations reported by [16], who found that the dietary supplementation of grape seeds to quails at 1% and 3% did not have any significant influence on dressed meat, percent giblet, and Ready-to-Cook yield. El-Din et al [17] found that feeding chickens with grape seeds @ 5, 10, and 15% had no significant influence on carcass parameters. While, the results were dissimilar to observations of [4] in broilers receiving 20g grape seeds showed significantly ($P < .05$) higher carcass yield, dressing percent, and gizzard percent.

3.4 Serum Biochemicals

Graded levels of grape seed powder (5, 10, and 15g/kg feed) significantly ($P < .05$) increased serum total protein and albumin level in broilers than control. The serum globulin levels were comparable. In agreement with present findings, Cao et al [9] reported significantly ($P < .05$) higher serum total protein and albumin in birds fed with grape seeds. While, [4,5,18] reported that feeding grape seed to broilers had no significant influence on serum total protein, albumin and globulin. Significantly ($P < .05$) higher AST and ALT were recorded in group D supplemented with 15g/kg GSP than other groups. The raised values of AST and ALT are indicators of liver dysfunction [19]. According to Ebrahimzadeh et al [18] the increased levels of serum AST and ALT may suggest that polyphenols present in grape seeds caused the hepatocellular necrosis, which leads to an increase in cell membrane permeability due to transaminase enzyme which is released in bloodstream. In contrast [5,16] reported a decrease in AST and ALT by feeding birds with grape seed. The results indicated that, it is beneficial to use 5 and 10g/kg GSP in broiler diet.

3.5 Antioxidant Activity

The dietary supplementation of 10 and 15g GSP per kg diet significantly ($P < .05$) increased the SOD activity compared to 5g/kg per kg diet and control (Table 2). In agreement with [5] GSP supplementation in broiler diet increased activities of SOD. The active compounds present in grape seeds are polyphenols and flavonoids which are effective antioxidants and enhance the plasma's potential by activating the antioxidant enzyme system against the oxidative stress caused by reactive oxidative species [20]. According to Puiggross et al [21] the procyanidins which are one of the polyphenols present in grapes, possess potential to modulate the gene expression of various antioxidant enzymes, which leads to an increase in SOD activity by scavenging reactive oxygen species like superoxide radicles. The results of the study indicated that the inclusion of GSP was beneficial in increasing antioxidant capacity.

3.6 Ileal Lactobacillus spp. and Escherichia Coli Study

The $\log_{10}$ values of cfu/g of ileal content for *E. coli* did not reveal significant ($P < .05$) differences among the different groups (Table 2). On the other hand, graded levels of GSP in broiler diet significantly increased *Lactobacillus* spp. count. Supplementation of 1 and 3% GSP [1] in Japanese Quails, 40g grape seed/kg feed [16] in Cobb-500 broilers, significantly ($P < .05$) increased the population of *Lactobacillus* spp. Lactobacilli possesses the ability to metabolize the phenolic compounds that supply the energy to cells and positively alter bacterial metabolism [22], which may be the reason for the significant increase in the population of *Lactobacilli* spp with the increase in GSP supplementation.

3.7 Economics

Net cost of production per bird (Table 3) for group A, B, C and D were 227.29, 229.29, 239.94 and 241.11, respectively. The per bird return on sale for groups A, B, C and D were 235.235, 245.154, 250.614 and 235.235, respectively. The net profit per bird for groups A, B and C was 7.94, 15.86 and 10.66, respectively. The group D has incurred the loss of Rs. 5.88 due to poor performance and higher feed cost due to higher inclusion level of grape seed powder. The net profit per kg live weight for groups A, B, and C were 3.07, 5.89, and 3.87, respectively. Both the net profit per kg and net profit per bird was maximum for the birds supplemented with grape seed powder @5g/kg feed followed by birds fed grape seed powder @ 10g/kg diet and control while, group D which is supplemented with grape seed powder @ 15g/kg diet incurred loss. El-Kelawy et al. [5] reported that supplementation of grape seeds @ 0.5 and 1g/kg of feed in broilers showed higher economic efficiency than the control group. The

Table 2. Effect of dietary supplementation of GSP on serum biochemical, SOD activity and ileal Escherichia coli and Lactobacillus spp. count

Parameters	A	B	C	D	P value	CD
Total Proteins, g/dL	4.14 ^{b} ± 0.03	4.22 ^{ab} ± 0.0	4.27 ^{a} ± 0.04	4.33 ^{a} ± 0.05	0.012	0.11
Albumin, g/dL	2.03 ^{b} ± 0.04	2.20 ^{a} ± 0.03	2.24 ^{a} ± 0.04	2.31 ^{a} ± 0.04	0.000	0.12
Globulin, g/dL	2.10 ± 0.04	2.03 ± 0.01	2.04 ± 0.03	2.02 ± 0.02	0.173	NS
AST, IU/L	55.75 ^{b} ± 0.88	54.03 ^{b} ± 1.22	56.14 ^{b} ± 0.57	60.28 ^{a} ± 0.46	0.000	2.47
ALT, IU/L	59.97 ^{b} ± 0.32	60.95 ^{b} ± 0.24	60.06 ^{b} ± 0.42	117.36 ^{a} ± 10.18	0.000	15.04
SOD concentration (ng/mL)	95.58 ^{c} ± 1.19	102.87 ^{c} ± 2.50	115.72 ^{b} ± 08.30	127.95 ^{a} ± 117.88	0.000	8.25
Escherichia coli (cfu/g)	6.98 ± 0.25	7.10 ± 0.15	6.98 ± 0.08	6.71 ± 0.07	0.356	NS
Lactobacillus spp. (cfu/g)	7.70 ^{c} ± 0.03	7.81 ^{c} ± 0.11	8.21 ^{b} ± 0.05	8.42 ^{a} ± 0.02	0.000	0.19

Means bearing different superscripts within the row differed significantly (P < .05)

Table 3. Economics of broilers fed with different levels of garpe seed powder

Particulars	A	B	C	D
Net cost of production	227.29	229.29	239.94	241.11
Average Body Weight (Kg)	2.585	2.694	2.754	2.585
Return on sale of bird @ Rs.91/kg	235.235	245.154	250.614	235.235
Net Profit/bird (Rs)	7.94	15.86	10.66	-5.88
Net Profit/kg (Rs)	3.07	5.89	3.87	-2.27

inclusion of grape seed powder @ 5 g/kg feed in broiler diets improves the net profit of broiler production.

4. CONCLUSIONS

The supplementation of grape seed powder @ 5, 10 and 15 g/kg feed did not affect body weight, weight gain and feed intake and carcass parameters. Grape seed powder @ 5g/kg diet was beneficial to improve feed efficiency in broilers. Graded levels of grape seed powder (5, 10, and 15g/kg feed) significantly ($P < .05$) increased serum total protein and albumin level in broilers without affecting serum globulins. Grape seed powder @ 10, and 15g/kg feed helps to maintain liver health and improve antioxidant capacity in terms of serum SOD activity. Grape seed powder significantly improved beneficial *Lactobacillus* spp. count. The inclusion of grape seed powder @ 5 g/kg feed in broiler diets improves the net profit of broiler production. Hence it is recommended to use grape seed powder @ 5g/kg of feed in broiler diet for improving feed efficiency, gut health and net profit without altering growth and carcass parameters.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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