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Research Article

The Influences of Black Pepper, Turmeric and Fennel Essential Oils Supplementation in Feed on Egg Quality Characteristics of Layers

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Abstract | Poultry eggs are a well-balanced sole source of protein in terms of nutrition. Concerns over the extensive use of Antibiotic Growth Promoters (AGPs), which may have driven consumer demand for antibiotic-free animal yield, have necessitated research towards a safer natural alternative, such as the use of phytobiotic essential oils (EOs). The purpose of this study was to determine the effect of phytobiotic EOs on laying hen performance, physical characteristics, and egg cholesterol content. A total of 280 birds aged 70 days were enrolled in the study, which included seven experimental diets supplemented with EOs of black pepper, turmeric, and fennel at rates of 0%, 1%, and mixed diet at 0.5%. Hen-day egg production, feed efficiency, egg volume, and shell weight were significantly improved ($p<0.05$) with the mixed diet supplemented with black pepper and turmeric. The egg shape index was considerably enhanced ($p<0.05$) while the egg cholesterol level was reduced ($p<0.05$) with 1% black pepper supplementation. However, the mixed dietary EOs treatment did not affect ($p>0.05$) albumin index and shell thickness. The results of this study showed that a 0.5% combination diet of black pepper, turmeric, and fennel increased the performance of laying birds, overall egg quality, and reduced egg cholesterol levels as well as LDL levels.

Keywords | Phytobiotics, Egg quality, Cholesterol, Impact, Feed intake

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INTRODUCTION

Poultry production is an incumbent livestock industry that contributes to the country's economy by providing unadulterated protein and essential minerals for human health and nutrition. Poultry farmers used to employ Antibiotic Growth Promoters (AGPs) to boost growth and yield in a short period, which result in an extremely stressful situation for the birds. Antibiotics are used in the poultry industry, and the quality of their eggs is questionable due to antibiotic residues in the eggs (Gilani et al., 2021, Samantaray and Nayak, 2022). According to projections from the United Kingdom, by 2050, around 10 million people would have died as a result of antibiotic-resistant bacterial infection and antibiotic residues in meat and eggs. In the United States and Europe, antimicrobial resistance is responsible for 50,000 fatalities per year. A study on the increased number of instances of antibiotic resistance cases in Asia has already been published by the World Health Organization (Alkindi et al., 2019; WHO, 2017). Also, Dietary cholesterol levels have an impact on a person's risk of atherosclerosis, cardiovascular disease, and coronary artery disease. In poultry eggs, the yolk is considered to be the richest source of cholesterol. In light of the well-known consequences, we must employ natural, safer alternatives to AGPs. (Sugiharto et al., 2020; Zhuang et al., 2020).

Essential oils (EOs) of phytobiotics as natural alternatives, could be utilized to maintain a high level of animal productivity while also decreasing cholesterol levels in poultry eggs. Thyme, garlic, ginger, black pepper, and amla have all been shown to lower cholesterol and lipoproteins (Kumar et al., 2019, Rabelo-Ruiz et al., 2021). As a result, several researchers have been encouraged to develop better egg quality with lower cholesterol content, which might be beneficial to human health. The yolk cholesterol content in poultry birds can be reduced with a targeted diet. There are few studies on the impact of black pepper, turmeric, and fennel in varied combinations and concentrations on egg cholesterol and quality (Natsir et al., 2021).

We investigated that, phytobiotics can influence the quality of poultry bird eggs. We used EOs of three commonly available phytobiotics, namely black pepper, turmeric, and fennel, in the diet of layer chicken (*Gallus gallus*) at 1% concentration and mixed EOs at 0.5% concentration each. The work aimed to determine the effects of oral dietary phytobiotic EOs on egg quality and laying bird performance.

