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Effects of Black Pepper, Turmeric, and Fennel on Essential and Non-essential Chemical Contents of Egg

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ABSTRACT

The use of essential oils (EOs) in animal feeding has gained attention as a potential antibiotic growth promoter replacement over the past two decades. The current study aimed to determine the impact of three feed additives, namely black pepper, turmeric, and fennel, on the productivity of laying hens, the chemical composition, and macro- and microelement content in layer eggs. A total of 280 chicks aged 75 days were randomly divided into 7 treatments (5 replicates of 8 chicks). One treatment group was provided as the unsupplemented control. The other six treatment groups, namely D0 (basal diet [BD] control), D1 (BD + 1% of black pepper), D2 (BD + 1% of turmeric), D3 (BD + 1% of fennel), D4 (BD + 0.5% of black pepper + 0.5% of turmeric), D5 (BD + 0.5% of black pepper + 0.5% of fennel), D6 (BD + 0.5% of turmeric + 0.5% of fennel) were supplemented with varying levels of phytobiotics. The result of the study indicated that the egg weight, Hen-day-production (HDP), egg quality, and haugh unit significantly improved with a combined supplementation of phytobiotics (D4, D5, and D6 diets) when compared with the control. However, there were no significant differences in the chemical composition of eggs. The X-ray fluorescence spectrometer analysis of eggs revealed the presence of 17 significant elements, including phosphorous, sulfur, chlorine, potassium, calcium, manganese, iron, copper, zinc, and bromine. The study findings showed that the combined supplementation of phytobiotics lowered K and Cl, whereas Zn, Ca, S, and Cu contents positively increased in hen eggs by including phytobiotic in the diet. In conclusion, the EOs of phytobiotics as dietary supplementation at 1% and 0.5% could improve the HDP, egg weight, and egg mass, including nutrient elements in the egg.

Keywords: Egg, Essential oil, Hen, Mineral, Phytobiotic

INTRODUCTION

Poultry eggs are a worthy source of essential nutrients, proteins, vitamins, and minerals for human beings. However, the use of conventional antibiotic feed additives or antibiotic growth promoters in poultry feed has caused numerous complications in public health, such as antibiotic residue in poultry products and the development of antibiotic resistance in consumers (Saeed et al., 2018). Consumer anxiety about food safety could stimulate the animal feed production revolution and enhance the use of phytobiotic feed additives (Abd El-Ghany, 2020). Herbs belong to phytobiotic feed additives and contain secondary metabolites or bioactive compounds such as alkaloids, carotenoids, phenols, and flavonoids (Mohanraj et al., 2018; Ivanisova et al., 2020). These phytogenic attributes play a key role in producing healthy and safe poultry eggs, ultimately contributing to the maintenance of consumer health (Samantaray and Nayak, 2022).

The biological, physical, and chemical properties of poultry eggs are well known as they are a rich source of protein, fat, vitamins, and minerals. However, the investigations on the effect of phytobiotic feed additives and the possibility of positive impact on poultry eggs are scanty (Mirzaei et al., 2022). The objective of the present study was to evaluate the effect of three herbs, namely black pepper, turmeric, and fennel, on poultry eggs. These herbs are widely distributed and commonly used in Indian households as spices due to their considerable importance for human consumption in ayurvedic science and also rich in secondary metabolites like phytopolyphenols, polypropanoids, and flavonoids (Kumar et al., 2017). As previous studies noted, phytochemicals, such as capsaicin (Liu et al., 2021), curcumin (Yadav et al., 2020), caffeoylquinic acid, and rutin (Castaldo et al., 2021) add a positive impact on the quality of egg and health of layer chickens.

Eggs are a good source of minerals that are macro elements, microelements, and trace elements. Elements, such as phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), and calcium (Ca) belong to macro elements (Welch and Graham, 2004). Ions in less amount include manganese (Mn), bromine (Br), sodium (Na), copper (Cu), rubidium (Rb), strontium (Sr), zinc (Zn), selenium (Se), fluoride (F), titanium (Ti) and chromium (Cr); Dobrzanski et al., 2020). Aliu et al. (2021) classified heavy metals into the essential (Mn, Iron [Fe], Cu, and Zn) and non-essential groups (Rb, Br, and Sr). Gombart et al. (2020) classified Fe, Zn, Cu, and Mn micronutrients as essential for humans. A slightly different classification of the trace element is given by Rehault-Godbert et al. (2019), that Ca, Cu, Fe, Mn, P, K, and Zn are found in raw whole eggs, egg yolk, and egg white. In contrast, Lim and Schoenun (2010) include Cu, Mn, Zn, and Cr as heavy metals with toxic properties depending on their doses consumed by the consumer.

