Pumpkin and garden cress seed oils as feed additives to improve the physiological and productive traits of laying hens

Amina S. El-Saadany, Amal M. El-Barbary, Effat Y. Shreif, Alaa Elkomy, Ayman M. Khalifa and Karim El-Sabrout

Poultry Breeding Research Department, Animal Production Research Institute, Agriculture Research Center, Giza, Egypt; Poultry Nutrition Research Department, Animal Production Research Institute, Agriculture Research Center, Giza, Egypt; Livestock Research Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications (SRTA-City), Borg El Arab, Egypt; Faculty of Desert and Environmental Agriculture, Matrouh University, Matrouh, Egypt; Poultry Production Department, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt

ABSTRACT
This study aimed to investigate the effects of adding pumpkin (PK) and garden cress (GC) seed oils to laying diets, as nutraceuticals, on the physiological and productive performance of layers. A total of 360 hens, at 24 weeks of age, were randomly and equally assigned into four groups of nine replicates each (10 birds/replicate). The 1st group was fed the basal diet and was served as a control. The 2nd, 3rd, and 4th groups were fed diets supplemented with 0.5% PK oil, 0.5% GC oil, and 0.25% PK oil + 0.25% GC oil, respectively. The experiment lasted eight weeks. According to the results, PK (0.5%) and GC (0.5%) seed oils, as well as their combination (PK 0.25% + GC 0.25%), had significant positive effects (p < 0.05) on several biochemical parameters, such as cholesterol and triglycerides, as well as some productive traits, such as egg production and egg mass of laying hens. They also positively affected (p < 0.05) birds’ blood antioxidant parameters, such as superoxide dismutase (SOD) and Malondialdehyde (MDA), and improved (p < 0.05) some immunological response parameters, such as globulin, lymphocytes, and heterophils. Additionally, they enhanced (p < 0.05) egg yolk fatty acids, such as oleic and linoleic. Therefore, we recommend adding PK and GC seed oils to laying diets as natural feed additives, either separately (0.5%) or in combination (0.25% + 0.25%), to improve several physiological functions and productive traits of birds.

HIGHLIGHTS
- PK and GC seed oils are natural feed additives for laying hens.
- PK and GC seed oils can improve birds’ antioxidative and immunological status.
- PK and GC seed oils addition has positive impacts on egg production and quality.

Introduction

To confront the world’s increasing population and the demands of the times, there is a growing need to increase productivity and improve the quality of the poultry industry. The continued use of conventional feed additions, either from plant or animal sources, in poultry diets has become a critical issue due to the high consumption competition among livestock species, which has the greatest impact on the price increase of these components and livestock products. Therefore, it is necessary to find other readily available feed additions to protect the poultry industry, particularly in developing countries (El-Deek et al. 2020).

Nutraceuticals are biologically active substances found in natural products. They can be added to poultry diets to provide nutrition and health benefits to the birds (Alagawany et al. 2021a, 2021b). Pumpkin (Cucurbita maxima) (PK) and garden cress (Lepidium sativum) (GC) seeds, as nutraceuticals, are important economic plant seeds that have recently attracted global interest due to their nutritional and therapeutical properties, resulting in novel sources of feed additives that can fortify various poultry diets. These plant seeds comprise a good amount of protein, fat, vitamins, minerals, fibres, and phytochemicals, which are incorporated in bird’s biological functions (Bryan et al. 2009;...
Due to their nutritional, antioxidant, and antimicrobial properties, they can improve bird's growth and productivity, as well as enhance health and immune response (Adamu and Boonkaewwan 2014; Singh and Paswan 2017; Achilonu et al. 2018; Al-Sayed et al. 2019; Mathewos et al. 2019). Additionally, PK and GC seed oils are essential aromatic oils (rich with essential fatty acids), which have several nutritional and medicinal features for poultry farming. The PK seed oil is rich in β-carotene and vitamin E, which have strong antioxidant and anti-cancer effects (Stevenson et al. 2007; Mitra et al. 2009; Srbinoska et al. 2012; Abbas et al. 2016). It contains L-tryptophan, omega-3, and omega-6 fatty acids (Hashemi 2013). It also contains trace minerals such as zinc (Kôna et al. 2007). Martínez et al. (2012) concluded that incorporating 10% of PK into laying hen diets is recommended to increase the content of ether extract and beneficial fatty acids, as well as reduce the total cholesterol and the harmful fatty acids in the eggs. Garden cress (also known as pepper grass) is an annual herb that primarily grows in the Middle East countries and Europe (Poy et al. 2015). The GC seed oil contains tocopherol (a natural antioxidant), carotenoid, oleic acid, and α-linolenic acid, and it can reduce various types of radicals (Diwakar et al. 2010; Zia-Ul-Haq et al. 2012). According to Shawle et al. (2016), GC can be included as a feed additive at a level of 0.75% in the total ratio of the broiler's diet for better biological performance and health status. Hassan and El Shoukary (2019) also reported that GC supplementation (1%) could significantly improve broiler body weight, weight gain, blood total protein, blood globulin, feed consumption, behaviour, and economic efficiency.

Despite their numerous benefits, PK and GC seed oils have received insufficient attention, particularly in laying chickens. Therefore, the purpose of this study was to investigate the effects of PK and GC seed oils as dietary supplements on the physiological and productive performance of layers.

Materials and methods

Animal ethics

All procedures and husbandry guidelines were performed according to the experimental animal care committee ethics of Animal Production Research Institute and Alexandria University, Egypt (2020/3).