MATERIALS AND METHODS

The research was carried out from August to December 2021 at Centurion University of Technology and Management, Bhubaneswar, Odisha. The 70-day-old layer-type chicks (n=280) were purchased from a local hatchery in the month of August. After being weighed, the birds were randomly placed in a poultry house and separated into seven groups. Each group was further divided into four replicates (each with 10 birds) in a climate-controlled environment. They were given 5 birds/ 1.5m^2 of floor space. Chicks were fed a conventional balanced diet (Table 1), as recommended by the National Research Council in 1994. Birds were given an appropriate room temperature and a 14-L: 10-D natural light cycle during the experiment. The Centurion University Ethics Committee for Experiments with Animals approved all experimental procedures related to use of live animals.

Layer eggs were collected for forty five days, from the 180th to the 225th day of age, during October to December 2021. Laying hen performance, feed consumption, laid eggs, and the number of mortality of birds were recorded every week. One group (D0) was provided a control diet and the other six groups were provided with three oral dietary phytobiotic supplements of 6 different combinations. The details is as follows: (1) D0- basal diet with no additive (control) (2) D1- basal diet plus 1% of black pepper (10g/Kg of feed), (3) D2- basal diet plus 1% of turmeric (10g/Kg of feed), (4) D3- basal diet plus 1% of fennel (10g/Kg of feed), (5) D4- basal diet plus 0.5% of black pepper plus 0.5% of turmeric (10g/Kg of feed), (6) D5- basal diet plus 0.5% of black pepper plus 0.5% of fennel (10g/Kg of feed), (7) D6- basal diet plus 0.5% of turmeric plus 0.5% of fennel EOs (10g/Kg of feed).

Egg quality parameters

The eggs from all seven experimental groups were freshly collected from the age of 180th to 225th, and each egg was labelled with the group name, number, and date of lay. The eggs were kept at 4^0 C, and the weight of the eggs, yolk, albumin, and shell were all measured separately in an analytical balance (Aczet, CY 224 by HPFS instruments India LLP, Vasai). Feed intake was determined daily in g/day. For each of the seven experimental groups, physical parameters were assessed using 10 eggs from each group in 4 replicates. The length and width (cm) of eggs, yolk width (cm), and albumin width of all eggs were measured with a vernier caliper (Mitutoyo 532, stainless steel, Ahemdabad, India) (Wijedasa et al., 2020). While egg shape index (%) was calculated using following formula

$$\text{Egg shape index (\%)} = 100 \times \frac{Width\ of\ egg}{Length\ of\ egg}$$

Egg volume was determined by the water displacement method. All eggs were broken into a plain white surface board to measure interior physical quality metrics. The thick albumen height (cm) and yolk height (cm) were measured with a tripod micrometer (Baras Spherometer, Saran SC, Delhi) (Wijedasa et al., 2020; Nuraini et al., 2019) that were used to calculate yolk and albumin indices using the following formulae.

$$\text{Yolk index} = \frac{Height\ of\ yolk}{Width\ of\ yolk}$$

$$\text{Albumin index} = \frac{Height\ of\ albumin}{Width\ of\ yolk}$$

The shell thickness was measured at three distinct locations (the upper and lower ends, and the middle) using a screw gauge (25mm, Smartlabs, India) (Kowalska et al., 2021). The Haugh Unit (HU) was determined (Lordelo et al., 2020) by using following formula i.e. described by Haugh (1937), where

$$\text{HU} = 100 \times \log(H - 1.7W^{0.37} + 7.57)$$

(H refers to the height of thick albumin (mm), W refers to the weight of egg (g))

At the end of the study, 8 eggs per replicate (32 eggs per experimental group) were collected for analysis of egg cholesterol, high density lipoprotein (HDL)-cholesterol, and low density lipoprotein (LDL)-cholesterol concentrations. For analysis, commercial test kits for cholesterol (Coral diagnostic cholesterol crest Biosystems clinical systems), HDL (Coral clinal system HDL cholesterol Ppt. Set), and LDL (Coral LDL-D cholesterol kit) were used. Absorption was recorded with a spectrophotometer (CL-1320 UV-VIS spectrophotometer, Chemiline) at 505nm according to the procedure described by Ukachukwu et al. (2017) for cholesterol, HDL and LDL concentrations.