The accumulation of essential and non-essential chemical trace elements in poultry eggs suggests that the composition and quantities of feed additives, as well as their chemical form, bioavailability, and bioactive substances or secondary metabolites, have a significant impact on the quality of poultry eggs (Abduljaleel, 2016; Ahmad et al., 2017; Heflin et al., 2018). This research was designed to address concerns with consumer health, egg quality, and the impacts of black pepper, turmeric, fennel, and their different combinations as a feed supplement in layer (*Gallus gallus*) diets, and the traceability of essential and non-essential nutrients in poultry eggs.

MATERIAL AND METHODS

Ethical approval

All experimental methods involving the use of live animals were conducted with the approval of the Centurion University, Jatni, India, Ethics Committee for Experiments with Animals.

Study design

The research was carried out from May 2022 to November 2022 at Centurion University of Technology and Management in Bhubaneswar, India. A total of 280 75-day-old chicks (*Gallus gallus*) with an average weight of 826.67 gm were purchased from the local hatchery of Jatni, India. After being weighed, the chicks were divided into one control and six experimental groups and randomly placed in a poultry house. Each group was further separated into five replicates, with eight chicks in each under controlled climate conditions. In the poultry house, an adaptation period of 2 weeks was maintained. Hens began laying eggs at week 25 of age, and 17 eggs were randomly chosen from each group for the study. Ever four chickens were roomed in 2 m^2 of floor area. The chicks were fed a conventional balanced diet as per NRC (1994) standard recommendations. An appropriate room temperature of daily high (29-32^{o}C) and low (25-22^{o}C) were regulated by an electronic control panel (Temptron-607 AgroLogic), and 14 hours of light and 10 hours of dark schedule for natural light were maintained throughout the experiment. The relative humidity was 65-76% throughout the investigation.

The daily feed consumption of layer chickens was calculated as g/day. To calculate feed consumption, the difference between the leftover feed and the given feed/week was determined (Abou-Elkhair et al., 2018). The percentage of hen-day production was calculated from week 25 of age using daily egg production records (Adebiyi et al., 2018).

Data on bird mortality, feed consumption, weight gain, and growth performance were recorded weekly. The chemical composition of the chickens' diet was analyzed according to the protocols of Cerrate et al. (2019), Barzegar et al. (2019), and AOAC (2005), in the Department of Applied Science, CUTM, BBSR, Odisha, India (Table 1). A control diet was given to the control group (D0), and three oral dietary phytobiotic essential oils (Eos) supplements with varied compositions were given to the other six groups. The chickens in the D0 received a basal diet with no additive (control), D1 was fed a basal diet plus 1% of black pepper (10 g/Kg of feed), D2 chickens were subjected to a basal diet plus 1% of turmeric (10g/Kg of feed), D3 received basal diet plus 1% of fennel (10g/Kg of feed), chickens in D4 were fed basal diet plus 0.5% of black pepper plus 0.5% of turmeric (10g/Kg of feed), D5 received basal diet plus 0.5% of black pepper plus 0.5% of fennel (10g/Kg of feed), and D6 was given basal diet plus 0.5% of turmeric plus 0.5% of fennel (10g/Kg of feed).

Table 1. Composition and chemical content of diets fed to layers for 6 months

Items (g/kg)	Control diet	Experimental groups (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[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Metabolizable energy (MJ/kg)[3]							
Crude protein	176	181	178	177	180	179	176
Lysine	8.4	8.5	8.5	8.5	8.6	8.5	8.4
Methionine	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Threonine	6.5	6.6	6.6	6.5	6.5	6.6	6.5
Calcium	39.0	40.1	40.2	39	40.1	40.1	39
Total protein	3.2	3.2	3.1	3.4	3.1	3.3	3.3
Sodium	1.8	1.8	1.9	1.9	1.8	1.9	2.0
DEB[4] (mEq)	175	177	178.1	178	177	179	179

[1]D0: Basal diet plus control diet, D1: Basal diet plus 1% of black pepper, D2: Basal diet plus 1% of turmeric, D3: Basal diet plus 1% of fennel; D4: Basal diet plus 0.5% of black pepper plus 0.5% of turmeric, D5: Basal diet plus 0.5% of black pepper plus 0.5% of fennel, D6: Basal diet plus 0.5% of turmeric plus 0.5% of fennel. [2]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; 8 mg 11 mg d-pantothenic acid; 1.1 mg folic acid; 0.13 mg biotin; 12,300 IU vitamin A, 4,500 IU vitamin D3; [3]Calculated according to (Barzegar et al., 2019; Cerrate et al., 2019) as a sum of ME content of components; [4]Dietary Electrolyte Balance.

