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EFFECT OF DIETARY SUPPLEMENTATION OF POT MARIGOLD FLOWER POWDER AND EXTRACT (*CALENDULA OFFICINALIS*) ON NUTRIENT DIGESTIBILITY, PERFORMANCE, SERUM BIOCHEMISTRY, ANTIOXIDANT PARAMETERS, IMMUNE RESPONSE AND SOME GUT BACTERIAL COUNT OF LAYING JAPANESE QUAIL

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ABSTRACT: The aim of this study was to investigate the influence of *Calendula officinalis* flowers either as a powder (COP) or as an extract (COEx) on productive, digestion enzymes, nutrient digestibility coefficients, egg yolk cholesterol, serum metabolites, gut bacterial content, antioxidant capacity and immunological traits in laying Japanese quail. 504 hens (168 males and 336 females), at 9th weeks of age were randomly distributed to seven experimental treatments, with six replicates of 12 birds each (four males and eight females). The first treatment was received a basal diet (control), groups from two to four fed on the basal diet supplemented with 0.6, 0.9 and 1.2 % COP, respectively. While, groups from five to seven were fed on basal diet supplemented with 150, 200 and 250 ppm COEx, respectively. Hens fed with 1.2% COP, 200 and 250 ppm COEx levels had significantly ($p \leq 0.001$) higher egg number, egg production %, egg weight, and egg mass with the best feed conversion ratio without holding significant effect on feed intake. Egg yolk cholesterol was significantly ($p \leq 0.001$) decreased in quails treated with 250 ppm COEx 1.2 % COP and 200 ppm COEx, respectively. Serum biochemistry (total cholesterol, TG, HDL and LDL), liver functions, antioxidant parameters, immune response and some gut bacterial count (*Lactobacillus*) were significantly ($p \leq 0.001$) improved by some treatments. Digestive enzymes were significantly ($p \leq 0.001$) improved by treatments with COP and COEx especially at 250 pm COEx. Finally, quails fed diets containing 0.6 % COP and 250 ppm COEx had significantly ($p \leq 0.001$) the highest nutrient digestibility percentage. The present study concluded that the dietary supplementation with 200 and 250 ppm COEx to laying quail improved digestive enzymes, digestion of nutrients, productive performance, serum biochemistry, immunological parameters, antioxidant capacity, and gut microbial content.

Key words: Quail; laying period, *Calendula officinalis*, performance; serum biochemistry.

1. INTRODUCTION

Products of poultry such as meat and eggs provide substantial potentials to bridge the protein gap, the continuous increase in feed cost has resulted in shortage of animal protein supply, which has encouraged the rearing available and cheap animal as feed resources to make the future of poultry production safe (Obuzor and Ntui, 2011). Recently, alternative poultry source such as Quail farming has been introduced into many countries such as Egypt, as it was found to have outstanding meat and egg features due to its numerous nutritive and economic because meat and eggs of quail are well-known for their high quality of protein, high biological value and low calories (Odugbo, 2004).

Since 2006, the use of antibiotic as growth promoter was prohibited by the European Union, because of the proliferation of resistant bacteria by the food chain and the increasing concern over the transmission. Therefore, the current commercial additives obtained from plants (extracts of aromatic plant and purified components) have been studied as part of alternative feed strategies for the future. Such products are residue-free, generally recognized as safe, used in the food industry, and have various features over commonly used commercial antibiotics (Varel, 2002). Herbs and their mixture are able to enhance the bird's performance by improving the function of the digestive system, the anti-inflammatory and physiological effects (Hernandez et al., 2004). Braun and Cohen, (2015) showed that calendula has been used for decades to treat inflammations as well as gastrointestinal disorders such as gastric and duodenal ulcers and wounds. Furthermore, calendula contains a variety of active components such as beta carotene, oils, polyphenolic compounds, tannins, saponins, and polysaccharides that have antibacterial, antiviral, antitumoral, liver protective, kidney protective, antifungal, free radical scavenging, and antioxidant activities (Preethi *et al.*, 2009, Khalil *et al.*, 2007 and Chandran and Kutten, 2008). Regarding animal feeds, Calendula officinalis flower extract is regarded as a natural alternative for enhancing xanthophyll content and brightness in chick eggs and skin (Skrivan *et al*., 2015 and Wang *et al*., 2017); nevertheless, less attention has been given to marigold's antioxidant properties in farm animals. According to Diaz Carrasco et al. (2019), all plant extracts have additional properties that may be beneficial to animal health and production, through positive effects on gut ecology and enhance physiological functions, immune response and enhancing beneficial microbial content. Nowadays, modern chicken strains need a substantial quantity of food, which tends to make the digestive system vulnerable to impaired functionality (Svihus, 2014). In gut intestinal, villus length and crypt thickness are connected with nutrient absorption ability and are important indicators of gut health (Yazdani *et al.*, 2013). Active ingredients are acknowledged as improving healthy gut both in the direct and indirect ways by influence intestinal microbiota and increasing useful bacteria (Diaz Carrasco *et al*., 2019 and Oviedo-Rondón, 2019). Almost, no research has been published on the effects of calendula extract on digestive health. In addition, a few trials have been conducted to investigate the effect of adding calendula flowers or extract in broiler diets and its effect on nutrient digestibility and gut ecology (Shafey *et al.*, 2013 and Leskovec *et al.*, 2018).

As our knowledge, the available data are not sufficient to know the effects of COP and COEx during egg production on laying hens. Therefore, the current research aimed to investigate the effects of adding COP and COEx to diets of laying Japanese quail on digestive enzymes, nutrient digestibility, egg production, egg mass, feed conversion ratio, yolk cholesterol content, serum biochemistry, microbial content, antioxidant parameters and immunity.