Feed consumption were determined from the difference between the left over feed and given feed/week (Abou-Elkhair et al., 2018). The % hen-day production was determined by daily egg production records (Adebiyi et al., 2018).

$$\text{\% Hen-day production} = \frac{Total\ no.of\ egg\ laid}{No.of\ birds \times no.of\ days} \times 100$$

$$\text{Egg mass} = \frac{Egg\ production \times egg\ weight}{100}$$

$$\text{Feed efficiency} = \frac{Feed\ intake\ (g)}{Egg\ mass} \times 100$$

Essential Oils

The EOs of black pepper, turmeric, and fennel were extracted at the Centurion University Research and Development Laboratories in Paralakhemundi using the supercritical CO_2 extraction method. The dried phytobiotics were ground into fine powders with a grinder (SS Pulverizer, 3HP, India) and stored in an airtight vacuum-sealed bag. To decrease the loss of essential oils during the milling process, the phytobiotic powders were cooled (at 10 °C) in a refrigerator (SSU-168, India) for 2 hours before milling. Each phytobiotic powder (1000g) was put to the high-pressure equilibration vessel in a known proportion. A reciprocating pump was used to charge liquid CO_2 into the system at a constant flow rate of 10 ml/min, and it was compressed to the extraction pressures of 60 °C and 300 bar in 3 hours for black pepper (Tran et al., 2019, Shityakov et al., 2019), 60 °C and 250 bar for 2.5 hours for turmeric (Neves et al., 2020, Gopalan et al., 2000), and 32 °C (Hammouda et al., 2013). For each phytobiotic essential oil, the precipitated fractions were collected in a trapping flask. The collected samples were individually stored in amber bottles at 4 ^{0}C for further use. Gravimetric analysis was used to determine extraction yields.

Statistical analyses

All analysis were subjected to statistical analysis using one-way ANOVA by using SAS- Statistical Analysis System (SAS Institute Inc., 2012). Mean values were compared using Tukey's test at the significance level of $P < 0.05$. Each analysis was performed in triplicate, and the average values were used for representation.

RESULTS AND DISCUSSION

This research examined the effects of EOs from black pepper, turmeric, and fennel in the diet of *Gallus gallus*. The HDP (Hen Day Production) of birds fed the D5 (0.5% black pepper and 0.5% fennel) and D4 (0.5% black pepper and 0.5% turmeric) diets were significantly higher ($p<0.05$) than that of other experimental groups (Table 2). The results for HDP were higher than those reported by Nuraini et al. (2019). Birds fed with D4, D5, and D2 (1% turmeric) diets showed significant effects ($p<0.05$) on egg mass values, but diet D0 had the lowest HDP and egg mass (Table 2), which is supported by Phuoc et al. (2019). Egg weight and volume are two important characteristics that influence egg quality. Birds fed with D4 and D5 diets recorded the highest egg weight and volume in the current study (Table 3). The average feed intake of the birds and their egg weight increased with diets D2, D6, and D4. But experimental diets did not affect feed conversion ratio or egg production rate, which is supported by Phuoc et al. (2019) and Elnaggar et al. (2021).

Albumin weight has been shown to influence internal egg quality, eggs having thicker and higher albumin content being considered to be better (Kowalska et al., 2021). Birds fed D2 (1% turmeric), and D4 (0.5 % black pepper and 0.5 % turmeric) diets had higher albumin content (at $p<0.05$) than the other experimental groups, which is consistent with Abou-Elkhair et al. (2018). Weight, texture, hardness, and smell are all indicators of yolk quality (Wijedasa et al., 2020). Table 3 shows that the yolk weight of the eggs of the birds fed with the D4 diet was found to be (18.87±0.01g) at a significant level ($p<0.05$) with superior yolk quality, which is supported by Nuraini et al. (2019). In comparison to the D0 diet, the D6 (0.5% turmeric and 0.5% fennel) diet group had no significant influence on yolk weight, and the current study is also supported by Souza et al. (2020).