Experimental design

A total of 360 hens at 24 weeks of age from Mandarh strain (improved Egyptian strain), with an initial body weight of 1570 g on an average, were used to investigate the effect of using pumpkin (PK) and garden cress (GC) seed oils, as feed additives, on the productivity and health of laying hens. Hens were randomly assigned into 4 groups (treatments) of 9 replicates (each 10 hens) in floor pens with a 16-hr light:8-hr dark cycle. Group (1) was fed the basal diet and served as a control group. Group (2) was fed the basal diet supplemented with 0.5% PK oil/kg basal diet, while group (3) was fed the basal diet supplemented with 0.5% GC oil/kg basal diet. The 4th group was fed the basal diet supplemented with 0.25% PK oil + 0.25% GC oil. The experiment period lasted 8 weeks. The levels of supplementation have been chosen according to the previous studies, such as Stevenson et al. (2007), Kulaitiene et al. (2007), Srbinoska et al. (2012), Bardaa et al. (2016), Abbas et al. (2016), and Achilonu et al. (2018). PK and GC seed oils were obtained from El-Hawag factory for the extraction of natural oils and cosmetics in Badr City, Egypt.

The experiment was conducted at El-Sabahia Poultry Research Station (Alexandria), Animal Production Research Institute, Agricultural Research Centre, Egypt during the spring season (25°C temperature, 70% humidity rate, 17 km/h wind speed, as averages). Birds were fed a diet, formulated on dry basis, containing 2700 kcal/kg ME and 16% crude protein (Table 1). The methods used for calculated chemical analysis of the basal diet were according to the Association of Official Analytical Chemists (AOAC 2000), while the method used for ME calculation was according to Bourdillon et al. (1990). Feed and water were provided ad libitum throughout the experimental period.

Productive and egg quality parameters

Daily egg production (%), egg weight (g), and egg mass (g of egg/hen/day) were recorded for each group. In addition, daily feed intake (g/hen/day) and feed conversion ratio (g feed/g egg) were estimated. Twenty eggs were randomly chosen from each group (four eggs from each replicate) and used to determine eggshell, albumin, and egg yolk weights as a percentage of egg weight. The eggshell thickness (mm) was measured by a micrometer after removing the inner eggshell membrane, and yolk colour intensity was assessed based on the standard colour of the yolk.
using a Roche yolk colour fane with a score range of 1–15 from light yellow to the dark yellow.

**Hematological and biochemical parameters**

At the end of the experimental period, nine blood samples from each group were collected from the birds’ wing veins to obtain serum and plasma for hematological and biochemical analyses. The red blood cells count (RBCs), white blood cells count (WBCs), haemoglobin (Hb), and packed cell volume (PCV) were the hematological parameters determined. The RBCs were counted on an acridine orange (AO) bright-line haemocytometer using a light microscope at 400x magnification, while the WBCs were counted on an AO bright-line haemocytometer using a light microscope at 100x magnification after diluting blood samples 20 times with a diluting fluid (1% acetic acid solution with a little of Leishman’s stain), and their fractions (lymphocytes and heterophils) were determined according to Altan et al. (2000). The Hb was determined by the cyanomethemoglobin method as cited by Coles (1986), while wintrobe haematocrit tubes were used for determining the PCV as a percentage.

Plasma was obtained by centrifuging the blood at 3500 xg for 20 minutes and it was stored at −20°C for biochemical analysis. Total protein concentration (g/dL) was determined according to Henry et al. (1974), while albumin concentration (g/dL) was estimated using Doumas et al. (1971) method. Globulin concentration (g/dL) was calculated by subtracting total protein from albumin. Plasma total cholesterol concentration (mg/dL) was determined according to Bogin and Keller (1987). High-density lipoproteins (HDL) cholesterol and low-density lipoproteins (LDL) cholesterol concentrations (mg/dL) were determined according to the methods of Lopes-Virella et al. (1977) and Wieland and Seidel (1983), respectively. Plasma triglycerides concentration was measured by the method of Sugiu et al. (1977). Plasma glucose concentration was measured according to the method of Trinder (1969) using the instructions of a specific kit (Diamond Diagnostics Chemical Company, Egypt). Liver enzymes activities (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) were assayed in plasma by the method of Reitman and Frankel (1957) using a specific kit (Diamond Diagnostics Chemical Company, Egypt).

**Antioxidant parameters**

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were estimated according to Misra and Fridovich (1972) and Chiu et al. (1976). Malondialdehyde (MDA), as a lipid peroxidation biomarker, was assayed in the blood plasma according to Conti et al. (1990).

**Yolk’s fatty acid analysis**

At the end of the experiment, nine eggs from each studied group were randomly selected for yolk fatty acid analysis. Yolk samples (approximately 2 g) were extracted in 125 mL with 2 parts chloroform and 1-part methanol for 1 to 2 hours. The contents were filtered to remove the insoluble components, and the filtrate was brought to volume in a 50 mL volumetric flask. A sample of 0.2 mL aliquot was analysed for cholesterol according to Allain et al. (1974) method using commercial kits produced by Diamond Diagnostics Company, Egypt. Total lipids extraction was performed according to Fisher and Leveille (1957). Preparation of fatty acids methyl esters from total lipids samples was performed according to the procedure of Radwan (1978) by shaking off a solution of 0.2 g of oil and 3 mL of hexane with 0.4 mL of 2N methanolic potassium hydroxide. A sample of total lipids (50 mg) was transferred into a Screw-Cap vial, then 2 mL benzene and 10 mL H2SO4 (1%) were added in absolute methanol. The vial was placed in a stream of nitrogen gas before being heated in an oven at 90°C.
for 90 minutes. The volume of 10 mL of distilled water was added to the cooled vial and the methyl esters in each vial were extracted with 5 mL of petroleum ether three times. The three petroleum ether extracts were combined and concentrated to their minimum volume by using a stream of nitrogen. Fatty acids were determined by gas-liquid chromatography (GLC) according to the method described by Metcalfe et al. (1966). A standard mixture of methyl esters was analysed under identical conditions prior to running the samples. The fatty acids were identified by matching their retention times with those of their corresponding standards. The proportions of methyl esters were calculated by the triangulation method.