2. MATERIALS AND METHODS

The current study was carried out at the Center of Poultry Research, Faculty of Agriculture, Fayoum University, Egypt. Research on live animals met the guidelines approved by the Institutional Animal Care and Use Committee in Egypt (Code No. of the proposal: AEC 2208).

2.1. Experimental design, birds and diets

A total number of 1000 of quail chicks one-day old was brooded in electrically heated battery and fed on a basal diet which contained 24% CP with 2900 Kcal/ME/Kg for six weeks of age according the requirements published by NRC (1994). At the beginning of laying period, a total number of 504 birds were randomly assigned to seven equal treatments, each treatment containing 72 birds in six replicates of 12 birds each (8 females plus 4 males). At 7th week of age, hens were given a diet containing 20% CP and 2900 Kcal/Kg until the end of experiment at 25th week of age.

At the start of 9th week, all birds were housed in laying cages with dimensions 50 × 60 ×25 cm^3. Birds were reared under the normal environmental conditions of Fayoum University Poultry Farms. Birds were exposed to light 16-h/ day, feed and water were *ad libitum* all over the experimental period.

The 1st group was fed without any studied additives (control), the 2nd, 3rd and 4th groups were fed on the control diet supplemented with 0.6, 0.9 and 1.2 % COP, respectively. While, the 5th, 6th and 7th groups were fed on the control diet supplemented with 150, 200 and 250 ppm COEx, respectively**.** Table 1 demonstrates the feed ingredients and proximate chemical analyses of the experimental diets. Pot marigold dried flower was obtained from Al-Rehab Company for the processing and export of medicinal and aromatic plants (Ibshawi, Fayoum, Egypt) in February 2019. *Calendula officinalis* flowers were ground by grinder contains sieve with 2 mm. Thus, it stored in suitable environment till used.

2.2. Chemical composition of Calendula Officinalis

Calendula officinalis flowers (air-dried) contain 9.90%, 8.40%, 12.20%, 43.10%, 8.40%, 18.00%, moisture, ash , crude protein, nitrogen-free extract (NFE), crude fat and crude fibre, respectively (Abd El-Wahab et al., 2022).

2.3. Preparation of the Calendula Officinalis flower extract

The *Calendula Officinalis flower* extract was prepared, as shown by Abd El-Wahab et al., (2022)

2.4. Laying hens performance

Feed consumption was recorded weekly, egg production and weight were recorded daily. Thereafter, feed conversion ratio, hen-day egg production, egg mass output and mortality were calculated.

2.5. Blood constituents

At 25 weeks of age, 12 birds (6 males and 6 females) were slaughtered according to

Islamic ritual to obtain blood samples (5 mL per bird), then it's were kept individually in clean dry centrifuge tubes. Samples were centrifuged at 3000 rpm for 20 minutes to take serum samples and stored in a tube of Eppendorf at −20℃ till analysis. According to James (2001), lipid profile including total cholesterol (Chol), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TG) were estimated. Thiobarbituric acid-reactive substances (TBARS), glutathione peroxidase (GPx) and total antioxidant capacity were measured according to Paglia and Valentine (1967). Liver function including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Reitman and Frankel (1957). Amylase and lipase enzymes were assayed by Friedman and Young (2001) and according to Bovine Trypsin ELISA Kit MBS706461 trypsin enzyme assayed. Immunoglobulins of birds including IgM, IgG and IgA were assayed according to Erhard et al., (1992).

2.6. Microbial analysis

After slaughter, the intestinal content was immediately collected in sterile glass containers, digesta was evacuated and mixed. Samples (1 g of the mixed fresh mass) were taken into sterile test tubes. Microbial counts (*E. coli, Salmonella spp., and Lactobacilli count*) were determined according to (Reda et al., 2020 and Xia et al., 2004). The microbial counts were estimated as colony-forming units per gram (cfu/g) of the sample.

2.7. Egg yolk cholesterol

After 28 days from the start of the experiment, eight eggs were taken randomly from each pen to determine yolk cholesterol. The yolk was dried in a forced-air oven at 70°C for 3 days. Extraction of yolk cholesterol was done using the modified method of Washburn and Nix (1974).

2.8. Nutrient digestibility

At 25 weeks old, 42 quail males (one per replicate) were reared individually to study the nutrients digestibility. Over a six-day collection period, the digestibility experiment was carried out **(**Ratriyanto et al. 2014). During the digestibility trial, procedures were implemented according to Ratriyanto and Prastowo (2019).

Nutrients Proximate analyses were analyzed as summarized by the Association of Official Analytical Chemists (AOAC, 2001). Separation of urinary nitrogen from fecal nitrogen was carried out according to Jakobsen et al., (1960). Calculation of nutrient digestibility coefficients was implemented (Emamzadeh and Yaghobfar 2009), as follows:

$$\text{Nutrient digestibility (\%)} = \frac{\text{Nutrient intake } g - \text{nutrient excreted } g}{\text{Nutrient intake } g} \times 100$$

2.9. Statistical analysis

The results were analyzed using analysis of variance by the statistics tools of Infostat (Di Rienzo, 2017). The model used was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where: Y_{ij}: observation of traits, μ: overall mean, T_i: treatment effect, e_{ij}: experimental random error. According to Duncan (1955), the multiple range test was used to compare all means. The significance level was at 0.05.

3. RESULTS

3.1. Digestive Enzymes and Digestibility Coefficients

According to Table 2, the data showed that digestibility coefficients (DC) of all nutrients digestibility were influenced by COP and COEx especially at levels 0.6 %

COP and 250 ppm COEx, respectively. Where, quail hens fed diets that contain 0.6 % COP had the highest DC ($p \leq 0.001$) of crude protein, ether extract, organic matter, dry matter and nitrogen free extract, being 90.66, 85.87, 77.88, 75.64, and 73.69%, respectively. While the group fed with COEx at 250 ppm COEx recorded 68.7, 73.88, 89.29, 82.43 and 66.28%, respectively. Lastly, quails fed with 250 ppm COEx had significantly ($p \leq 0.001$) higher DC of crude fiber (31.91) than that fed control diets (22.07). Moreover, the treatments had affected significantly on digestive enzymes. The groups fed diets supplemented with 250 ppm COEx showed significantly ($p \leq 0.001$) higher lipase and trypsin (97.54 and 118.50, respectively) compared with the control diets (58.48 and 70.88, respectively). While, groups fed 0.9 % COP had the highest amylase (758.25) ($p \leq 0.001$) compared with the control and other groups.

