



Effect of pomegranate and orange oils supplementing as antioxidant on productive and physiological performance of aged developed chickens

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Abstract

This study was conducted to investigate the effects of dietary Pomegranate oil and Orange oil supplementation on egg production traits, semen quality and blood constituents of Golden Montazah (Egyptian local developed strain) laying hens. A total number of 270 laying hens + 27 cock, 52 weeks old were randomly taken to be similar in body weights (1440.70 ± 2.23), which were randomly divided into nine experimental groups, (30 hens + 3 cocks in each). Each group was contained three replicates (10 hens+1 cock in each). The first group was fed the basal diet as control group (without any supplementation), the second and third groups were fed the basal diet and supplemented with Pomegranate seed oil (PO) levels (150 or 300 ml /kg diet), the fourth and fifth groups were supplemented with Orange oil (OO) levels (150 or 300 ml /kg diet), the sixth group was supplemented with (150 ml PO +150 OO /kg diet), the seventh group was supplemented with (150 ml PO +300 OO /kg diet), the eighth group was supplemented with (300 ml PO +150 OO /kg diet) and the ninth group was supplemented with (300 ml PO +300 OO /kg diet), respectively, during the experimental periods lasted three months from 52 to 64 weeks of age. The obtained results showed that supplementing with either pomegranate or orange oil levels or mixtures improved significantly ($P < 0.5$) in body weight change (BWC, %) and ($P < 0.01$) egg production (EP, %); egg mass (EM, g) and feed conversion ratio (FCR) as g feed /g eggs when compared to hens control group, during period 52 at 64 weeks of age. Semen ejaculate volume, sperm motility (%) and sperm-cell concentration ($\times 10^9/\text{ml}$) of Golden Montazah cocks treated with supplemented of PO or OO and their mixtures significantly ($P < 0.05$) and ($P < 0.01$) increased compared with the other treatment groups, while dead spermatozoa (%), sperm abnormalities (%) and acrosomal damage (%) of layer treated with PO or OO and their mixtures significantly ($P < 0.01$) decreased compared with the control group. Supplementing layer diets with pomegranate or orange oil, or their combinations, led to significant increases ($P < 0.01$) in total protein, globulin, serum cholesterol, total antioxidant capacity, SOD, GPX, and testosterone levels in roosters compared to the control group.

Conclusively, the findings suggest that incorporating 300 ml/kg of either pomegranate or orange oil into the diets of Golden Montazah laying hens is more effective in improving productive performance, as well as enhancing biochemical, immunological blood parameters, and semen quality.

Keywords: Pomegranate oil, Orange oil, Productive performance, Biochemical & Hematological Parameters, Immunology, Semen quality, laying hen.

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1. Introduction

Poultry production plays a major role in providing a large and cheap source of animal protein in Egypt. beside pure Egyptian breeds there were some local developed strains that established for both meat and egg production. The Golden Montazah was developed by cross breeding a Rode Island Red with Dokki 4, using systems of breeding coupled with selection.

Phytogenic additives are substances derived from medicinal plants or spices, such as essential oils, which have

positive effects on production and health of animals. Beneficial results of phytogenic additives are attributed to the presence of diverse classes of active substances conferring antimicrobial actions and stimulus to digestion and promoting production of endogenous enzymes in animals [1,2,3,4,5]. There is also evidence that essential oils can impact pathogen concentrations in the intestine and improve feed digestibility [6].

Pomegranate fruits (*Punica granatum*, Punicaceae) are rich sources of many valuable biologically active substances besides punicalic acid, e.g. flavonoids

(anthocyanidins and catechins), tannins, polyunsaturated fatty acids, vitamins, polyphenols, minerals and one is flavone – genistein [7]. Pomegranate seed oil has a very interesting fatty acid profile for a component of a functional food, as its components are cis-9, trans-11, cis-13 octadecatrienoic (punicic) acid and one conjugated linolenic acid (CLnA). Pomegranate seed oil also has strong antioxidant properties [8].