Statistical analysis
Data were statistically analysed according to the SAS program (2004). All values were presented as least-square means with an overall standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA. Significant differences among treatment groups were subjected to Tukey test. Results were considered significant at $p \leq 0.05$. The statistical model used was as follows:

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

where $x_{ij}$ is the value of the measured variable, $\mu$ is the overall mean, $T_i$ is the effect of treatment ($i=4$ treatments), and $e_{ij}$ is the residual error.

Results
PK and GC seed oils' fatty acids are presented in Table 2. It was observed that PK and GC seed oils have high concentration of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). As a general result, PK (0.5%) and GC (0.5%) seed oils, as well as their combination (PK 0.25% + GC 0.25%), had significant effects on several biochemical parameters and productive traits of laying hens. They also positively affected bird's blood antioxidant capacity, immunological response, and egg yolk fatty acids.

Productive and egg quality parameters
The results of Table 3 showed that the supplementation of PK and GC seed oils, as well as their combination, in laying diet significantly ($p \leq 0.05$) affected the egg production, egg mass, and feed conversion ratio, but it did not affect the egg weight and the feed intake. Additionally, this supplementation did not significantly ($p > 0.05$) affect egg quality traits such as yolk weight, yolk colour, albumin percentage, and eggshell thickness (Table 4).

Table 2. Major fatty acid contents (%) in pumpkin and cress seed oils.

| Fatty acids     | PK seed oil | GC seed oil |
|-----------------|-------------|-------------|
| Palmitic C16:0  | 15.32       | 10.09       |
| Palmitoleic C16:1 | 0.2        | 0.21        |
| Stearic C18:0   | 5.49        | 3.17        |
| Oleic C18:1     | 42.07       | 24.08       |
| Linoleic C18:2n6 | 36.39      | 12.20       |
| Linoleic C18:3n3 | 0.13       | 33.65       |
| Arachidic C20:0 | 0.40        | 3.34        |
| Gadoleic C20:1  | –           | 13.08       |
| SFA$^a$         | 21.21       | 16.6        |
| MUFA$^b$        | 42.27       | 37.37       |
| PUFA$^d$        | 36.52       | 45.85       |

$^a$The total not equal 100% as trace amount of some fatty acids was not identified. $^b$SFA: Saturated fatty acids; $^c$MUFA: Monounsaturated fatty acids; $^d$PUFA: Polyunsaturated fatty acids. PK: Pumpkin; GC: Garden cress. The fatty acid values are presented as averages according to Stevenson et al. (2007), Kulaitiene et al. (2007), Srbinoska et al. (2012), Bardaa et al. (2016), and Achilonu et al. (2018). The fatty acid concentrations are variable and depend on several factors such as the part from which the oil is extracted. The metabolised energy value of PK was 8571 kcal, and the metabolised energy value of GC was 8620 kcal, according to the manufacturer (El-Hawag Factory for the extraction of Natural Oils and Cosmetics in Badr City, Egypt).

Hematological and biochemical parameters
The differences in hematological traits (blood picture) between the supplemented groups and the control group were not significant ($p > 0.05$) except for WBCs and their differential (lymphocytes and heterophils %) (Table 5).

Table 6 showed the effects of PK and GC seed oils supplementation on the biochemical traits (blood chemistry) of laying hens. The differences in cholesterol, HDL, LDL, triglycerides, glucose, globulin, AST, and ALT levels between the supplemented and control groups were significant ($p \leq 0.05$), while the differences in total protein and albumin levels were not significant ($p > 0.05$).

Antioxidant parameters
The antioxidant parameters (SOD, GPx, and MDA) were significantly ($p < 0.05$) improved in all groups supplied with PK or/and GC compared to the control group (Table 7).

Yolk’s fatty acid analysis
Yolk’s fatty acids in laying hen eggs, such as myristic, palmitic, stearic, oleic, linoleic, and docosahexaenoic, were significantly ($p \leq 0.05$) affected by the supplementation of PK and GC seed oils, as well as their combination (Table 8). With the supplementation of
PK seed oil 0.5% GC seed oil 0.5% SEM p-value
Egg weight (g) 50.27 a 51.85 b 52.01 a 51.68 b 2.22 0.081
Egg production (%) 60.07 a 69.01 b 67.01 b 73.33 a 1.89 <0.001
Egg mass (g/h/d) 30.18 a 35.77 b 34.85 b 37.89 a 0.46 0.020
Feed intake (g/h/d) 119.78 a 127.65 b 125.71 b 126.11 b 4.31 0.550
Feed conversion ratio (g feed/ g egg) 3.97 a 3.56 b 3.55 b 3.33 b 0.15 0.020

Table 3. Egg quality traits of laying hens as affected by the supplementation of PK and GC seed oils and their combination.

Table 4. Hematological traits of laying hens as affected by the supplementation of PK and GC seed oils and their combination.

Table 5. Biochemical traits of laying hens as affected by the supplementation of PK and GC seed oils and their combination.

Table 6. Antioxidant parameters of laying hens as affected by the supplementation of PK and GC seed oils and their combination.