3.2. Laying Performance

Results in Table 3 show egg number (EN), egg weight (EW), egg mass (EM), egg production % (EP), feed consumption (FC) and feed conversion ratio (FCR) of quails fed different treatments. In the current experiment, there was no significant difference in feed consumption between all groups. The higher EN, EP, EW, EM, with the best FCR, were significantly ($p \leq 0.001$) recorded by group that received 200 ppm COEx (0.9, 89.62, 13.6, 12.19 and 2.05, respectively), followed by 250 ppm COEx (0.88, 88.08, 13.44, 11.83 and 2.07, respectively), and 1.20 % COP, (0.86, 85.92, 13.21, 11.35 and 2.4, respectively), in comparison with the control and other groups. In the current study, the diet supplemented with COEx had highly significant ($p \leq 0.001$) effect on EM, EN, EW, EP and FCR; but had insignificant impact on feed consumption.

3.3. Egg yolk cholesterol

Data found in Table 4 showed the effect of COP and COEx on the egg yolk cholesterol. Results showed that dietary COP and COEx especially 250 ppm COEx and 1.2 % COP had a significant ($p \leq 0.001$) influence on egg yolk cholesterol. The lowest content values were obtained from 250 ppm COEx (134.88) and 1.20 % COP (137.63), respectively compared to the control and other groups.

3.3. Serum Biochemistry

The treatments has affected all studied serum biochemical indices (Table 4). Quail hens fed a diet supplemented with 250 ppm COEx showed significantly ($p \leq 0.001$) lower Tri G (149.88), LDL (128.50) and ALT (2.75). While, groups received 200 ppm COEx showed significantly ($p \leq 0.001$) lower total Chol (195.50) and AST (184.50). Finally, hens fed on a diet supplemented with 1.2 % COP showed significantly ($p \leq 0.001$) higher HDL (54.63). Results also showed that quail hens fed diets supplemented with 250 and 200 ppm COEx, and 1.2 % COP recorded lower total Chol, Tri G, LDL, ALT and AST with higher HDL than the control**.**

3.4. Antioxidant Capacity and Immunological Parameters

The treatments has affected significantly all antioxidant parameters and immune responses studied. Quail hens fed the diet with 250 ppm COEx had the highest significantly ($p \leq 0.001$) GPx (1176.75), IgG (1120.86) and IgM (203.09) with the lowest TBARS (0.83). While, hens fed the diet with 1.2 % COP had the highest significantly ($p \leq 0.001$) IgA (105.53) and lymphocyte (91.15), but the highest T-AOC (1.46) was recorded by those received the supplemented diet with 200 ppm COEx, whereas both the control group and other groups showed an opposite trend (Table 5).

3.5. Microbial Content

Data presented in Table (6) represented useful and harmful intestinal bacteria. Results indicated that dietary levels of 250, 200 ppm COEx and 1.2 % COP supplementation significantly ($p \leq 0.001$) increased *Lactobacillus* count (6.60, 6.57 and 6.47, respectively) while decreased both *E. coli* (5.06, 5.56 and 5.13, respectively) and *Salmonella* (4.90, 4.94 and 5.15, respectively) counts compared with those fed the control diet. The lowest significant ($p \leq 0.001$) number of *E. coli* and *Salmonella* counts were showed for the group fed the diet supplemented with 250 ppm COEx (5.06 and 4.90, respectively), whereas the control group had the highest harmful intestinal bacteria (Table 6).

4. DISCUSSION

Results in the current research showed that digestibility coefficients (DC) of all nutrients were influenced by dietary supplementation of COP and COEx especially at levels 0.6 % COP and 200 ppm COEx, respectively. Additionally, the group of chicks fed 250 ppm COEx supplemented diets showed higher lipase and trypsin and groups fed 0.9 % COP had the highest amylase compared with the control diet. Our results correspond with those of Abd El-Wahab et al., (2022), who noticed that quails received diets supplemented by COP and COEx enhanced digestive enzymes and DC during the growth period. Additionally, Al-Sultan et al., (2016) showed that natural ingredients may enhanced utilization of energy, protein, minerals, and vitamins from diets, which improves feed efficiency by enhancing useful gut microbial population, gut safety, pancreatic enzymes, appetite stimulation, and immune response. Herbal such as COP and COEx are found to be safe, friendly to the environment with no side effects, and costing effectively so, their presence in the bird's diet should be encouraged to improve feed utilization and the performance. Herbal plants can participate in the animals nutrient requirements, endocrine system stimulation and improve nutrient metabolism (Kumar et al., 2014). Besides, the antioxidant activity, extracts of plant were found to have other useful activities for the health and productivity of animal. One of the most important effects is improvement of intestinal health, including morphological and physiological functions, developed barrier functions, efficient immune response, metabolism of tissue, adequate microbiota, and continuous inflammatory balance (Diaz Carrasco *et al.*, 2019). Using the extracts from Marigold (*Calendula officinalis L*.) in broilers improved feed digestibility (Hernandez et al., 2004). In contrast, nitrogen balance was reduced by supplementation with the *Calendula officinalis* extract compared to the control treatment (Leskovec, et al., 2018).