The addition of pomegranate seed oil to the chickens' diet has a positive effect on the lipid profile of meat and eggs, and their cholesterol content [9,10]. Pomegranate which belongs to the family Punicaceae. It's used widely as a traditional medicine because of its curative features. Many studies reported the useful effect of pomegranate on mans' health, pomegranate has proven to rise testosterone levels which have a main role in preservation of secondary sexual features and spermatogenesis [11]. Pomegranate is possessing a high level of polyphenol, tannins, and flavonoids like pedunculagin, punicalin, ellagic acid, and gallagic acid., it's also contains a high level of glucose, anthocyanins, ascorbic acid, caffeic acid catechin, numerous minerals, particularly amino acids and iron [12,13].

Pomegranate consumption increases significantly sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats [14].

Orange oil (essence) is an essential oil produced by cells in peel of orange fruit (*Citrus sinensis* fruit). Compared to numerous essential oils, orange oil is extracted as a by-product via centrifugation in production of orange juice and produced as cold pressed oil. A great majority of its components consists of d-limonene (more than 90%) and a very little part of them is β -myrcene (2-2.1%) [15]. In some studies, conducted recently, orange peel essential oil (OEO) was used as antiparasitic [12], antifungal [16], antioxidant, antimicrobial, and growth regulator agent [17]. [18] illustreated that the treatments of broiler chicks with (0, 100, 200, 300, and 400 mg kg⁻¹ diet) orange essential oil showed feed intake and weight gain of all birds linearly increased, while feed conversion decreased.

The aim of this study to evaluate supplementation of Pomegranate seed oil and Orange peel oil in the diet on productive performance semen quality, some antioxidant enzymes and Testosterone of the developed laying hens and cocks.

2. Materials and Methods

2.1. Birds, management and experimental design

The present study was carried out in a private farm in Sharkia Governorate, Egypt, during the period from March to June 2022.

A total number of 270 Golden montazaha (Egyptian local developed strain) laying hens+ 27 cocks, 52 weeks old were randomly taken from the farm flock, to be similar in body weight (1440.70 \pm 2.23). Birds were randomly divided

into nine treatment groups (30 hens + 3 cocks in each group) and then each treatment group was divided into three replicates (10 hens+ 1 cock /replicate). The first group was fed the basal diet as control group (without any supplementation), the second and third groups were fed the basal diet and supplemented with Pomegranate seed oil (PO) levels (150 or 300 ml /kg diet), the fourth and fifth groups were supplemented with Orange peel oil (OO) levels (150 or 300 ml /kg diet), the sixth group was supplemented with (150 ml PO +150 OO /kg diet), the seventh group was supplemented with (150 ml PO +300 OO /kg diet), the eighth group was supplemented with (300 ml PO +150 OO /kg diet) and the ninth group was supplemented with (300 ml PO +300 OO /kg diet), respectively, during the experimental period from 52 to 64 weeks of age.

All birds were housed individually in layer's rooms and maintaining in similar managerial and conditions environment with a photoperiod length of 17 h daily. Feed and water were provided *ad libitum* throughout the experimental period (52 - 64 weeks of age). Experimental diets were formulated to be *iso nitrogenous* and *iso-caloric* to cover the nutrients requirements as recommended by [19] as shown in Table 1.

2.2. Measurements

Body weight (BW) of bird at 52 and 64 weeks of age and change body weight (%) was recorded. Daily and total egg number and egg weight (g) were recorded for each hen/day in each group, while daily and total feed intake were recorded, during the experimental period.

Egg production rate (%) was calculated for four weeks intervals, during the production periods as egg number/hen/period x100 for each replicate and calculated the average of the whole experimental period.

Egg mass was calculated by multiplying egg number X average egg weight. Feed conversion (g feed/ g eggs) was calculated as Kg feed consumption produced number of eggs for four weeks intervals and the whole experimental period from 52 to 64 weeks of age.