Discussion
The prospect of using new natural materials as dietary supplements in poultry diets has been recently

PK and GC seed oils, yolk's saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids (n-3 and n-6) were significantly (p ≤ 0.05) increased.
investigated. These materials must be capable of producing safe and high-quality food (Açık-kgoz et al. 2005; El-Hanoun et al. 2020). Nutraceuticals have several biological functions in the bird’s body and may help birds in enhancing their welfare and producing high-quality products (Sugiharto 2016; Alagawany et al. 2021a, 2021b). Plants’ essential oils, as a kind of these nutraceuticals, have been routinely used in chicken farms to keep birds healthy and improve their productive performance (El-Husseiny et al. 2008; Abo Ghanima et al. 2020).

In this study, we investigated the effects of PK and GC seed oils as nutraceutical dietary supplements on the physiological and productive performance of laying hens. PK and GC seeds are rich sources of phenolics, polyphenols, flavonoids, antioxidants, and PUFA. These active substances have different biological activities in bird’s body, including stimulating growth genes, metabolism processes, white blood cell proliferation, cytokine synthesis, and improving final product quality by modulating cholesterol concentration and increasing PUFA (Du et al. 2016; Parham et al. 2020; Bilal et al. 2021; Alagawany et al. 2022).

Data showed that laying hens treated with PK and GC seed oils were significantly (p ≤ 0.05) better in some hematological and biochemical parameters, productive traits, antioxidant activity, immunological response status, and egg yolk fatty acids profile compared to the control group, as well as birds treated with the combination (PK 0.25% + CG 0.25%) had the best value, as a general. The positive effects of PK and GC supplementation in laying diets could be attributed to their nutritional and therapeutical properties. PK and GC seeds are rich in protein (25–30%) and an almost equal amount of healthy fats. They also have a good content of vitamins (such as A, C, and B12), minerals (such as calcium, copper, and iron), fibres (>15%), and phytochemicals, which are essential for bird’s physiological and biological functions (Bryan et al. 2009; Mitra et al. 2009; Srbinoska et al. 2012; Deshmukh et al. 2017; Wafar et al. 2017; Al-Sayed et al. 2019; Lotfi et al. 2021). They can enhance birds’ growth and health due to their nutritional, antioxidiant, and antimicrobial effects (Adamu and Boonkaewwan 2014; Singh and Paswan 2017; Wafar et al. 2017; Achilonu et al. 2018; Al-Sayed et al. 2019; Mathewos et al. 2019).

PK and GC seed oils also contain a high concentration of vitamin E, Zinc, L-tryptophan, omega-3, and omega-6 fatty acids (Koña et al. 2007; Stevenson et al. 2007; Elfyky et al. 2012; Hashemi 2013; Deshmukh et al. 2017; Al-Sayed et al. 2019), which can improve bird’s productivity and immunity, as well as physiological functions including the antioxidant (Alagawany et al. 2021b). Furthermore, GC seeds contain lepidine (a diuretic and imidazole compounds) that acts as antihypertensive (Al-Sayed et al. 2019), as well as glucosinolates and semilipidinoside that act as anticarcinogenic and antiasthmatic, respectively (Jain et al. 2016).

### Table 8. Fatty acids profile of laying hen’s egg yolk as affected by the supplementation of PK and GC seed oils and their combination.

| Fatty acids (µg/g yolk) | Control | PK seed oil 0.5% | GC seed oil 0.5% | PK seed oil 0.25% + GC seed oil 0.25% | SEM | p-value |
|------------------------|---------|------------------|------------------|---------------------------------------|-----|--------|
| Myristic | C14:0 | 0.13a | 0.22a | 0.26a | 0.17a | 0.00 | <0.001 |
| Pentadecanoic | C15:0 | 0.05ab | 0.05ab | 0.06a | 0.04ab | 0.00 | 0.010 |
| Palmitic | C16:0 | 26.20c | 32.67a | 37.66a | 31.37c | 0.35 | 0.001 |
| Heptadecanoic | C17:0 | 0.21b | 0.24a | 0.25a | 0.23ab | 0.01 | <0.001 |
| Stearic | C18:0 | 12.50a | 17.57b | 17.57b | 19.20b | 0.28 | 0.001 |
| Behenic | C22:0 | 0.00b | 0.12a | 0.11a | 0.11a | 0.01 | <0.001 |
| ΣSFA | 39.16a | 50.92b | 55.91a | 51.12b | 5.12b | 2.02 | 0.001 |
| Palmitoleic | C16:1 | 2.20b | 1.90c | 2.77a | 1.80c | 0.11 | 0.001 |
| Heptadecenoic | C17:1 | 0.07b | 0.07b | 0.11a | 0.09b | 0.00 | <0.001 |
| Oleic | C18:1 | 27.07c | 34.73b | 38.01a | 35.00b | 0.30 | 0.001 |
| Eicosanoic | C20:1 | 0.18a | 0.25b | 0.29a | 0.25a | 0.00 | <0.001 |
| ΣMUFA | 29.52a | 36.96c | 41.17a | 37.14b | 37.14b | 0.78 | 0.001 |
| Linoleic | C18:2n6 | 12.30b | 16.70a | 14.17ab | 11.60b | 0.80 | 0.001 |
| Y-linolenic | C18:3n6 | 0.11b | 0.17a | 0.18a | 0.11a | 0.01 | 0.001 |
| Eicosadienoic | C20:2n6 | 0.28b | 0.37a | 0.26b | 0.29ab | 0.02 | 0.011 |
| Eicosatrienoic | C20:3n6 | 0.33a | 0.51a | 0.53a | 0.48a | 0.05 | 0.001 |
| Arachidonic | C20:4n6 | 6.83c | 11.10a | 12.07b | 12.60a | 0.65 | 0.001 |
| Σn-6 PUFA | 19.88c | 28.85b | 27.20ab | 25.08b | 25.08b | 0.73 | 0.001 |
| α-linolenic | C18:3n3 | 0.18a | 0.21bc | 0.34a | 0.24b | 0.01 | 0.001 |
| Eicosatrienoic | C20:3n3 | 0.06b | 0.06b | 0.09a | 0.08b | 0.00 | <0.001 |
| Eicosapentaenoic | C20:5n3 | 0.03b | 0.05b | 0.04b | 0.05a | 0.00 | <0.001 |
| Docosahexaenoic | C22:6n3 | 2.77a | 5.40c | 8.87a | 7.37c | 0.38 | 0.001 |
| Σn-3 PUFA | 3.04a | 5.71b | 9.33a | 7.73b | 7.73b | 0.30 | 0.001 |
| n-6:n-3 ratio | 6.67a | 5.37b | 2.93c | 3.24c | 3.24c | 0.41 | <0.001 |