In the current trial, there was no significant difference in feed intake due to dietary COP and COEx treatments compared with the control group. The highest EN, EP, EW, EM and the best FCR were significantly ($p \leq 0.001$) obtained by the group received 200 mg COEx/kg, followed by 250 mg COEx/kg and 120g COP/kg, compared with the control and other groups. These findings are in agreement with those of Agiang et al. (2011), who found that daily egg production was enhanced in quails consumed a 5% aqueous extract of bush calendula leaves. The same results were obtained by Nuraini and Djulardi (2016) who mentioned that the levels of marigold flower extract (MFE) in the diet affected ($p<0.05$) the hen-day egg production of Japanese quails. While, increasing MFE (5, 10 and 15 ppm) levels in the diets improved the hen-day production by 74.33-80.69%. This improvement could be due to improvement in the nutrients digestibility coefficients and higher trypsin, amylase and lipase activity and this agree with Abd El-Wahab et al. (2022) who showed that quail chicks fed on the diet contained 200 ppm COEx had statistically higher digestive enzymes compared to the control group. Moreover, Sirri et al. (2007) noticed a substantial rise improvement in FCR for birds fed with diets augmented by calendula extract, also had a positive impact on digestive enzymes production. They added that the egg mass production of Japanese laying quails was influenced by diets supplemented with MFE. Skřivan *et al*. (2015) reported that hen-day egg production and egg mass (g/hen/day) were augmented through the addition of 150 mg/kg diet of marigold flower extract to diet. Also, EP of quails was improved by dietary supplementation of MFE (Mirzah and Djulardi, 2017). In contrast, Hasin et al., (2006) found that layers fed orange skin and marigold obtained slightly higher EP but the differences among treatments were recorded to be statistically non-significant compared with the control. Also, the use of 4% orange skin and 4% marigold in the diet of laying pullets had a useless effect on the rate of EP (Sikder *et al.*, 1998). Englmaierova and Skrivan (2013) utilized lutein, that found in extract of marigold, in diets for laying hens at 250 mg/kg and they noticed no effects on the hens productivity. Also, Rowghani et al., (2006) indicated that the dietary treatments supplemented with levels of marigold flower (0.49, 0.80 or 1.2 %) did not affect significantly the EP and EW in high line white layers. Rezaei et al., (2019) found that the use of the marigold flower extract had no adverse effects on egg production when the laying hens fed 20 or 40 ppm from the marigold flower extract.

In the current study, the dietary COEx had a significant improvement in FCR, but had no significant impact on FI. Our results on feed consumption and FCR were in the same line with Hasin et al. (2006) who indicated that the addition of 4% marigold in the diet did not affect feed consumption of pullets that could be due to feed palatability. Where, the highest efficient utility of feed was obtained by birds that received 4% marigold while, the bad obtained with the control group. Also, Rowghani, et al., (2006) indicated that the dietary treatments supplemented with levels of marigold flower (0.49, 0.80 or 1.2 %) did not significantly affect daily feed consumption and feed conversion ratio in high line white layers. Rezaei et al., (2019) illustrated that using calendula flowers extract pigments had no negative impacts on FC. Additionally, Nuraini and Djulardi (2016) reported that the marigold flower extract levels (5, 10 and 15 mg/kg diet) significantly influenced the laying quails feed consumption. They showed that increasing the level of extract in the diet significantly improved FC and FCR of Japanese laying quails.

In the present research, dietary supplementation of COP and COEx especially 250 ppm COEx and 1.2 %

COP had a significant influence on egg yolk cholesterol. The lowest content of egg yolk cholesterol was obtained from 250 ppm COEx and 1.20 % COP, respectively compared to the control and other groups. These findings are in the same line with McNaughton (1978) who displayed that the cholesterol of egg yolk was decreased by the alfalfa meal in the laying hens. Khajali et al. (2007) have also reported similar findings. Saponins cause hypocholesterolemia by forming unabsorbable cholesterol-saponins compounds at the site of intestinal absorption, resulting in minimal absorption of both exogenous and endogenous cholesterol or enhanced bile acid excretion (Jenkins and Atwal, 1994). Likewise, Güçlü et al. (2004) presented that the addition of alfalfa meal (90 g/kg) to the diet of laying quail depressed egg yolk cholesterol and serum lipids without adverse effect on performance. On the other hand, some researchers described that meal of alfalfa had no impact on cholesterol of egg yolk (Mourao et al., 2006) or saponins (Sim et al., 1984) in laying hens.

Quail birds supplied with diets containing COEx at 250 ppm COEx, 200 ppm COEx and 1.2 COP had dramatically lower Chol, Tri G, LDL, ALT, and AST with higher HDL compared to control. Calendula flowers contain lycopene, carotenoids, and flavonoids, which had a beneficial impact on blood lipids, hepatic, and renal processes. This could be due to the antioxidant properties impacts, which enhanced the health of hens (Abd El-Wahab et al., 2022). The effects of antioxidants that are found in medicinal herbs may be due to the phenolic substances concentration for instance (phenolic terpenes, flavonoids, proanthocianidins hydrolysable tannins and phenolic acids) and some vitamins (C, A and E). *Calendula officinalis* contains various phytochemical constituents being flavonoids like quercetin, alkaloids, lupeol, carotenoids, protocatechuic acid, isorhamnetin, etc. and triterpenoids which help in increasing the antioxidant effect (Matysik et al., 2005). Ševčikova et al. (2008) observed that the plasma lipid profile (the higher content of HDL cholesterol and lower content of LDL cholesterol) was positively affected by dietary lycopene. According to Hamzawy et al. (2013), groups treated with high and low levels of calendula extract (1000 and 500 mg/kg b.w) were similar to the control in all biochemical measurements. In contrast, Mishra et al. (2018) observed that animals treated with 2.5 mL/kg body weight of *calendula officinalis* oil, their biochemical changes were insignificant compared to the control group. Where, animals treated with doses of 5 and 10 mL/kg of *calendula officinalis* oil had significantly ($p < 0.01$) increasing in AST, ALT and triglycerides. Furthermore, blood serum biochemical analysis revealed that the activities of ALT and AST were not various among the MFEx treatments when compared to the other treatments (Pirman, et al., 2020).