2.3. Semen quality

Semen samples were collected randomly from 27 cocks (3 cocks of each treatment) at 64 weeks of age using the abdominal massage method. Semen samples were examined according to [20], to determine ejaculate volume (ml), advanced motility score, alive sperm (%), died sperm %, sperm concentration (10⁹/ ml) and sperm abnormality %. The ejaculate volume was determined to the nearest 0.01 ml. using 1.00 ml. tuberculin syringe. Sperm concentration was determined by using Thomes– Zeishaemocytometer. Mass motility score (from 1 to 5 grades). Total live sperm/ejaculate x 10⁹ = (sperm concentration x live sperm % / 10). Total abnormal sperm/ejaculate x 10⁹ = (sperm concentration x abnormal sperm % / 10). Total live sperm/ejaculate x 10⁹ = (sperm concentration x live sperm % / 10).

Table 1: Composition and chemical analysis of the basal diet

Ingredients	(%)
Yellow corn	63.15
Soybean meal (44%)	23.29
Corn gluten meal (60%)	3.02
Mono calcium phosphate	1.39
Lime stone	8.40
NaCl	0.40
Vitamins and minerals mixture*	0.30
DL-methionine	0.05
Total	100.00
<i>Determined analysis**</i>	
Crude protein (%)	17.00
Crude fiber (CF)	3.09
Available phosphorus (%)	0.72
Calcium (%)	3.41
Lysine (%)	0.868
Methionine (%)	0.377
Methionine+ Cysteine (%)	0.666
Metabolizable energy (Kcal ME/kg diet)***	2748
Values (AOAC, 1998) Analyzed	
Dry matter, %	90.73
Crude protein, %	16.97
Ether extract, %	2.45
Crude fiber, %	3.96
Ash, %	6.37
Nitrogen free extract, %	60.98

*Each 3 kg of Vitamins and Minerals mixture * contains: Vit. A 10000,000 IU; Vit.D3 2000,000 IU; Vit. E 10,000 mg; Vit.K3 1000 mg; Vit.B1 1000 mg; Vit.B2 5000 mg; Vit.B6 1500 mg; Vit. B12 10 mg; Pantothenic acid 10,000 mg; Niacin 30,000 mg; Folic acid 1000 mg; Biotin 50 mg; Choline 250,000 mg; Manganese 60,000 mg; Copper 4,000 mg; Iron 30,000mg; Iodine 300 mg; Cobalt 100 mg; CaCO₃ to 3,000gm.

***According to NRC (1994)

2.4. Blood biochemical analysis

At the end of experiment (64 weeks of age), blood samples were collected. Three hens from each treatment group were randomly selected for taking blood samples (3cm /hen) from the right brachial vein using a sterilized syringe; in heparinized test tubes then centrifuged at 3000 rpm for 20 minutes. The biochemical characteristics of blood serum total protein, albumin, Cholesterol, HDL, LDL, antioxidant enzymes (GPX and SOD), total antioxidant capacity and testosterone were determined calorimetrically, using commercial chemical kits as previously described by [21].

2.5. Statistical analysis

The experiment was conducted as a Completely Randomized Design with 9 treatment groups in a one-way arrangement and were analyzed according to [22] using ANOVA procedures of SAS [23]. The following model was used to study the effect of test materials on parameters investigated as follows: $Y_{ijk} = \mu + T_i + e_{ijk}$.

Where: Y_{ijk} = Observation for each dependent variable, μ = Overall mean, T_i = Treatment effects ($i = 1, 2, \dots$ and 9), R_j = Replicates ($j = 1, \dots, 3$), e_{ijk} = Random error. Significant difference among means of treatment groups was detected by [24] multiple range test procedures. The differences were considered significant at ($P \leq 0.05$).

The percentage values were subjected to be sine transformation before performing the analysis of variance. Means were present of after recalculated from the transformed value to percentages.

3. Results and Discussion

3.1. Productive performance traits

The effect of either pomegranate or orange oils and their mixture on productive performance for the whole experimental period (52-64 weeks of age) are shown in (Table 2).

Results obtained revealed that dietary supplementation pomegranate or orange oil levels and their

mixture were significantly ($P < 0.01$ or $P < 0.05$) improved egg production (EP, %); daily egg mass (DEM, g) feed conversion ratio (FCR); as g feed /g eggs and body weight change (BWC, g) when compared to control groups. Similar results were obtained by. The effects of dietary pomegranate by-products on the performance of laying hens were variable in previous reports. [25] reported increased feed intake, egg production, and egg mass as a dose-dependent form of supplementation of pomegranate seed oil to laying hens' diet. Similarly, [26] observed an improvement in egg production and feed intake of Japanese quails supplemented with pomegranate peel powder. However, [27] recorded no effect of dietary supplementation of 1% pomegranate peel

powder on egg production in laying quails until 10 weeks of the study, whereas 2% supplemented birds were lower in egg production than the control birds in the same research.