The yolk index is an indicator of the freshness of an egg; a higher yolk index suggests that the interior egg quality is much better (Abou-Elkhair et al., 2018). Table 3 shows that birds fed the D5 diet had a greater yolk height of eggs, followed by birds fed the D4 and D2 diets. Table 3 shows that adding different phytobiotics in different concentrations and mixtures did not affect the yolk index or albumin index. In this study, albumin index values differed consid

Table 1: Composition and chemical content of diets used in the experiment, g/kg

Items	Control diet	Experimental diet(D_{1}- D_{6})					
	D_0	D_1	D_2	D_3	D_4	D_5	D_6
Maize	355	353	352	353	351	355	347
Wheat	254	252	252	251	250	249	252
Soybean meal	244	242	243	243	245	243	245
Millet	40	39	40	39	40	39	38
Peanut meal	81	78	77	77	77	78	82.6
Black pepper	-	10	-	-	5	5	-
Turmeric	-	-	10	-	5	-	5
Fennel	-	-	-	10	-	5	5
DL-methionine	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Dicalcium phosphate	16.0	16.0	16.0	16.0	16.0	16.0	16.0
Sodium chloride	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin-mineral complex[1]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Metabolisable energy (MJ/kg)[2]							
Crude protein	176	181	178	177	180	179	176
Lys	8.4	8.5	8.5	8.5	8.6	8.5	8.4
Met	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Thr	6.5	6.6	6.6	6.5	6.5	6.6	6.5
Ca	39.0	40.1	40.2	39	40.1	40.1	39
Total P	3.2	3.2	3.1	3.4	3.1	3.3	3.3
Na	1.8	1.8	1.9	1.9	1.8	1.9	2.0
DEB[3] (mEq)	175	177	178.1	178	177	179	179

[1]The supplied premix/kg of diet: 3.45 mg retinyl acetate; 2 mg menadione (K3); 20 mg DL-alpha-tocopheryl acetate; 0.075 mg cholecalciferol; 2 mg thiamine; 2 mg riboflavin; 0.015 mg cyanocobalamin; 25 mg niacin; 8mg 11 mg d-pantothenic acid; 1.1 mg folic acid; 0.13 mg biotin; 12,300 IU vitamin A, 4,500 IU vitamin D3: [2]Calculated according to (Cerrate *et al.*, 2019, Barzegar *et al.*, 2019) as a sum of ME content of components; [3]Dietary Electrolyte Balance.

Table 2: Effect of Phytobiotics of varying combinations on laying bird performance

Parameters	Control diet	Experimental diet[1]					
	D_0	D_1	D_2	D_3	D_4	D_5	D_6
Average daily feed intake (g/bird)	94.67±0.12^{ab}	95.3±0.17^{ab}	96.87±0.03^{a}	92.78±0.09^{b}	97.81±0.1^{a}	98.15±0.09^{a}	94.57±0.08^{ab}
Feed efficiency	3.62±0.01^{a}	2.94±0.01^{b}	2.71±0.012^{bc}	2.91±0.011^{b}	2.51±0.01^{c}	2.53±0.011^{c}	2.87±0.013^{b}
Average egg weight (g)	34.12±0.17^{c}	40.33±0.11^{b}	42.72±0.12^{ab}	39.71±0.14^{b}	44.8±0.02^{a}	42.92±0.09^{ab}	40.85±0.07^{b}
Hen day production (%)	76.67±0.19^{d}	80.1±0.011^{c}	83.33±0.1^{b}	80.13±0.12^{c}	86.67±0.13^{ab}	90.34±0.14^{a}	80.4±0.12^{c}
Egg mass (g/day/hen)	26.15±0.45^{c}	32.33±0.21^{b}	35.72±0.12^{ab}	31.81±0.17^{b}	38.82±0.2^{a}	38.77±0.14^{a}	32.84±0.17^{b}

[1]D_0- Basal diet(BD)/ control diet; D_1- BD+1% of Black pepper; D_2- BD+1% of Turmeric; D_3- BD+ 1% of Fennel; D_4- BD+ 0.5% of Black pepper+ 0.5% of Turmeric; D_5- BD+0.5% of Black pepper+0.5% of Fennel; D_6- BD+0.5% of Turmeric+ 0.5% of Fennel.
[a-d]Different letters in the same row differ by Tukey test indicates P < 0.05 probability level.