In the present experiment, quail hens received diet with 250 ppm COEx had the highest significantly ($p \leq 0.001$) GPx, IgG and IgM with the lowest TBARS. While, hens fed the diet with 1.2 % COP had the highest significantly IgA and lymphocyte, but the highest T-AOC recorded by those received the supplemented diet with 200 ppm COEx. The fact that calendula flowers involve polyphenolic compounds and beta carotene, which have antioxidative effects on inhibition of fat oxidation and reducing oxidative stress, that may be related to the enhancement of immune response (IgG, IgA, IgM, and lymphocyte) and oxidation enzymes (TBARS, T-AOC, and GPx) (Abd El-Wahab et al., 2022). Bioflavonoids can decrease the oxidative stress and enhance the performance of numerous farm animals (Hager-Theodorides et al., 2014). Moreover, extract of *Calendula officinalis* displays activity against reactive nitrogen species and reactive oxygen species (ROS) with an effective activity even at low concentrations (Braga et al., 2009). As reported by Sabir et al. (2015), Calendula officinalis anti-oxidant activity with flower extract displaying higher anti-oxidant activity than extract of the leaf. The extract of calendula decreases kidney damage due to its antioxidant effectiveness. The augmented activity of antioxidant enzymes like catalase, glutathione peroxidase, superoxide dismutase, and in extract-treated group resulted in protection against cisplatin-induced damage of renal (Preethi et al., 2009). The marigold antioxidant properties (Ukiya *et al.*, 2006), originate from its carotenoids and polyphenols (Miliauskas *et al.*, 2004), and from its sterols, essential oils, flavonoids, triterpene alcohols, carotenoids, tannins, saponins, polysaccharides, resin, mucilage, and a bitter principle (Khalil *et al.*, 2007).

In this concern, the *in vitro* studies showed that the various differently extracts prepared for Marigold flowers have an effective radical capacity (Miliauskas et al., 2004), also, supplementation with marigold extract (0.075%, 0.15%, 0.30%, and 0.60% marigold extract) significantly augmented GSH-PX and T-AOC of liver in all treatment groups comparing to the control group. Plants and spices rich in carotenoids, vitamin C and flavonoids are useful for the immune system. The herbs containing molecules caused possess immune-stimulatory properties. These herbs can improve natural killer cells, the activity of lymphocytes and macrophages; they stimulate the interpheron synthesis or phagocytosis (Frankic et al., 2009). As showed by Lavinia et al. (2009), the immune response can be improved by medicinal plants essential oils and also are able to cause changes of mucosa of the duodenal with favorable effects for the animal. The MFEx displayed complete inhibitory influence on the lymphocytes proliferation in the presence of mixed lymphocyte reaction and phytohemagglutinin (Amirghofran, et al., 2000). Furthermore, Rajput et al. (2012) stated that the beneficial influences of calendula extract on immune function may be attributed to augmented DNA integrity, which also aids in the improvement of antibody levels against Newcastle and influenza viruses.

In the current study, supplementation with 250, 200 ppm COEx, and 1.2 % COP substantially increased *Lactobacillus* numbers while lowering *Escherichia coli* and *Salmonella* numbers compared to those fed the normal diet (control). The existing results agree with those of Balenovi et al. (2018), who discovered that faecal samples used for microbiological checking for the appearance of *Escherichia coli*, they showed a reduction in E.coli Colony - forming units number in all test group augmented with marigold at levels of 1% and 2% to laying chickens of the Tetra SL line in comparison to the normal treatment (control). This reduction in harmful bacteria and increment in helpful bacteria could be related to the antimicrobial properties of polyphenolic compounds found in calendula flowers, which modify the pH of the intestines and enhance the conditions within the gastrointestinal tract (Abd El-Wahab et al., 2022). In addition, *Lactobacilli* bacteria may additionally utilize these polyphenols compounds to generate energy for their cells and metabolic activity (Abd El-Wahab et al., 2022). In this regard, polyphenolic compounds found in medicinal herbs may have an antibacterial properties within intestinal tract and restrict the harmful bacteria, where this herbal derivatives have essential factors that can reduce pH (7.0 to 5.5) in the intestinal system (Viveros et al., 2011). The majority of medicinal herbs additives exert antibacterial benefits through their impact on the microbial cell walls structure, causing denaturing and coagulating proteins. The cytoplasmic membrane's absorption of H^+ and K^+ ions was altered by the essential oils. Therefore, the microbes will die because of the loss of chemiosmotic regulation and the disruption of important cellular functions such as enzyme-dependent reactions, electron transport, oxidative phosphorylation, and protein translocation (Dorman and Deans, 2000). The bacterial cytoplasmic membrane disruption is owing to the essential oils lipophilic nature that accumulate in the membranes (Dorman and Deans, 2000). According to Elmi et al. (2020), who suggested that using *L. acidophilus* with herbal flower aqueous extracts could enhance *Lactobacillus spp.* and restrict colonies harmful bacteria like *Salmonella spp.* in the digestive tract of chickens.

Likewise, marigold flowers extract has antimicrobial action against a number of bacteria. *In vitro*, the oil of calendula flowers reduced the development of gram-negative bacteria like *Escherichia coli*, as well as gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* (Hamad et al., 2011).

5. CONCLUSION

From the previous results mentioned above, it could be concluded that diet supplemented with COEx (especially, 250 and 200 ppm COEx) to layer Japanese quail can enhance productive performance, digestive enzymes, lipid profiles, antioxidant capacity, liver functions, immunological parameters, microbial content, digestion of nutrients and physiological parameters.