abcdMeans in the same row having different superscripts are significantly different (p ≤ 0.05). PK: pumpkin; GC: garden cress; SEM: standard error of mean; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
Some botanical seeds, such as PK and GC, are rich in essential oils and essential fatty acids, which have multiple benefits on the animal’s digestive system, such as activating the digestive enzymes, restoring microbiota balance, and increasing nutrient absorption (Mountzouris et al. 2011; Zhang et al. 2020), and this may be one of the reasons behind birds’ production and health improvements. The up-regulated expression of some nutrient transporters, such as sodium-glucose cotransporter 1 and glucose transporter 2, maybe another reason for birds’ production and health improvements (Su et al. 2018).

**Productive and egg quality parameters**

The results of Tables 3 and 4 showed that PK and GC seed oils supplementation in layer diets has positive effects on birds’ feed conversion ratio, egg mass, and egg production; and their combination showed the best values compared to the control group for the egg mass (+20%) and egg production (+16%), PK and GC, as sources of plant oils in laying diets, have positive impacts on some productive traits of hens, such as increased feed conversion ratio and egg production (El-Husseiny et al. 2008; Abbas et al. 2016; Achilonu et al. 2018). These positive effects can be due to the capability of PK to enhance birds’ health by maintaining a balanced microflora in the digestive system (promote bird’s gut health) (Sugiharto 2016; Achilonu et al. 2018) and making nutrients more available for absorption (El-Husseiny et al. 2008). A reduced number of pathogenic (such as microbes) in the gastrointestinal tract may enhance the proliferation ability of epithelial cells and thus improve intestinal absorptive capacity (Zeng et al. 2015). Moreover, several chicken studies have documented positive effects of essential oils on the digestive enzyme (pancreatic α-amylase and intestinal maltase) secretion and intestinal mucosa (Jamroz et al. 2006; Jang et al. 2007). The dietary essential oils supplementation induced gut lesions and increased crypt depth in the ileum (Du et al. 2016). It also down-regulated the claudin-1 and occludin mRNA expression, up-regulated the mRNA expression of interleukin-1β, and tended to increase toll-like receptor (TLR) 2 mRNA expression in the ileum (Du et al. 2016).

The addition of PK and GC oils as sources of essential fatty acids, particularly polyunsaturated fatty acids, can has a positive effect on the function of some egg production hormones in laying hens (El-Husseiny et al. 2008). Polyunsaturated fatty acids significantly increase the mRNA levels of gonadotropin-releasing hormone, luteinizing hormone, and follicle-stimulating hormone (Alagawany et al. 2019). Additionally, PK and GC seed oils are particularly rich in omega-3 (such as docosahexaenoic acid) and omega-6 (such as linoleic acid) fatty acids, which may be associated with improved several physiological functions such as the metabolism process for birds (Alagawany et al. 2021b). Thus, plant essential oils can be added to laying diets to enhance feed utilisation (decrease the feed conversion ratio) and therefore, achieve better production and financial results.

On the other hand, the supplementation of PK and GC seed oils and their combination in laying diet did not affect the feed intake, the egg weight, the egg yolk, and the eggshell thickness. In agreement, Abbas et al. (2016) found that dietary supplementation with PK seed oil (1% and 2%) had no significant effect on feed intake and body weight, egg and yolk weight, shell weight and thickness of laying quails. Suitable levels of plant oils can generally promote calcium absorption in the gut and increase shell thickness (El-Husseiny et al. 2008). On the contrary, the current study did not show a significant effect of PK and GC addition on eggshell percentage. It could be due to differences in oil sources or levels used.

**Hematological and biochemical parameters**

The results of Table 5 showed that supplementing PK and GC seed oils in layer diets has a positive effect on hens’ WBCs and their differential (lymphocytes and heterophils %), and their combination (PK 0.25% + CG 0.25%) showed the best values. Data of Table 6 showed that PK and GC seed oils supplementation also has a positive effect on some hens’ biochemical traits such as cholesterol (−17%), HDL (±12.5%), LDL (−28%), triglycerides (−11%), globulin (±7%), AST (−10%), and ALT (−28%) levels; and their combination showed the best values compared to the control group. The significant ($p \leq 0.05$) decrease in plasma cholesterol, LDL, triglycerides, AST, and ALT of treated groups, as well as the significant ($p \leq 0.05$) increase in plasma HDL and globulin, are positive indicators for treated hens with PK and GC seed oils. These beneficial effects can be due to the high antioxidant capacity of PK and GC. PK and GC seed oils are particularly rich in linoleic acid, a type of omega-6 fatty acid, that may be associated with improved several hematological and biochemical parameters in birds (Diwakar et al. 2010; Abbas et al. 2016; Bardaa et al. 2016). However, plant seed oils can be added to laying diets to improve metabolism, kidney and liver
functions, and egg production quality (Alagawany et al. 2019; Gao et al. 2021). Egg lipid profile can be modified by feeding hens plant seed oils (such as flaxseed and canola) to reduce the content of n-6 fatty acid while increasing the content of n-3 fatty acid (Leskanich and Noble 1997; Grobas et al. 2001). Furthermore, the significant reduction in hens’ plasma cholesterol can have a great effect on decreasing cholesterol levels in eggs, as well as triglycerides (Çelik et al. 2011; Vlaicu and Panaite 2022). Bad cholesterols reduction may be related to HMG-CoA reductase activity inhibition, which is one of the enzymes involved in regulating cholesterol metabolism, and thus cholesterol synthesis reduction (Çiftci et al. 2010). On the other hand, the significant increase in plasma globulin reflects a positive impact on bird’s immunity. Polyunsaturated fatty acids consumption by laying hens can increase serum lysozyme activity and enhance immune response (Gao et al. 2021). Total protein and albumin levels did not differ between groups when PK and GC oils were supplemented, which is agreement with the findings of Abbas et al. (2016).