These findings are consistent with studies reporting positive effects of different doses of Orange Peel Essential Oil (OPEO) on egg production in poultry [5]. [28] reported that the supplementation of Orange Peel Essential Oil (OPEO) in the layer quail diet significantly increased egg production ($p < 0.05$). Similarly, the addition of an essential oil mixture to the diets of laying chickens has been shown to increase egg production [29,30]. However, [31] reported no increase in egg production with the addition of essential oil to hen diets.

Table (2): Effect of either pomegranate oil or orange oil and their mixtures supplementation on some productive performance parameters of laying hens from 52 to 64 weeks of age

Items	Egg production %	Egg weight (g)	Egg mass (g/d)	Feed intake (g/d)	Feed conversion (g feed/ g egg)	Body weight change(g)
Control (Basal diet)	48.29 ^d	48.91	23.61 ^d	104.68	4.45 ^a	64.33 ^b
Pomegranate oil (150 ml) PO1	50.91 ^c	48.88	24.88 ^c	104.38	4.20 ^b	75.17 ^{ab}
Pomegranate oil (300 ml) PO2	51.67 ^c	48.89	25.25 ^{bc}	104.47	4.14 ^{bc}	80.50 ^a
Orang oil (150 ml) OO1	51.39 ^c	48.90	25.13 ^{bc}	104.55	4.16 ^b	75.17 ^{ab}
Orang oil (150 ml) OO2	52.78 ^{abc}	48.93	25.82 ^{abc}	104.63	4.06 ^{bc}	75.33 ^{ab}
PO1+OO1	53.69 ^{abc}	48.84	26.22 ^{ab}	104.78	4.00 ^{bc}	78.83 ^a
PO1+OO2	52.30 ^{bc}	48.92	25.59 ^{abc}	104.96	4.11 ^{bc}	80.67 ^a
PO2+OO1	53.57 ^{ab}	49.56	26.56 ^a	104.80	3.96 ^c	82.50 ^a
PO2+OO2	54.64 ^a	49.00	26.77 ^a	105.52	3.95 ^c	85.47 ^a
SEM	0.58	0.17	0.33	0.35	0.06	3.44
Sig. test	**	NS	**	NS	**	*

SEM: Mean at standard error; a,b,c: Means in the same column with different superscripts, differ significantly ($p < 0.05$)

* $P < 0.05$, N.S: Not Significant, ** $P < 0.01$

3.2. Semen quality

The addition of either pomegranate or orange oil levels supplementation and their mixture in cocks diets were significantly ($P < 0.05$ and $P < 0.01$) improved on semen ejaculate volume, sperm motility (%), dead sperm (%), sperm abnormalities, acrosomal damage (%) and sperm cell concentration ($\times 10^9$) as compared with control group (Table 3).

It could be noticed that within addition each of pomegranate oil, orange oil in cock's diets with any level improved semen characteristics and this improved means were increased gradually by increasing dietary pomegranate and orange oils supplementation from 150 to 300 mg /kg diet (Table 3).

The significant improvements in the semen volume may be attributed to higher concentrations of pomegranate and orange oil which increases testosterone synthesis and therefore accessory gland secretions.

Pomegranate consumption increases significantly sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats [14].

The improvement in overall ejaculate volume, sperm concentration and therefore total sperm output ($P < 0.001$) observed in this study is consistent with antioxidant effects on semen quality. This can be explained by the findings of [14] and who reported that treating rats with pomegranate juice resulted in increased epididymal sperm concentration, spermatogenic cell density and diameter of seminiferous tubules and germinal cell layer chickens. The enhancement observed in sperm motility could be partially attributed to the concomitant induction in semen fructose [32]. In addition to the previous beneficial effects of pomegranate and orange oils on aged males' reproductive status, pomegranate and orange oils treatments were able to significantly reduce dead sperm concentrations, which may be attributed to suppression of oxidative stress.