Table (1): Composition and analysis of experimental diets containing *Calendula Officinalis* flower powder (COP) and extract (COEx) during the laying period in Japanese quail

Ingredients %	Control 0.0	0.6 % COP	0.9 % COP	1.20 % COP	150 ppm COEx	200 ppm COEx	250 ppm COEx
Corn Yollow	54.90	54.90	54.90	54.90	54.885	54.88	54.875
Soybean Meal (44%)	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean Full fat (37%)	9.10	9.10	9.10	9.10	9.10	9.10	9.10
Corn Gluten Meal (60%)	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Wheat Bran	3.00	2.40	2.10	1.80	3.00	3.00	3.00
Soybean oil	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Lime Stone	6.05	6.05	6.05	6.05	6.05	6.05	6.05
Mono Calcium Phosphate	1.30	1.30	1.30	1.30	1.30	1.30	1.30
COP	0.00	0.6	0.9	1.2	0.00	0.00	0.00
COEx	0.00	0.00	0.00	0.00	150	200	250
DL-Methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Salt, (NaCl)	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix *	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis**							
Dry Matter	88.56	88.09	87.86	87.62	88.56	88.56	88.56
Crude Protein	20.22	20.27	20.29	20.32	20.22	20.22	20.22
Ether Extract	4.05	4.13	4.18	4.22	4.05	4.05	4.05
Crude Fiber	3.00	2.94	2.90	2.87	3.00	3.00	3.00
Calcium	3.52	3.52	3.52	3.52	3.52	3.52	3.52
Methionine	0.48	0.47	0.47	0.47	0.48	0.48	0.48
Lysine	1.14	1.14	1.14	1.13	1.14	1.14	1.14
Methionine + Cystine	0.86	0.86	0.86	0.86	0.86	0.86	0.86
Available Phosphorus	0.34	0.39	0.38	0.38	0.34	0.34	0.34
M.E. Kcal/Kg	2880.00	2872.00	2868.00	2864.00	2880.00	2880.00	2880.00
Determined Analysis:							
Dry Matter %	90.670	89.700	89.310	88.350	87.050	90.410	89.510
Crude Protein %	20.100	20.460	20.700	20.400	20.170	20.110	20.190
Ether Extract %	3.990	4.012	4.101	3.970	3.895	3.690	3.970
Ash %	9.232	9.181	9.164	9.232	9.232	9.232	9.232
crude fiber %	2.802	2.499	2.299	2.201	2.912	2.983	2.854
NFE %	54.546	53.548	53.046	52.547	50.841	54.395	53.264

*Each 3.0 kg of premix supplies one ton of the diet with: Vit. A, 12000000 I.U; Vit. E, 10g; Vit. D3, 2500000 I.U; Vit. K3, 2.5g; Vit.B1,1g; Vit.B2,5g; Vit.B6,1.5g; Vit.B12,10g; Biotin 50mg; Folic acid, 1g; Nicotinic acid, 30g; Capantothenate, 10g; Zn, 55g; Cu, 10g; Fe, 35g; Co,250mg; Se, 150mg; I, 1g; Mn,60g; and antioxidant, 10g.
**According NRC, 1994.

Table (2): Effect of *Calendula Officinalis* flower powder (COP) and extract (COEx) on digestive enzymes and nutrients digestibility (%) during Laying period in Japanese quail

Items Treat.	Control 0.0	0.6 % COP	0.9 % COP	1.20 % COP	150 ppm COEx	200 ppm COEx	250 ppm COEx	SEM	P-value
Digestive Enzymes (U/L)									
Amylase	534.75 ^{b}	615.38 ^{ab}	758.25 ^{a}	673.63 ^{a}	736.13 ^{a}	669.38 ^{a}	694.88 ^{a}	44.90	0.0196
Lipase	58.48 ^{b}	66.71 ^{b}	88.91 ^{a}	91.88 ^{a}	93.58 ^{a}	96.90 ^{a}	97.54 ^{a}	3.97	0.0001
Trypsin	70.88 ^{c}	95.63 ^{b}	102.25 ^{ab}	98.13 ^{b}	94.75 ^{b}	101.63 ^{ab}	118.50 ^{a}	6.55	0.0008
Digestibility Coefficients									
DM	61.06 ^{c}	75.64 ^{a}	64.97 ^{ab}	63.99 ^{bc}	63.73 ^{bc}	61.69 ^{bc}	68.70 ^{ab}	2.19	0.021
OM	66.23 ^{bc}	77.88 ^{a}	68.12 ^{bc}	65.51 ^{c}	66.45 ^{bc}	66.61 ^{bc}	73.88 ^{ab}	2.44	0.005
CP	87.28 ^{b}	90.66 ^{a}	88.58 ^{ab}	88.24 ^{b}	87.55 ^{b}	89.29 ^{ab}	89.29 ^{ab}	0.7	0.027
EE	73.37 ^{b}	85.87 ^{a}	71.55 ^{b}	82.66 ^{ab}	78.5 ^{ab}	75.76 ^{b}	82.43 ^{ab}	3.06	0.05
CF	22.07 ^{b}	22.07 ^{b}	29.84 ^{ab}	25.09 ^{ab}	30.45 ^{a}	29.06 ^{a}	31.91 ^{a}	2.22	0.039
NFE	59.88 ^{bc}	73.69 ^{a}	61.58 ^{bc}	56.72 ^{c}	58.58 ^{bc}	59.21 ^{bc}	66.28 ^{ab}	3.29	0.008

$^{a-c}$: Means within the same row with different superscripts are significantly different ($P \leq 0.05$) , DM: Dry Matter, OM: Organic Matter, CP: Crude Protein, EE: Ether Extract, CF: Crude Fiber, NFE: Nitrogen Free Extract, SEM: Standard Error Mean.