Essential oils

Black pepper, turmeric, and fennel were purchased from the local market of Jatni, India, in March 2022. The EOs of the three phytobiotics were extracted using the supercritical CO_2 extraction method at the Centurion University Research and Development Laboratories in Paralakhemundi, India. The dried phytobiotics were processed to a fine powder using a grinder (SS Pulverizer, 3HP, India), and stored in an airtight vacuum-sealed bag. To reduce the loss of essential oils during the process, the phytobiotic powders were cooled (at 10°C) in a refrigerator (SSU-168, India) for two hours before grinding. Each phytobiotic powder (1000 g) was added to the high-pressure equilibration vessel. Liquid CO2 was supplied into the system via a reciprocating pump at a constant flow rate of 10 ml/minute and compressed to extraction pressures of 60°C and 300 bar for black pepper (Shityakov et al., 2019; Tran et al., 2019), 60°C and 250 bar for turmeric (Gopalan et al., 2000; Neves et al., 2020), and 32°C and 300 bar for fennel (Hammouda et al., 2013). For each phytobiotic EO, the precipitated fractions were collected in a trapping flask. The collected samples were kept in individual amber bottles at 4°C for further use. The extraction yields were calculated using gravimetric analysis.

X-Ray fluorescent spectrometer

The X-Ray fluorescent spectrometer operates on the principle that individual stimulated atoms by X-ray photons, wavelength, or external energy can count the number of photons released by the sample at each energy level (Figure 1). The elements can be measured and classified based on the excited characteristic x-ray emission rate. Freshly laid hen (*Gallus gallus*) eggs were procured from all experimental groups for 6 consecutive days at the pick (about 90%) of laying at week 30 of the age. Undamaged eggs (n = 17) of each experimental group with a weight between 42 and 44 g were randomly chosen, and each egg was labeled with the group name, number, and lay date (Figure 2).

The eggs were kept at 4°C and weighed using an analytical balance (Aczet, CY 224 by HPFS instruments India LLP, Vasai). Eggs were broken into a sheet of a plain whiteboard, and the edible part of the whole egg (albumin and yolk combined) was well stirred in a glass vessel. A metal container was dried to a constant weight in a hot air oven at 100°C. The egg samples were placed in these metal containers and heated to 100°C for 24 hours until the sample could gain a constant weight. The dried egg samples were crushed with a mortar and pestle and kept in cleaned plastic bags. The X-ray fluorescence spectrometer (Epsilon 1 PAN analytical B. V., Netherlands) was used to quantify the elements present in samples. The X-Ray beam ranged 40-60 KV and 50-300 W (Jamaluddin et al., 2018).

Statistical analysis

The obtained data were analyzed using the Statistical analysis system (SAS, 2012). The significance of the findings was tested using one-way ANOVA. The Tukeys test was used to compare mean values at a significance level of $p < 0.05$. Box-whisker charting was chosen as the most appropriate descriptive statistical analysis approach for the comparative analysis of the data sets of macro element measurements. Box plots were used in this study to represent the means of various data sets as well as the range between the 25 and 75 percentiles. In contrast, outliers were considered to be insignificant and were not considered.

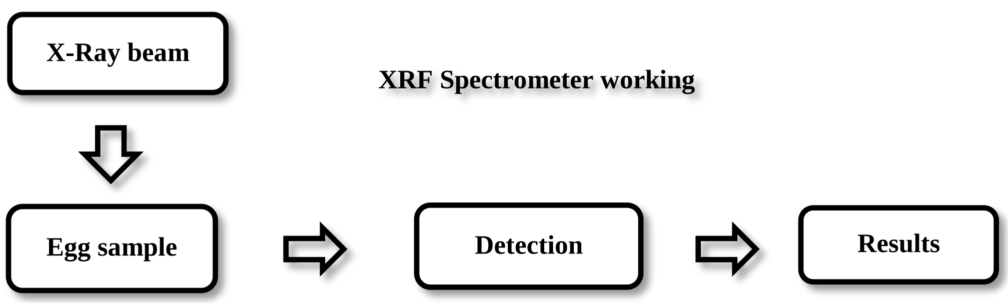

Figure 1. Working of the X-Ray fluorescent spectrometer (Epsilon-1) for the analysis of hen egg mineral contents