Antioxidant parameters

The results of Table 7 showed that supplementing PK and GC seed oils in layer diets has a positive effect on birds’ antioxidant parameters (SOD, GPx, and MDA); and their combination showed the best values compared to the control group for SOD (+19%), MDA (~37%), and GSH-px (+47%). PK and GC seed oils are rich in phytochemicals and vitamin E, which have a powerful antioxidant action (Abbas et al. 2016; Al-Sayed et al. 2019). Total phenolic compounds, one of the phytochemicals, are natural antioxidants that are also responsible for the high antioxidant activity of PK and GC (Stevenson et al. 2007; Al-Sayed et al. 2019; Vlaicu and Panaite 2022). Essential oil phenolic compounds increased catalase activity, which detoxifies hydrogen peroxide and converts lipid hydroperoxides to non-toxic substances (Fki et al. 2005). Furthermore, PK seed oil supplementation prevented changes in plasma lipids and has shown antiperoxidative properties (Elfiky et al. 2012; Vlaicu and Panaite 2022).

Yolk’s fatty acid analysis

Yolk’s fatty acids profile of laying hens supplemented with PK and GC seed oils and their combination is overviewed in Table 8. The results showed that the supplementation of PK and GC seed oils and their combination in layer diets has a positive effect on egg yolk fatty acids profile. It is an indicator of increasing the nutritive value of egg production, which is agreement with the findings of Martinez et al. (2012) working on PK. Referring to the saturated fatty acids, the supplemented groups had higher (p ≤ 0.05) yolk myristic, palmitic, and stearic values than the control, with 69%, 29%, and 45%, respectively. Yolk’s fatty acids are mainly synthesised by the liver (during the vitellogenesis process under oestrogens control), which is strongly influenced by the fatty acid composition of the diet. PK and GC seed oils are excellent sources of some saturated fatty acids such as palmitic acid (Diwakar et al. 2010; Bardaa et al. 2016). Furthermore, plant seed oils such as PK and GC improved metabolism, enzyme activities, and liver functions (Alagawany et al. 2019; Gao et al. 2021).

Yolk’s polyunsaturated fatty acids (n-3 and n-6) were particularly increased with the supplementation of PK and GC seed oils, which is considered a desirable effect because these kinds of fatty acids have several beneficial effects in the body, such as improving metabolism and liver function, and enhancing the immunity and the product quality, besides their anti-inflammatory and anti-cancer influences (Simopoulos 2000; Çelik et al. 2011; Gao et al. 2021; Alagawany et al. 2021b). This increase in PUFA, especially omega-3 and 6 fatty acids, may be due to the increase of desaturase enzymes activity and the availability of essential fatty acids, as well as the antioxidant effect of PK and GC, which are involved in the synthesis of these fatty acids.

PK and GC seed oils are excellent sources of unsaturated fatty acids, such as linoleic acid and oleic acid, tocopherols, and sterols (Diwakar et al. 2010; Bardaa et al. 2016). They also have a high content of saturated fatty acids such as arachidic acid and palmitic acid (Diwakar et al. 2010; Bardaa et al. 2016). Furthermore, PK and GC seed oils can provide birds with two types of omega-3: eicosapentaenoic acid and docosahexaenoic acid, besides providing them with omega-6 fatty acids. They also contain α-linolenic acid, which can be transformed into eicosapentaenoic acid and docosahexaenoic acid in the body (Richardson 2006). In general, yolk’s fatty acids are mainly synthesised by the liver, which is strongly influenced by the fatty acid composition of the diet. The addition of plant oils, such as PK and GC, to the laying diets increases the essential fatty acids contents, such as arachidonic acid and docosahexaenoic acid, in the egg yolk (El-Husseiny et al. 2008; Vlaicu and Panaite 2022). Various PUFA, such as linolenic acid and arachidonic acid, are required to maintain the integrity of the cell
membrane structure and function (Du et al. 2000). As a result, the prominent bioactive components and pharmacological substances of PK and GC seed oils can increase the nutritive value of poultry products, such as eggs, when they are added to their diets.

Conclusions

The use of botanical seeds in poultry farms is gradually gaining popularity due to their nutritional value and therapeutic properties, as well as leaving no residue in poultry products. The present study focussed on the potential of PK and GC seed oils as nutraceutical feed additives used in laying farms. Based on the findings, PK and GC seed oils can be used as natural egg production promoters, antioxidants, and immunostimulants. They can be added to the poultry diet separately (0.5%) or in combination (0.25%+0.25%) to improve the physiological, antioxidative, and immunological status of birds, which has the greatest impact on quantitative (egg production) and qualitative (egg quality) productivity.