Table (3): Semen quality of Golden Montazah cocks as affected by different levels of dietary of Pomegranate oil, Orange oil and their mixtures at 64 weeks of age

Items	Semen Ejaculate Volume (ml)	Sperm motility (%)	Dead Sperm (%)	Sperm abnormalities (%)	Acrosomal damage (%)	Sperm cell consent. (X109 \ ml)	Total Sperm-Output (X109 \ ej)
Control (Basal diet)	0.47 ^b	75.00 ^a	19.33 ^a	14.00 ^a	12.33 ^a	2.02 ^c	1.50
Pomegranate oil (150 ml) PO1	0.57 ^{ab}	81.67 ^b	12.00 ^b	11.67 ^b	10.00 ^b	3.01 ^b	1.73
Pomegranate oil (300 ml) PO2	0.60 ^a	83.33 ^b	8.00 ^{cd}	7.33 ^{de}	9.00 ^{bc}	3.37 ^{ab}	2.03
Orang oil (150 ml) OO1	0.56 ^{ab}	83.33 ^b	9.67 ^c	9.33 ^c	8.67 ^{bc}	3.69 ^a	1.98
Orang oil (150 ml) OO2	0.59 ^{ab}	83.33 ^b	7.33 ^d	8.00 ^{cd}	7.67 ^{cd}	2.03 ^c	2.02
PO1+OO1	0.63 ^a	86.67 ^{ab}	6.67 ^{de}	8.33 ^{cd}	7.33 ^{cd}	2.25 ^c	1.93
PO1+OO2	0.62 ^a	86.67 ^{ab}	7.33 ^d	9.00 ^{cd}	7.67 ^{cd}	2.89 ^b	1.93
PO2+OO1	0.65 ^a	91.67 ^a	5.00 ^{ef}	6.00 ^{ef}	6.00 ^{de}	3.44 ^{ab}	2.07
PO2+OO2	0.69 ^a	93.33 ^a	4.33 ^f	5.00 ^f	5.33 ^e	3.64 ^a	2.17
SEM	0.04	1.99	0.67	0.57	0.58	0.15	0.13
Sig.test	*	**	**	**	**	**	NS

Means having different letters at the same column are significantly ($P \leq 0.05$) different * = $P < 0.05$; ** = $P < 0.01$; NS= Not significant

3.3. Some blood parameters

Table (4). Showed that the layer feeding both pomegranate or orange oil levels and their mixtures caused significantly ($P \leq 0.01$) increased serum total protein and globulin, cholesterol as compared with control group. However, serum albumen, HDL and LDL its not significant effects.

Pomegranate seed oil consists of 65-80% conjugated fatty acids which is the most important is 9-trans, 11-cis, 13-trans, called punicic acid [33]. It has been shown that triglycerides and total cholesterol levels in plasma increased significantly with conjugated linoleic acid (CLA) in broilers [34] and pigs [35] diets. So, the reason for the increased plasma cholesterol level in pomegranate oil treated layers in current study could be related to the changes in enzyme activities associated with lipid metabolism in the liver through increasing liver lipogenesis, as showed by [34].

The addition of either pomegranate and orange oils and their mixtures supplementation on total antioxidant capacity, some antioxidant enzymes (GPX and SOD) and testosterone it's significantly ($P \leq 0.01$) increased as compared with control group (table 5).

In addition, [36] noted that broilers fed the pomegranate peel diet had higher serum SOD and GPx concentrations than those fed the low-level pomegranate peel powder, although the development rate was not statistically significant. The high concentration of SOD and CAT in the body leads to improved protection of cell membranes against oxidative stress [37]. Pomegranate peel contains phenolic and antioxidant compound [26], which may improve the antioxidant status of broilers by increasing antioxidant enzyme activity [38, 39]. [40] reported that herbal products include antioxidant compounds that directly inhibit lipid oxidation in tissues. Antioxidant enzymes can neutralize the free radicals of different types of oxygen. hence, the use of pomegranate by-products in diets reduces protein and DNA damage in the body, which probably prevents the production of oxygen free radicals in broilers [41].