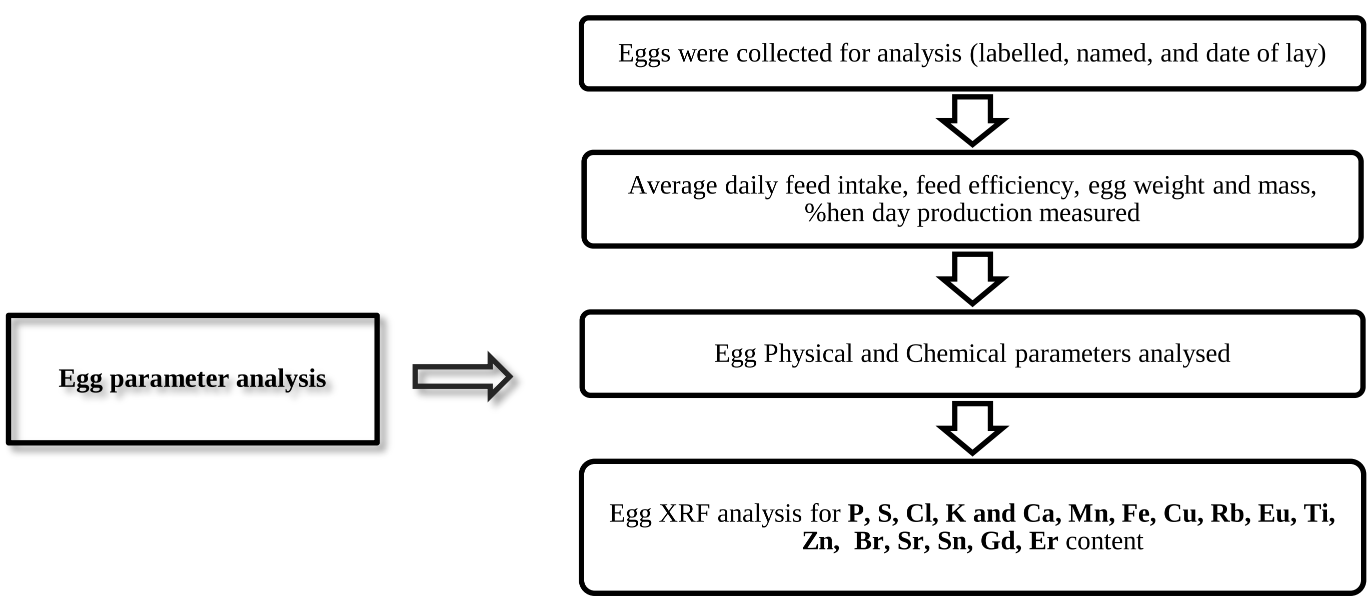

Figure 2. The schematic diagram for hen egg parameter analysis

RESULTS AND DISCUSSION

The Hen day production (HDP) of the layer chickens fed with D5 was 90.34% (0.5% black pepper and 0.5% fennel), and 86.67% for D4 (0.5% black pepper and 0.5% turmeric), which were significantly higher than that of the other experimental groups ($p < 0.05$). The obtained results were higher than those reported by Nuraini and Djulardi (2019). As can be seen in Table 2, D1, D3, and D6 groups were found to have a similar range of HDP and egg mass;

however, D0 (control) had the lowest HDP of 76.67% and egg mass. The D4, D5, and D2 diets substantially affected egg mass values, as Phuoc et al. (2019) supported.

The average egg weight was considerably higher in D4 when compared to the other experimental groups and the control ($p < 0.05$). Egg weight is a well-known factor that affects egg quality. Regarding the egg weight, the highest (44.80±0.02 g) was for D4 (Table 2). The findings indicated that the treatment-related values in D2 and D5 were statistically equivalent. In contrast, D1 and D6 have been proven to have a significantly similar effect on egg weight compared to D0, D3, and D4 diets ($p < 0.05$). There were no significant differences in feed efficiency with diets D1, D3, and D6, compared to D0, D2, and D5 ($p > 0.05$).

It has been demonstrated that albumin weight affects internal egg quality, with thicker and higher albumin content regarded as superior quality (Kowalska et al., 2021). Compared to the other experimental groups, the chickens fed the D2 and D4 diets indicated significantly greater albumin contents ($p < 0.05$), which is consistent with Abou-Elkhair et al. (2018). Indicators of the yolk quality include weight, texture, hardness, and scent (Wijedasa et al., 2020). According to Table 3, the yolk weight of the eggs from chickens in the D4 diet was found to be 18.87 ± 0.01 g, indicating higher yolk quality when compared to D0 (control) diet ($p < 0.05$), which is supported by Nuraini and Djulardi (2019). The D6 diet group showed no significant effect on yolk weight ($p > 0.05$), compared to the D0 (control) diet. The current findings are supported by Souza et al. (2020).