Table (3): Effect of dietary *Calendula Officinalis* flower powder (COP) and extract (COEx) on laying Performance of Japanese quail

Items Treat.	Control 0.0	0.6 % COP	0.9 % COP	1.20 % COP	150 ppm COEx	200 ppm COEx	250 ppm COEx	SEM	P-value
EN	0.80 ^{c}	0.85 ^{b}	0.85 ^{b}	0.86 ^{b}	0.86 ^{b}	0.90 ^{a}	0.88 ^{ab}	0.01	0.0001
EP (%)	79.76 ^{c}	85.23 ^{b}	84.97 ^{b}	85.92 ^{b}	85.73 ^{b}	89.62 ^{a}	88.08 ^{ab}	1.09	0.0001
Av. EW	12.86^{abc}	12.69^{bc}	12.40^{c}	13.21^{ab}	13.10^{abc}	13.60^{a}	13.44^{ab}	0.25	0.0229
EM	10.29^{d}	10.82^{cd}	10.55^{cd}	11.35^{abc}	11.23^{bc}	12.19^{a}	11.83^{ab}	0.30	0.0006
FC	28.31	27.18	25.66	27.14	26.05	24.89	24.43	1.05	0.1430
FCR	2.78^{a}	2.52^{ab}	2.45^{ab}	2.40^{bc}	2.32^{bcd}	2.05^{d}	2.07^{cd}	0.11	0.0005

EN: Egg Number (per bird/day), EP (%): Egg production (%), Av. EW: Average egg weight (g), EM: Egg mass (g/hen/day), FC: Feed consumption (g feed/bird/day), FC: feed conversion ratio (kg feed/kg egg mass), $^{a-c}$: Means within the same row with different superscripts are significantly different ($P \leq 0.05$), SEM: Standard Error Mean.

Table (4): Effect of dietary Calendula Officinalis flower powder (COP) and extract (COEx) on lipid profile, liver functions and yolk cholesterol in laying Japanese quail

Items Treat.	Control 0.0	0.6 % COP	0.9 % COP	1.20 % COP	150 ppm COEx	200 ppm COEx	250 ppm COEx	SEM	P-value
lipids profile (mg/dL)									
T. Chol	270.50 ^{a}	265.50 ^{ab}	224.00 ^{bc}	205.63 ^{c}	211.88 ^{c}	195.50 ^{c}	200.13 ^{c}	12.08	0.0003
TG	210.25 ^{a}	162.50 ^{b}	149.88 ^{b}	158.50 ^{b}	178.13 ^{ab}	156.88 ^{b}	149.88 ^{b}	12.04	0.0101
HDL	46.88 ^{b}	49.88 ^{ab}	51.88 ^{a}	54.63 ^{a}	50.75 ^{ab}	52.25 ^{a}	54.25 ^{a}	1.50	0.0110
LDL	191.88 ^{a}	171.38 ^{ab}	190.50 ^{abc}	145.75 ^{bc}	177.75 ^{ab}	134.13 ^{c}	128.50 ^{c}	11.76	0.0019
Liver Function (U/L)									
ALT	5.75 ^{a}	4.13 ^{b}	3.75 ^{bc}	2.50 ^{d}	3.13 ^{cd}	3.25 ^{cd}	2.75 ^{d}	0.25	0.0001
AST	265.13 ^{a}	241.50 ^{ab}	251.50 ^{ab}	230.25 ^{ab}	235.75 ^{ab}	184.50 ^{c}	201.50 ^{bc}	17.69	0.0328
Egg yolk Chol (mg/dL)									
Y.Chol	154.53 ^{a}	150.54 ^{b}	146.78 ^{c}	137.63^{de}	149.29 ^{bc}	140.41 ^{d}	134.88 ^{e}	1.19	0.0001

T. chol: Total cholesterol, TG: triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, Y.Chol: Yolk Cholesterol mg/dl, $^{a-e}$: Means within the same row with different superscripts are significantly different(P≤0.05), SEM: Standard Error Mean.

Table (5): Effect of dietary *Calendula Officinalis* flower powder (COP) and extract (COEx) on antioxidant parameters and immune response in laying Japanese quail

Items Treat.	Control 0.0	0.6 % COP	0.9 % COP	1.20 % COP	150 ppm COEx	200 ppm COEx	250 ppm COEx	SEM	P-value
Antioxidant Parameters (nmol /ml)									
T-AOC	0.91 ^{b}	0.95 ^{b}	1.16 ^{ab}	1.27 ^{ab}	1.17 ^{ab}	1.46 ^{a}	1.41 ^{ab}	0.10	0.0008
GSH-PX	570.25 ^{d}	698.00 ^{cd}	827.75 ^{bcd}	799.63 ^{bcd}	967.88 ^{abc}	1047.25 ^{ab}	1176.75 ^{a}	92.05	0.0004
TBARS µgg	1.57 ^{a}	1.21 ^{b}	1.13 ^{bc}	0.88 ^{d}	1.18 ^{b}	0.97 ^{cd}	0.83 ^{d}	0.06	.0001
Immune Response (mg/dl)									
IgG	864.23 ^{e}	998.33 ^{cd}	1050.03 ^{bc}	1091.17 ^{ab}	977.33 ^{d}	1074.00 ^{ab}	1120.86 ^{a}	20.86	0.0001
IgA	93.09 ^{d}	100.55 ^{bc}	103.93 ^{ab}	105.53 ^{a}	100.20 ^{c}	104.63 ^{ab}	105.34 ^{a}	1.41	0.0001
IgM	175.72 ^{b}	182.03 ^{b}	196.23 ^{a}	200.00 ^{a}	193.17 ^{a}	199.39 ^{a}	203.09 ^{a}	3.24	0.0001
Lymphocyte	76.59 ^{d}	81.53 ^{c}	89.14 ^{ab}	91.15 ^{a}	84.34 ^{bc}	89.93 ^{a}	89.09 ^{ab}	1.72	0.0001

T-AOC: Total Antioxidant Capacity, GSH-PX: Glutathione Peroxidase TBARS: Thiobarbaturic Acid- Reactive Substances, IgG: Immunglobin G, IgA: Immunglobin A, IgM: Immunglobin M, $^{a-e}$: Means within the same row with different superscripts are significantly different (P≤0.05), SEM: Standard Error Mean.