erably between the experimental diets D2 (1 % turmeric) and D6 (0.5 % turmeric and 0.5 % fennel) at p<0.05. Taherkhani et al. (2018) found no evidence of a substantial impact of fennel. However, the findings of Bugdayci et al. (2018) on egg quality were superior to ours. The results of this experiment demonstrated that when D3 and D4 diets are added to the diet, the thickness of the eggshell increases along with the weight of the shell, which is contradictory to the findings of Zacaria et al. (2021). The enhanced eggshell quality is due to increased laying bird feed intake, which releases serum calcium from plasma protein, allowing more Ca2+ from the blood to be involved in the production of eggshells, and our findings are superior to those of Liu et al. (2020).

Table 3: Effect of Phytobiotics of varying combinations on physical egg quality parameters

Egg parameter	Control diet	Experimental diet[1]					
	D_0	D_1	D_2	D_3	D_4	D_5	D_6
Shape index (%)	62.1±0.26^{c}	77.6±0.24^{a}	72.44±0.21^{ab}	77.67±0.22^{a}	69.68±0.2^{b}	73.25±0.09^{ab}	63.26±0.19^{c}
Egg volume (ml)	31.37±0.4^{c}	43.25±0.31^{b}	44.62±0.33^{b}	48.10±0.29^{a}	48.87±0.23^{a}	49.06±0.11^{a}	48.18±0.12^{a}
Yolk index	0.14±0.00^{b}	0.15±0.00^{b}	0.22±0.01^{a}	0.14±0.00^{b}	0.2±0.01^{a}	0.23±0.00^{a}	0.2±0.00^{a}
Yolk weight (gm)	14.25±0.07^{c}	16.61±0.04^{b}	17.56±0.03^{ab}	17.94±0.1^{a}	18.87±0.01^{a}	16.34±0.04^{b}	14.63±0.05^{c}
Albumin weight (gm)	19.7±0.1^{c}	22.23±0.12^{ab}	23.71±0.00^{a}	23.21±0.01^{a}	23.87±0.01^{a}	22.25±0.01^{ab}	21.37±0.00^{b}
Albumin index	0.08±0.00^{a}	0.07±0.00^{ab}	0.09±0.00^{a}	0.06±0.01^{b}	0.07±0.00^{ab}	0.08±0.00^{a}	0.08±0.01^{a}
Shell thickness	0.054±0.00^{a}	0.048±0.00^{ab}	0.044±0.01^{b}	0.041±0.00^{b}	0.052±0.01^{ab}	0.051±0.00^{ab}	0.055±0.00^{a}
Shell weight (gm)	3.41±0.01^{b}	4.12±0.00^{ab}	4.52±0.01^{a}	3.51±0.01^{b}	4.12±0.02^{ab}	4.55±0.02^{a}	4.55±0.00^{a}
Haugh Unit	70.88±0.1^{d}	79.99±0.20^{b}	88.24±0.14^{a}	75.38±0.11^{c}	78.64±0.1^{b}	81.33±0.12^{ab}	85.78±0.09^{a}

[1]D_0- Basal diet(BD)/ control diet; D_1- BD+1% of Black pepper; D_2- BD+1% of Turmeric; D_3- BD+ 1% of Fennel; D_4 - BD+ 0.5% of Black pepper+ 0.5% of Turmeric; D_5- BD+0.5% of Black pepper+0.5% of Fennel; D_6- BD+0.5% of Turmeric+ 0.5% of Fennel. [a-c]Different letters in the same row differ by Tukey test indicates P < 0.05 probability level.