All 17 trace elements (P, S, Cl, K, Ca, Mn, Fe, Cu, Rb, Eu, Ti, Zn, Br, Sr, Gd, Er) were quantitatively found in layer eggs of all experimental groups. Figure 3 displays the highest P, S, Cl, K, and Ca contents. Other macronutrients were found in lower concentrations in the D0 (control), while K was found in higher concentrations in the control group, compared to other experimental groups. Layer chickens fed D5 diet had the highest Fe, P, and Ca levels. Those fed D6 diet had greater levels of S and Cl than the other experimental groups.

The proximate composition of eggs was similar, regardless of the dietary treatments (Table 4). As seen in Table 3, Mn was only found in diets D2 and D6. According to Gilani et al. (2021), hens can use the calcium in calcium carbonate to produce bones and eggshells. Ca-phytate complex formation was accelerated by high Ca concentrations relative to P. All experimental diets had no significant effect on Cu, K, or Rb ($p < 0.05$). As shown in Figure 3, the present findings for Ca content were greater in D4 and D5 diets than those reported by Dobrzanski et al. (2020) and Rehault-Godbert et al. (2019). The results of the present study for K content were similar to those reported by Ahmad et al. (2017); however, they were lower than the suggested content by Rehault-Godbert et al. (2019). According to Table 3, diets D2, D4, and D6 contain titanium (Ti), higher than those measured by Dobrzanski et al. (2020). Regarding D2 and D6 diets, the presence of Eu was below detectable levels. The amount of Zn was found to be significantly higher in diet D5 than in the control ($p < 0.05$), which is considerably less than the reported value of Zn by Aliu et al. (2021). The mineral profiles for Gd and Er were only detected in egg samples given with the D1 and D6 diets, respectively.

The mineral content of eggs can be used to evaluate the mineral status of animals (Gilani et al., 2021). The results of this study revealed that phytobiotics significantly increased Fe, Se, and Zn concentrations in hen eggs ($p < 0.05$), which is similar to the findings of recent research (Bonos et al., 2022; Vlaicu et al., 2022). Phytobiotics enhance Fe absorption in the intestine, allowing Fe to enter cells via the clathrin-mediated endocytosis pathway (Chithrani and Chan, 2007). The Zn defends cell membranes from oxidative damage and is a crucial component of the body's antioxidant defense mechanism (Aliu et al., 2021). This could happen due to Zn scavenging free radicals through enhanced metallothionein production. Additionally, Zn might facilitate metallothionein and function as a P53 co-transcriptional factor to repair DNA damage brought on by oxidative stress (Yin et al., 2022). This could explain the advantages of increased Zn concentrations shown in hen eggs provided with the D5, D4, and D1 diets, which contained combinations of black pepper and turmeric, black pepper and fennel, and black pepper, respectively ($p < 0.05$).

The thyroid metabolism depends heavily on trace minerals (Abdelli et al., 2021). It was shown that Fe supplementation could enhance T3 levels while decreasing T4 levels in livestock, which was significant since serum T4 levels can influence fat metabolism by controlling the production and breakdown of cholesterol (Dilawar et al., 2021). The current investigation found that phytobiotics significantly lowered the serum T4 concentration in diets D5, D4, and D3, compared to other groups ($p < 0.05$). By supplementing livestock diets with EOs of phytobiotics, the increased Fe content may activate 5'-deiodinase activity and increase T3 to T4 conversion (Abdelli et al., 2021). Dietary trace elements, particularly Zn, can stimulate carboxypeptidase B and stabilize many proteins to facilitate protein conversion (Vlaicu et al., 2022). Incorporating phytobiotics may have enhanced performance by upregulating the quality of the eggs produced by the layers (Samantaray and Nayak, 2022).