These results suggest that the antioxidant metabolites of orange oil are functional nutrients that are highly absorbed at a cellular level and are utilized to support avian immune function.

Table (4): Effect of either pomegranate and orange oils and their mixtures supplementation on some blood parameters of laying hens at end of the experimental period at 64 weeks of age.

Items	Total protein (g/dl)	Albumen (g/dl)	Globulin (g/dl)	Cholesterol (mg/dl)	Hdl (mg/dl)	Ldl (mg/dl)
Control (Basal diet)	4.89 ^d	2.63	2.26 ^d	189.06 ^{ab}	67.08	74.87
Pomegranate oil (150 ml) PO1	5.08 ^{cd}	2.42	2.66 ^{bcd}	178.52 ^{cd}	62.98	71.56
Pomegranate oil (300 ml) PO2	5.25 ^{bcd}	2.55	2.70 ^{bcd}	176.17 ^{de}	66.97	71.67
Orang oil (150 ml) OO1	5.16 ^{cd}	2.45	2.71 ^{bcd}	169.14 ^e	64.71	70.57
Orang oil (300 ml) OO2	5.24 ^{bcd}	2.66	2.58 ^{cd}	194.92 ^a	64.81	72.11
PO1+OO1	5.48 ^{abc}	2.58	2.90 ^{abc}	189.45 ^{ab}	63.52	71.12
PO1+OO2	5.78 ^a	2.55	3.23 ^a	186.33 ^{abc}	64.71	70.14
PO2+OO1	5.67 ^{ab}	2.47	3.19 ^a	181.25 ^{bcd}	66.54	68.02
PO2+OO2	5.75 ^a	2.67	3.08 ^{ab}	185.94 ^{bc}	66.00	65.40
SEM	0.14	0.10	0.12	2.57	3.96	2.44
Sig.test	**	NS	**	**	NS	NS

Means having different letters at the same column are significantly ($P \leq 0.05$) different * = $P < 0.05$; ** = $P < 0.01$; NS= Not significant

Hdl = High – density Lipoproteins

Ldl = Low – density Lipoproteins

Table (5): Effect of pomegranate and orange oils and their mixture supplementation on total antioxidant capacity, some antioxidant enzymes and testosterone of cocks at end of the experimental period at 64 weeks of age

Items	TAOC (m mole/L)	SOD (U/mL)	GPX (U/mL)	Testosterone
Control (Basal diet)	0.75 ^c	179.67 ^d	153.67 ^c	2.07 ^d
Pomegranate oil (150 ml) PO1	0.81 ^{bc}	195.33 ^c	164.33 ^{bc}	2.22 ^c
Pomegranate oil (300 ml) PO2	0.84 ^b	219.00 ^{ab}	172.00 ^{ab}	2.36 ^a
Orang oil (150 ml) OO1	0.80 ^{bc}	198.00 ^c	167.33 ^b	2.22 ^c
Orang oil (150 ml) OO2	0.81 ^{bc}	211.33 ^b	183.33 ^a	2.25 ^{bc}
PO1+OO1	0.84 ^b	216.33 ^{ab}	175.67 ^{ab}	2.32 ^{ab}
PO1+OO2	0.82 ^{bc}	214.33 ^{ab}	171.33 ^{ab}	2.24 ^{bc}
PO2+OO1	0.83 ^b	220.33 ^{ab}	181.33 ^a	2.33 ^a
PO2+OO2	0.91 ^a	227.67 ^a	182.00 ^a	2.39 ^a
SEM	0.02	3.71	3.61	0.02
Sig. test	**	**	**	**

a,b,c: Means in the same column with different superscripts, differ significantly ($p < 0.05$)

TAOC = Total Anti – Oxidant Capacity

SOD&GPX = Some antioxidant enzymes

4. Conclusions

It can be concluded that, supplemental layer diets up to 300ml/ kg diet pomegranate oil and 300mg/ kg diet orange oil were effective in improving productive performance, biochemical, antioxidant blood parameters and testosterone values of Golden Montazah laying hens.

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