Table 4: Cholesterol, HDL, and LDL in egg yolk of birds under different experimental diets

Diet[1]	Cholesterol (mg/gm)	HDL[2] (mg/gm)	LDL[3] (mg/gm)
D_0	3.375±0.02^{a}	5.51±0.00^{b}	7.68±0.01^{a}
D_1	3.192±0.02^{b}	5.54±0.00^{a}	7.62±0.00^{b}
D_2	3.221±0.12^{ab}	5.57±0.002^{a}	7.63±0.00^{b}
D_3	3.365±0.03^{a}	5.51±0.01^{b}	7.67±0.003^{a}
D_4	3.218±0.00^{ab}	5.58±0.02^{a}	7.62±0.006^{b}
D_5	3.197±0.01^{b}	5.52±0.00^{b}	7.62±0.001^{b}
D_6	3.395±0.11^{a}	5.54±0.006^{a}	7.68±0.00^{a}

[1]D_0- Basal diet(BD)/ control diet; D_1- BD+1% of Black pepper; D_2- BD+1% of Turmeric; D_3- BD+ 1% of Fennel; D_4 - BD+ 0.5% of Black pepper+ 0.5% of Turmeric; D_5- BD+0.5% of Black pepper+0.5% of Fennel; D_6- BD+0.5% of Turmeric+ 0.5% of Fennel. [2]HDL-High density lipoprotein [3]LDL-Low density lipoprotein [a-b]Different letters in the same column differ by Tukey test indicates P < 0.05 probability level.

Melo et al. (2016), reported that black pepper improves egg quality, and our findings with black pepper inclusion were superior to their findings. In the current study, the egg shape index of laying hens fed with D1 (1 % black pepper) was greater than the other experimental groups, which is similar to Bugdayci et al. (2018). In comparison, the egg shape index of birds fed with diet D6 was not substantially different (p<0.05) from that of birds fed with control diet D0, and supported by Zacaria et al. (2021).

Table 4. shows that birds fed with D1 and D4 diets had the greatest hypocholesterolemic effects, which is supported by Elnagger et al. (2021). Our findings on cholesterol content contradict those of Bugdayci et al. (2018), who found that fennel had a positive effect on decreasing cholesterol levels in birds. Fennel in diets D3 (1% fennel) and D6 (0.5 % turmeric and 0.5 % fennel) did not affect cholesterol levels, while hens had higher cholesterol levels. These findings were similar to those of Abou-Elkhair et al. (2018), who found that the addition of a 0.5% fennel diet had no significant effect on egg yolk cholesterol levels.

HDL-C levels were substantially higher (p<0.05) in the D2 and D4 (0.5 % black pepper and 0.5 % turmeric) diets than in the D0 and D5 diets. These findings are comparable to those of Liu et al. (2020), who found that turmeric had a favorable effect on boosting HDL levels, and Elnaggar et al. (2021), who reported that a combination diet of turmeric and black pepper produces better effects than either turmeric or black pepper individually. The effect of D1 (1 % black pepper) on TC or Total Cholesterol level is superior to Melo et al. (2016) and Sidhu et al. (2017). The results of the LDL levels indicated a substantial (p<0.05) decrease due to the incorporation of the D1 and D4 diet in the egg yolk are reported to be similar to those of Elnagger et al. (2021).

CONCLUSION

The data presented in this study show that the addition of black pepper, turmeric, and fennel had no detrimental effect on laying *Gallus gallus* performance or egg quality. Diets including black pepper and turmeric (D4) and black pepper and fennel (D5) have been demonstrated to increase the performance and egg quality of laying birds. Incorporating black pepper and turmeric into the diets of laying hens can help to lower egg cholesterol and LDL levels. Because no expected effects of fennel as a feed supplement were seen in this study, more research is needed to determine if it is needed by health-conscious customers.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Ethics approval and consent to participate

Qualified veterinarians adhering to the regulations and guidelines on animal husbandry and welfare and performed all handling practices aimed at identification, and weighing of birds. No action involving pain or suffering was practiced. Hence approval of any ethical committee or subsidiary body thereof is not needed in this case.

AUTHORS' CONTRIBUTIONS

LS: Conceptualized the paper, fieldwork, contributed to writing, reviewed, and edited the manuscript.
YN: Compiled the information and prepared the draft for the manuscript. Both authors read and approved the final manuscript.

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