Copper (Cu) is recognized as essential and toxic to many biological processes. It can enter food materials through mineralization by crops, food processing, or environmental contamination (Afsana et al., 2004; Rehault-Godbert et al., 2019). The detected Cu means value in egg samples was 0.289 μg/gm with diet D5 and D1, and Cu values in egg samples analyzed in the presented study were close to the level reported in the above-mentioned studies. However, Cu levels in the D0 control and D6 diet were significantly lower than the other experimental diets ($p < 0.05$), and with no significant differences between the D2 and D4 diets ($p < 0.05$). Fe is an essential mineral, and it is well known that including enough Fe in the diet is essential for lowering the risk of developing anemia (Yin et al., 2022). In this investigation, Fe was substantially higher in the D5 diet when compared to similar values in D3 and D4 diets, and it is significantly lower in the D6 diet, compared to D5 ($p < 0.05$). The mean Fe concentrations showed a significant difference ($p < 0.05$) between the D5 and D0 diets, which was not beyond the standard limit reported by Rehault-Godbert et al. (2019). The daily intake of essential elements, such as Ca, P, Cl, Mn, Fe, and Zn, were compared to the provisional and recommended daily intake of minerals and FAA/WHO for certain foods (FAO/WHO, 2002; RDI, 2020). As shown in Table 3 and Figure 3, the calculated data of the average daily intake of Cl, Fe, Mn, Cu, Zn, and P from the egg sample did not exceed the standard limit. Compared to the other group elements, Cu and Fe came closer to the limit of the essential elements.

Table 2. The effect of different phytobiotic combinations on layer performance over a 6-month period

Egg parameter	Control diet	Experimental diet[1]					
	D0	D1	D2	D3	D4	D5	D6
Average egg weight (gm)	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^{a}	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}
Yolk weight (gm)	14.25 ± 0.07^{c}	16.61 ± 0.04^{b}	17.56 ± 0.03^{a}	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}
Haugh Unit	70.88 ± 0.1^{c}	79.99 ± 0.20^{ab}	88.24 ± 0.14^{a}	75.38 ± 0.11^{b}	78.64 ± 0.1^{b}	81.33 ± 0.12^{ab}	85.78 ± 0.09^{a}

[1]D0: Basal diet plus control diet, D1: Basal diet plus 1% of black pepper, D2: Basal diet plus 1% of turmeric, D3: Basal diet plus 1% of fennel; D4: Basal diet plus 0.5% of black pepper plus 0.5% of turmeric, D5: Basal diet plus 0.5% of black pepper plus 0.5% of fennel, D6: Basal diet plus 0.5% of turmeric plus 0.5% of fennel. [abc]Different letters in the same row indicate significant differences at $p < 0.05$.

Table 3. The effect of phytobiotics on the mineral content of the edible portion of the hen eggs (yolk and albumen)

Nutrients/Elements	Elements (μg/gm)						
	Control diet	Experimental diet[1]					
	D0	D1	D2	D3	D4	D5	D6
Iron (Fe)	6.37^{b} ± 0.001	6.72^{b} ± 0.01	5.99^{bc} ± 0.002	7.3^{b} ± 0.009	7.32^{b} ± 0.001	10.71^{a} ± 0.005	5.65^{c} ± 0.001
Zinc (Zn)	2.22^{b} ± 0.001	3.10^{a} ± 0.003	2.61^{ab} ± 0.002	2.85^{ab} ± 0.001	3.31^{a} ± 0.001	3.39^{a} ± 0.001	2.29^{b} ± 0.004
Manganese (Mn)	-	-	0.316^{a} ± 0.003	-	-	-	0.042^{b} ± 0.000
Copper (Cu)	0.204^{b} ± 0.0001	0.289^{a} ± 0.001	0.224^{b} ± 0.0001	0.251^{ab} ± 0.000	0.226^{b} ± 0.001	0.289^{a} ± 0.002	0.214^{b} ± 0.000
Rubidium (Rb)	-	0.740^{a} ± 0.01	0.683^{ab} ± 0.021	0.644^{b} ± 0.001	0.479^{c} ± 0.003	-	0.176^{d} ± 0.002
Europium (Eu)	0.761^{a} ± 0.001	0.741^{a} ± 0.000	-	0.599^{b} ± 0.001	0.630^{ab} ± 0.003	0.0001^{c} ± 0.000	-
Titanium (Ti)	-	-	0.338^{b} ± 0.008	-	0.293^{c} ± 0.002	0.380^{a} ± 0.004	0.266^{c} ± 0.005
Bromine (Br)	0.44^{a} ± 0.0001	0.235^{c} ± 0.000	0.292^{c} ± 0.001	0.363^{b} ± 0.001	0.317^{bc} ± 0.002	0.268^{c} ± 0.000	0.3^{bc} ± 0.002
Strontium (Sr)	0.206^{a} ± 0.001	-	0.095^{b} ± 0.004	-	-	-	-
Tin (Sn)	0.515^{a} ± 0.0001	-	-	0.368^{b} ± 0.000	0.395^{b} ± 0.001	0.545^{a} ± 0.003	0.364^{b} ± 0.005
Gadolinium (Gd)	-	1.32^{a} ± 0.001	-	-	-	-	-
Erbium (Er)	-	-	-	-	-	-	2.40^{a} ± 0.01