Acknowledgments

All the authors of this manuscript are grateful to their respective universities and institutes for their technical assistance and valuable support in completing this research.

Author contributions

A.El-S., A.El-B., and E.Sh. designed and supervised the study. A.El-S., A.El-B., E.Sh., and A.Kh. performed the experiments. A.El-S., A.El-B., E.Sh., A.Kh., A.E., and K.El-S. analysed the data. K.El-S., A.E., and A.Kh. wrote the draft with approval from all authors.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Karim El-Sabrout  http://orcid.org/0000-0003-2762-2363

References

Abbas RJ, Al-Shaheen SA, Majeed TI. 2016. Effect of supplementing different levels of pumpkin seed oil in the diets of spent laying Japanese quail (Coturnix coturnix japonica). Association of Genetic and Environmental Resources Conservation (AGERC). Proceeding of the 4th International Conference of Genetic and Environment, Cairo, Egypt, July 23rd–30th, 2016.

Abo Ghanima MM, Elsadek MF, Taha AE, Abd El-Hack ME, Alagawany M, Ahmed BM, Elshafie MM, El-Sabrouk K. 2020. Effect of housing system and rosemary and cinnamon essential oils on layers performance, egg quality, haematological traits, blood chemistry, immunity, and antioxidant. Animals. 10(2):245.

Achilolu MC, Nwafor IC, Umehsobi DO, Sedibe MM. 2018. Biochemical proximates of pumpkin (Cucurbitaeae spp.) and their beneficial effects on the general well-being of poultry species. J Anim Physiol Anim Nutr. 102(1):5–16.

Açıkgöz Z, Yücel B, Altan O. 2005. The effects of propolis supplementation on broiler performance and feed digestibility. Archiv für Geflugelkunde. 69(3):117–122.

Adamu M, Boonkaewwan C. 2014. Effect of Lepidium sativum L. (Garden Cress) seed and its extract on experimental Eimeria tenella infection in broiler chickens. Kasetsart J. 48:28–37.

Alagawany M, Elnesr SS, Farag MR, Abd El-Hack ME, Khafaga AF, Taha AE, Tiwari R, Yatoo ML, Bhatt P, Khurana SK, et al. 2019. Omega-3 and Omega-6 fatty acids in poultry nutrition: effect on production performance and health. Animals. 9(8):573.

Alagawany M, Elnesr SS, Farag MR, Abd El-Hack ME, Barkat RA, Gabr AA, Foda MA, Noreldin AE, Khafaga AF, El-Sabrouk K, et al. 2021a. Potential role of important nutraceuticals in poultry performance and health – a comprehensive review. Res Vet Sci. 137:9–29.

Alagawany M, Elnesr SS, Farag MR, Abd El-Hack ME, barkat RA, Gabr AA, Foda MA, Noreldin AE, Khafaga AF, El-Sabrouk K, et al. 2021b. Nutritional significance and health benefits of omega-3, -6 and -9 fatty acids in animals. Anim Biotechnol. 20:1–13.

Alagawany M, Elnesr SS, Farag MR, El-Naggar K, Madkour M. 2022. Nutrigenomics and nutrigenetics in poultry nutrition: an updated review. World’s Poult Sci J. 2022:1–20.

AIlain CC, Poon LS, Chan CSG, Richmond W, Fu PC. 1974. Enzymatic determination of total serum cholesterol. Clin Chem. 20(4):470–475.

Al-Sayed HMA, Zidan NS, Abdelaleem MA. 2019. Utilization of garden cress seeds (Lepidium sativum L.) as natural source of protein and dietary fiber in noodles. Inter J Pharm Res Allied Sci. 8(3):17–28.

Altan O, Altan A, Çabuk M, Bayraktar H. 2000. Effects of heat stress on some blood parameters in broilers. Turk J Vet Anim Sci. 24:145–148.

AOAC. 2000. Official methods of analysis of the association of official analytical chemists. International 18th ed.; Association of Official Analytical Chemists, Washington DC, USA.

Achilolu MC, Nwafor IC, Umehsobi DO, Sedibe MM. 2018. Biochemical proximates of pumpkin (Cucurbitaeae spp.) and their beneficial effects on the general well-being of poultry species. J Anim Physiol Anim Nutr. 102(1):5–16.

Açıkgöz Z, Yücel B, Altan O. 2005. The effects of propolis supplementation on broiler performance and feed digestibility. Archiv für Geflugelkunde. 69(3):117–122.

Adamu M, Boonkaewwan C. 2014. Effect of Lepidium sativum L. (Garden Cress) seed and its extract on experimental Eimeria tenella infection in broiler chickens. Kasetsart J. 48:28–37.

Alagawany M, Elnesr SS, Farag MR, Abd El-Hack ME, Barkat RA, Gabr AA, Foda MA, Noreldin AE, Khafaga AF, El-Sabrouk K, et al. 2021a. Potential role of important nutraceuticals in poultry performance and health – a comprehensive review. Res Vet Sci. 137:9–29.

Alagawany M, Elnesr SS, Farag MR, Abd El-Hack ME, barkat RA, Gabr AA, Foda MA, Noreldin AE, Khafaga AF, El-Sabrouk K, et al. 2021b. Nutritional significance and health benefits of omega-3, -6 and -9 fatty acids in animals. Anim Biotechnol. 20:1–13.

Alagawany M, Elnesr SS, Farag MR, El-Naggar K, Madkour M. 2022. Nutrigenomics and nutrigenetics in poultry nutrition: an updated review. World’s Poult Sci J. 2022:1–20.

AIlain CC, Poon LS, Chan CSG, Richmond W, Fu PC. 1974. Enzymatic determination of total serum cholesterol. Clin Chem. 20(4):470–475.