Table (6): Effect of dietary *Calendula Officinalis* flower powder (COP) and extract (COEx) on intestinal bacteria in laying Japanese quail

Items Treat.	Control 0.0	0.6 % COP	0.9 % COP	1.20 % COP	150 ppm COEx	200 ppm COEx	250 ppm COEx	SEM	P-value
E.coli *log 10 cfug*	7.65 ^{a}	6.56 ^{b}	5.56 ^{c}	5.13 ^{c}	6.42 ^{b}	5.56 ^{c}	5.06 ^{c}	0.23	0.0001
Salamonella *log 10 cfug*	7.36 ^{a}	5.76 ^{bc}	5.34 ^{bc}	5.15 ^{bc}	5.92 ^{b}	4.94 ^{bc}	4.90 ^{c}	0.31	0.0001
Lactobacillus *log 10 cfug*	5.25 ^{b}	6.24 ^{a}	6.31 ^{a}	6.47 ^{a}	5.59 ^{b}	6.57 ^{a}	6.60 ^{a}	0.19	0.0001

$^{a-c}$: Means within the same row with different superscripts are significantly different, **SEM:** Standard Error Mean

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Quail; laying period, *Calendula officinalis*, performance; serum biochemistry.

الملخص العربى

تأثير الوجبات المكملة بمسحوق أزهار الكلانديولا *Calendula officinalis* ومستخلصها على هضم العناصر الغذائية ، والأداء ، والكيمياء الحيوية في الدم ، ومضادات الأكسدة ، والمعايير المناعية ، وبعض البكتيريا المعوية في السمان الياباني خلال فترة إنتاج البيض

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الهدف من هذه التجربة هو النظر في تأثير مسحوق أزهار الكلانديولا ومستخلصها على الأداء الإنتاج وإنزيمات الهضم ومعاملات هضم العناصر الغذائية وكوليسترول صفار البيض والكيمياء الحيوية في الدم والمحتوى البكتيري للأمعاء والقدرة المضادة للأكسدة والمعايير المناعية في السمان الياباني البياض. في هذه التجربة ، تم أستخدام عدد 504 من السمان الياباني البياض بواقع (168 ذكر و 336 أنثى)، بعمر 9 أسابيع، حيث وزعت بشكل عشوائي على سبع معاملات تجريبية، وكل معاملة أحتوت على ستة مكررات في كل مكرر 12 طائرًا (أربعة ذكور وثماني إناث). تلقت المعاملة الأولى عليقة قاعدية بدون أي إضافات كمجموعة كنترول، في حين غذيت المجاميع من أثنين إلى أربعة على عليقة الكنترول مضافا إليها مسحوق أزهار الكلانديولا بنسب 0.6 و 0.9 و 1.2 %، على التوالي . بينما تغذت المجموعات من خمس إلى سبع على عليقة الكنترول مضافا إليها مستخلص أزهار الكلانديولا بمعدل 150 و 200 و 250 جزء في المليون على التوالي. أظهرت النتائج أن الدجاجات الذي تم تغذيتها على علائق تحتوى على مسحوق أزهار الكلانديولا بمعدل 1.2 % و مستخلص أزهار الكلانديولا بمعدل 200 و 250 جزء في المليون هى الأفضل معنويا في الأداء الإنتاجي حيث كانت الأعلى في عدد بيض، إنتاج البيض، وزن البيض، وكتلة البيض مع أفضل نسبة تحويل غذائي للعلف مع عدم وجود تأثير معنوي على كمية العلف المأكول. انخفض كوليسترول صفار البيض معنويا في السمان المعامل بـ 250 جزء في المليون من مستخلص أزهار الكلانديولا و 1.2 % من مسحوق أزهار الكلانديولاو 200 جزء في المليون من مستخلص أزهار الكلانديولا، على التوالي. تم تحسين الكيمياء الحيوية في الدم بشكل ملحوظ (الكوليسترول الكلي، الدهون الثلاثية، الكوليسترول عالى الكثافة، الكوليسترول منخفض الكثافة)، وظائف الكبد، معاملات مضادات الأكسدة ، الاستجابة المناعية وبكتيريا حامض اللاكتيك (Lactobacillus) المعوية. أيضا تحسنت إنزيمات الجهاز الهضمي بشكل ملحوظ للطيورالتى تم تغذيتها على علائق مضاف إليها مسحوق أزهار الكلانديولا ومستخلصها وبالأخص التي غذيت على المستخلص بمعدل 250 جزء في المليون. أخيرًا ، كان لدى السمان المغذى على العلائق المحتوية على 0.6 % من مسحوق أزهار الكلانديولا و 250 جزء في المليون من مستخلص أزهار الكلانديولا أعلى نسبة لمعاملات هضم العناصر الغذائية. خلصت الدراسة الحالية إلى أن العلائق التي تحتوي على 200 و 250 جزء في المليون من مستخلص أزهار الكلانديولا لكل كيلوجرام في السمان الياباني البياض أدت إلى تحسين إنزيمات الجهاز الهضمي ، ومعاملات هضم العناصر الغذائية ، والأداء الإنتاجي ، والكيمياء الحيوية في الدم ، والإستجابات المناعية ، والقدرة المضادة للأكسدة ، والمحتوى الميكروبي للأمعاء.

الكلمات الدالة: طائر السمان ؛ فترة إنتاج البيض، الكلانديولا، الأداء الإنتاجي، كيمياء الدم .