[1]D0: Basal diet plus control diet, D1: Basal diet plus 1% of black pepper, D2: Basal diet plus 1% of turmeric, D3: Basal diet plus 1% of fennel, D4: Basal diet plus 0.5% of black pepper plus 0.5% of turmeric, D5: Basal diet plus 0.5% of black pepper plus 0.5% of fennel, D6: Basal diet plus 0.5% of turmeric plus 0.5% of fennel. [abcd]Different letters in the same row indicate significant differences at $p < 0.05$.

Table 4. The effect of phytobiotics on the chemical composition of the edible portion of the hen eggs (yolk and albumen)

Chemical composition / Experimental groups[1]	Moisture Content (%)	Crude protein (%)	Crude fat (%)	Total ash (%)	Carbohydrate (%)	Energy (Kcal/100g)
D0	74.12 ± 0.350^{b}	13.54 ± 0.04^{a}	13.18 ± 0.066^{a}	0.82 ± 0.128^{b}	0.87 ± 0.33^{b}	186.59 ± 1.27^{c}
D1	75.79 ± 0.278^{a}	13.16 ± 0.075^{a}	13.38 ± 0.171^{a}	1.26 ± 0.040^{a}	0.85 ± 0.209^{b}	195.65 ± 1.64^{a}
D2	74.95 ± 0.31^{b}	12.35 ± 0.10^{b}	13.28 ± 0.11^{a}	0.89 ± 0.08^{b}	0.93 ± 0.27^{a}	189.12 ± 1.45^{bc}
D3	75.14 ± 0.060^{a}	13.56 ± 0.068^{a}	12.26 ± 0.075^{b}	0.9 ± 0.044^{b}	0.88 ± 0.048^{b}	185.9 ± 0.801^{c}
D4	74.64 ± 0.225^{b}	12.36 ± 0.136^{b}	12.52 ± 0.111^{b}	0.76 ± 0.051^{bc}	0.94 ± 0.202^{a}	190.1 ± 1.686^{b}
D5	75.50 ± 0.153^{a}	12.5 ± 0.170^{b}	12.42 ± 0.136^{b}	0.88±0.116^{b}	0.96 ± 0.224^{a}	193.92 ± 1.07^{ab}
D6	76.42 ± 0.14^{a}	13.80 ± 0.12^{a}	12.73 ± 0.10^{b}	0.88 ± 0.07^{b}	0.89 ± 0.15^{b}	193.30 ± 1.18^{ab}
D7	74.52 ± 0.11^{b}	12.56 ± 0.08^{b}	12.99 ± 0.06^{b}	0.68 ± 0.05^{c}	0.9 ± 0.04^{c}	188.55 ± 0.93^{bc}

[1]D0: Basal diet plus control diet, D1: Basal diet plus 1% of black pepper, D2: Basal diet plus 1% of turmeric, D3: Basal diet plus 1% of fennel, D4: Basal diet plus 0.5% of black pepper plus 0.5% of turmeric, D5: Basal diet plus 0.5% of black pepper plus 0.5% of fennel, D6: Basal diet plus 0.5% of turmeric plus 0.5% of fennel. [abcd]Different letters in the same row indicate significant differences at $p < 0.05$.