Al-Sayed HMA, Zidan NS, Abdelaleem MA. 2019. Utilization of garden cress seeds (Lepidium sativum L.) as natural source of protein and dietary fiber in noodles. Inter J Pharm Res Allied Sci. 8(3):17–28.

Altan O, Altan A, Çabuk M, Bayraktar H. 2000. Effects of heat stress on some blood parameters in broilers. Turk J Vet Anim Sci. 24:145–148.

AOAC. 2000. Official methods of analysis of the association of official analytical chemists. International 18th ed.; Association of Official Analytical Chemists, Washington DC, USA.
Metcalfe LD, Schmitz AA, Pelka JR. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal Chem. 38(3):514–515.

Misra HP, Fridovich I. 1972. The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 247(10):3170–3175.

Mitra P, Ramaswamy SH, Chang SK. 2009. Pumpkin (Cucurbita maxima) seed oil extraction using supercritical carbon dioxide and physicochemical properties of the oil. J Food Eng. 95(1):208–213.

Mountzouris KC, Paraskevas V, Tsirtsikos P, Palamidi I, Steiner T, Schatzmayr G, Fegeros K. 2011. Assessment of a phyto- genic feed additive effect on broiler growth performance, nutrient digestibility and caecal microflora composition. Anim. Feed Sci Tech. 168(3–4):223–231.

Parham S, Kharazi AZ, Bakhsheshi-Rad HR, Nur H, Ismail AF, Sharif S, RamaKrishna S, Berto F. 2020. Antioxidant, antimicrobial and antiviral properties of herbal materials. Antioxidants. 9(12):1309.

Poy D, Akbarzadeh A, Ghanei M. 2015. Garden cress: morphology, genetically and therapeutic properties. J Hortic Photon. 103:130–136.

Radwan SS. 1978. Coupling of two-dimensional thin layer chromatography with gas chromatography for the quantitative analysis of lipids classes and their constituent fatty acids. J Chromatog Sci. 16(11):538–542.

Reitman S, Frankel SA. 1957. Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 28(1):56–63.

Richardson AJ. 2006. Omega-3 fatty acids in ADHD and related neuro-developmental disorders. Int Rev Psychiatry. 18(2):155–172.

SAS. 2004. Institute SAS/STAT, User’s Guide, Version 9.1. Cary, NC: Inst. Inc.

Shawle K, Urge M, Animut G. 2016. Effect of different levels of Lepidium sativum L. on growth performance, carcass characteristics, hematology and serum biochemical parameters of broilers. SpringerPlus. 5(1):1441.

Simopoulos AP. 2000. Human requirement for n-3 polyunsaturated fatty acid. Poutl Sci. 79(7):961–970.

Singh CS, Paswan VK. 2017. The potential of garden cress (Lepidium sativum L) seeds for development of functional foods. Chapter 14: advances in seed biology. Intechopen Publisher. p. 280–290.

Srbinoska M, Hrabovski N, Rafajlovskva V, Sinadinovic-Fiser S. 2012. Characterization of the seed and seed extracts of Pumpkins Cucurbita maxima D and Cucurbita pepo L from Macedonia. Maced J Chem Chem Eng. 31(1):65–78.

Stevenson DG, Eller FJ, Wang L, Jane JL, Wang T, Inglett GE. 2007. Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. J Agric Food Chem. 55(10):4005–4013.

Su G, Zhou X, Wang Y, Chen D, Chen G, Li Y, He J. 2018. Effects of plant essential oil supplementation on growth performance, immune function and antioxidant activities in weaned pigs. Lipids Health Dis. 17(1):139.

Sugiharto S. 2016. Role of nutraceuticals in gut health and growth performance of poultry. J Saudi Soc Agric Sci. 15(2):99–111.

Sugiura M, Oikawa T, Hirano K, Maeda H, Yoshimura H, Sugiyama M, Kuratsu T. 1977. A simple colorimetric method for determination of serum triglycerides with lipoprotein lipase and glycerol dehydrogenase. Clin Chim Acta. 81(2):125–130.

Trinder P. 1969. Colorimetric method for the determination of blood glucose. Ann Clin Biochem. 6(1):24–27.

Vlaicu PA, Panaite TD. 2022. Effect of dietary pumpkin (Cucurbita moschata) seed meal on layer performance and egg quality characteristics. Anim Biosci. 35(2):236–246.

Wafar RJ, Hannison M, Abdullahi UU, Makinta A. 2017. Effect of pumpkin (Cucurbita pepo L) seed meal on the performance and carcass characteristics of broiler chickens. Asian J Adv Agric Res. 2(3):1–7.

Wieland H, Seidel D. 1983. A fatty enzymatic colourimetric determination of LDL-Cholesterol in serum. J Lipid Res. 24(7):904–907.

Zeng Q, Huang X, Luo Y, Ding X, Bai S, Wang J, Xuan Y, Su Z, Liu Y, Zhang K. 2015. Effects of a multi-enzyme complex on growth performance, nutrient utilization and bone mineralization of meat duck. J Anim Sci Biotechnol. 6(1):12.

Zhang R, Wang XW, Liu LL, Cao YC, Zhu H. 2020. Dietary ore- gano essential oil improved the immune response, activity of digestive enzymes, and intestinal microbiota of the koi carp, Cyprinus carpio. Aquaculture. 518:734781.

Zia-Ul-Haq M, Ahmad S, Calani L, Mazzeo T, Rio DD, Pellegrini N, De Feo V. 2012. Compositional study and antioxidant potential of Ipomoea hederacea Jacq. and Lepidium sativum L. seeds. Molecules. 17(9):10306–10321.