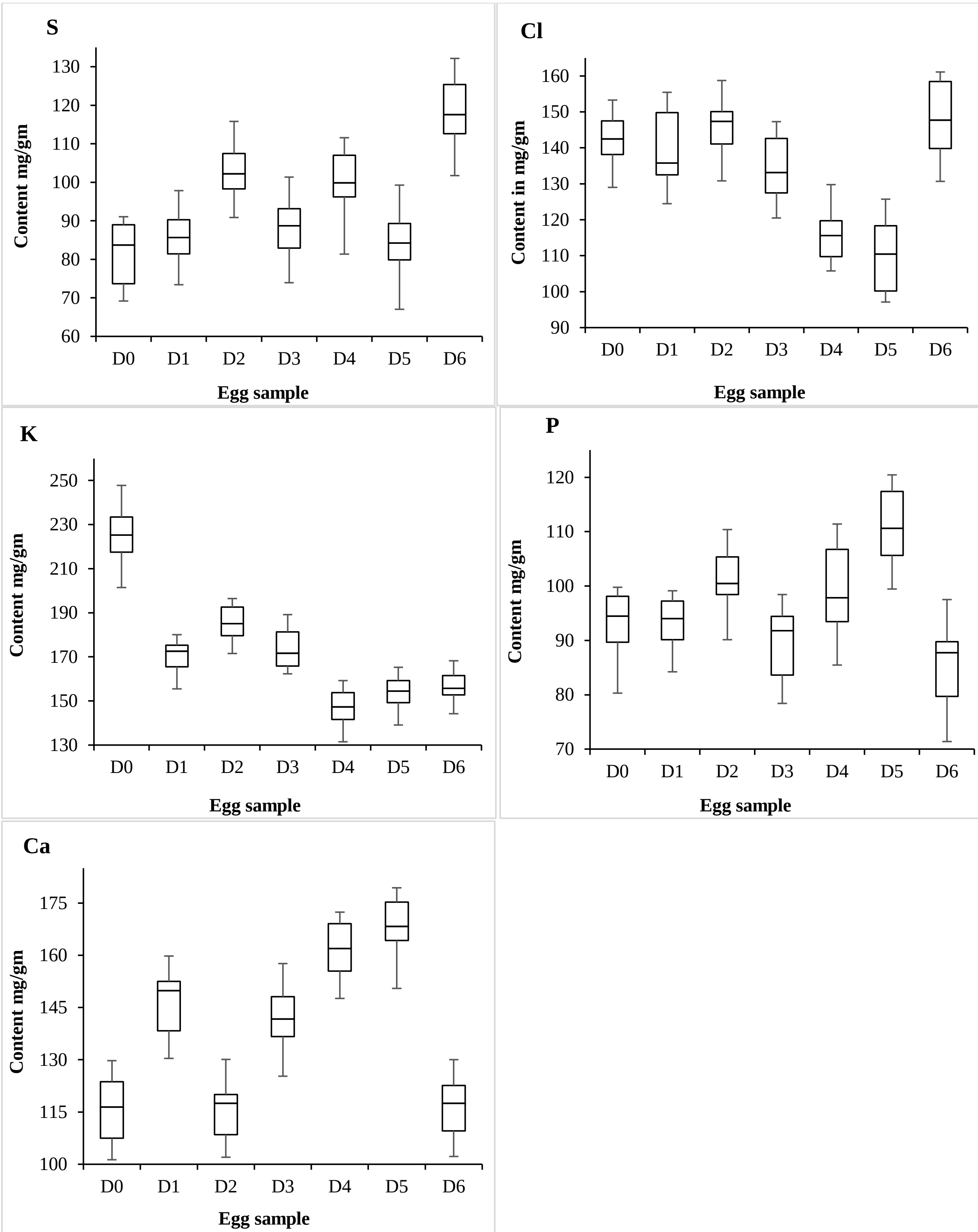

Figure 3. Box-whisker plots of (macro elements) phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), and calcium (Ca) content detected in hen eggs (week 30) with different experimental diets. D0: Basal diet plus control diet, D1: Basal diet plus 1% of Black pepper, D2: Basal diet plus 1% of turmeric, D3: Basal diet plus 1% of fennel; D4: Basal diet plus 0.5% of black pepper plus 0.5% of Turmeric, D5: Basal diet plus 0.5% of black pepper plus 0.5% of fennel, D6: Basal diet plus 0.5% of turmeric plus 0.5% of fennel.

CONCLUSION

The results of the present study revealed that when phytobiotics were added to layer feed, they positively impacted the *Gallus gallus* laying performance and nutrient content of eggs. Layer chickens that received combined phytobiotics diet D4 (0.5% black pepper and 0.5% turmeric), D5 (0.5% black pepper and 0.5% fennel), and D6 (0.5% turmeric and 0.5% fennel) had better egg quality than layers that did not consume phytobiotics. Therefore, Eos of black pepper, turmeric, and fennel could become ideal feed additives for layer chickens to elevate egg nutrient content. However, more study is required to determine the ideal phytobiotic concentration for layer chickens' diets as a potential replacement for antibiotic growth promoters.

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Authors' contributions

Lopamudra Samantaray conceptualized the paper, and fieldwork, contributed to writing, reviewed, and edited the manuscript and Yashaswi Nayak Compiled the information and prepared the draft for the manuscript. Both authors read and approved the final manuscript for publication in the present journal.

Competing interests

The authors declare no conflict of interest.

Ethical consideration

The authors thoroughly examined all ethical concerns surrounding plagiarism, consent to publish, misconduct, data fabrication, falsification, duplicate publishing or submission, and manuscript redundancy.

Availability of data and materials

This article includes all data generated or analyzed during this research.

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