

Research Article



Impacts of Enzymes and Probiotic in Improving the Utilization of Sieved Olive Pulp Meal on Productive Performance of Laying Hens

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Abstract | Nutritionists try to find suitable and cheap alternatives to replace traditional feed ingredients, which are also food items for humans. This study aimed to evaluate the effect of incorporation of sieved olive pulp (SOP) treated with Econase (E), dry yeast (DY), or both on productive performance of laying hens. Total of 147 Mandarrah laying hens (28-week old) were assigned to seven groups. The first group was fed a control diet, while six treatment groups were fed diets containing 15 or 20% SOP with Econase (0.1g/kg), DY (0.3g/kg), or both. The tested diets were iso-nitrogenous and isoenergetics (16% CP and 2750 kcal ME/kg). During a feeding period from 28 to 40 weeks of age, growth performance and egg production of hens were determined, and blood samples were taken for hematological and biochemical assays. Results showed significant ($P < 0.001$) increase in feed intake with all SOP diets compared with controls. Feed conversion ratio was improved ($P < 0.05$) in response to feeding hens on the diet containing SOP_{15%}+E compared to control. Number, daily mass, and production rate of eggs were increased ($P < 0.001$) by SOP_{15%}+E and SOP_{20%}+E or DY. Egg shape index and plasma cholesterol and triglyceride were improved ($P < 0.05$ or $P < 0.001$) by all SOP_{20%} diets. Shell percentage was increased ($P < 0.01$) by SOP_{20%}+E+DY. Haugh unit was increased ($P < 0.01$) by SOP_{15%}+E or E+DY, SOP_{20%}+E+DY. Egg surface area was improved ($P < 0.05$) by SOP_{15%}+E or DY, SOP_{20%}+DY. SOP_{20%}+DY diet showed the highest economic efficiency relative to the control followed by SOP_{15%} or SOP_{20%}+E diets. Overall, results demonstrated that sieved olive pulp could be incorporated in the diets of Mandarrah laying hens up to 15% with Econase or 20% with Econase or dry yeast to improve productive performance, egg production, and economic feed efficiency.

Keywords | Mandarrah laying hens, Sieved olive pulp meal, Enzyme, Probiotic, Egg characteristics.

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INTRODUCTION

The poultry industry has long been one of Egypt's most profitable livestock sectors, producing wholesome meat and eggs for human consumption (Mekled et al., 2019). Lately, one of the poultry industry's risks is the continuous rise in the price of their feed ingredients around

the world and/or a lack of feed ingredients. This has led nutritionists to find suitable alternatives to replace traditional feed ingredients, which may reduce the competition between humans and animals for these sources (Omenka and Anyasor, 2010), especially in Egypt. Traditional raw materials, such as corn meal and soybean, represent about 60-70% of the production cost of most poultry products

(Mallick et al., 2020). Agricultural by-products may be used after enzyme or biological treatment to enhance the utilization of these wastes to incorporate into poultry nutrition (Seidavi et al., 2018).

Olive pulp (OP) is one of the most important available agro-industrial by-products in Egypt, with production around 26 million tons per year. The use of olive pulp in poultry diets is restricted due to its low nutritive value, high fiber content, low contents of protein and energy, low degradability of cell wall components (Yansari et al., 2007), lower digestibility coefficient of nutrients, and condensed tannins (Martin Garcia et al., 2003). The use of enzymes in poultry diets is increasing the usage of alternative ingredients or by-products of processed grains to maintain the birds' productive performance and increase fiber digestibility or phytic phosphorus solubility (Tsuzuki et al., 2003; Al-Saffar et al., 2012). Dietary enzymes offer a number of benefits, such as enhanced bird performance and feed conversion and decreased feed costs (Perazzo Costa et al., 2015). A proper multi-enzyme preparation in the diet enhances animal performance by digesting starch, protein, fat, amino acids, and energy (Alagawany et al., 2018).

Probiotics are defined as live microorganisms that balance the microflora in the intestines to improve host health (FAO/WHO, 2002), maintain intestinal flora balance, produce digesting enzymes and antimicrobial peptides, decrease stress, and modify the host's protective immunity against various pathogens (Wang et al., 2018). The use of dry yeast, a type of probiotic, is a practical tool to improve the use of agricultural by-products in animal nutrition and reduce the cost of feeding and environmental pollution (Swiatkiewicz et al., 2014). Dietary probiotics improve animal performance, lower the number of pathogenic organisms, diminish the negative environmental effects of livestock production, and promote feed digestibility. Along with being a plentiful source of protein, lipids, vitamin B, and enzymes like phytase, cellulase, peroxidases, glutathione, superoxide dismutase, and catalase (Aluwong et al., 2013).

Extensive lignification of the cell wall causes poor digestion and reduced voluntary intake from olive pulp. Biological and enzyme treatments of olive pulp may enhance their nutritional value, particularly those that contain non-starch polysaccharides or phytic acid, to prevent metabolic disturbances (Coimbra et al., 1995).

Therefore, the present study aims to evaluate the impact of the incorporation of 15 or 20% sieved olive pulp (SOP) meal treated with the enzyme Econase (a thermostable endo-xylanase enzyme for monogastric animal feed), dry yeast, or their combination, as an alternative to a major amount of yellow corn and a minor amount of wheat bran,

on the productive and reproductive performance of Mandarah laying hens.

MATERIALS AND METHODS

The experimental work of this study was carried out at Research Station, Gimmizah, belonging to Animal Production Research Institute, Agricultural Research Center, Egypt. The handling and management of animals were conducted according to Directive 2010/63/EU for animal protection, used for scientific purposes (Official Journal of the European Union, 2010).

BIRDS

Total of 147 Mandarah laying hens having 28-week-old were randomly divided into 7 treatment groups, 21 hens in each, in a completely randomized design. All birds were housed in wire cages (40 x 35 x 60 cm) and managed under similar conditions. The lighting program was designed to give 16 hours of light daily throughout the experimental period from 28 up to 40 weeks of age.

EXPERIMENTAL DESIGN

Birds in the first group were fed the control diet (G1), while treatment groups included six groups fed diets containing 15 or 20% SOP treated with enzymes (0.1g Econase/kg), 0.3g dry yeast/kg, or a combination of the same level of Econase and dry yeast as the following:

G1: fed the basal diet (control).

G2: 15% SOP diet treated with Econase (SOP_{15%}+E).

G3: 15% SOP treated with dry yeast (SOP_{15%}+DY).

G4: 15% SOP treated with Econase and dry yeast (SOP_{15%}+E+DY).

G5: 20% SOP treated with Econase (SOP_{20%}+E).

G6: 20% SOP treated with dry yeast (SOP_{20%}+DY).

G7: 20% SOP treated with Econase and dry yeast (SOP_{20%}+E+DY).

The experimental birds were gradually adapted to fed the tested diets for two weeks before the main feeding period then all birds were fed their experimental diets *ad libitum* in mash form, while fresh water was available at all times. The experimental diets were isoenergetic (ME=2750 Kcal/kg) and isonitrogenous (CP=16 g/kg) and were formulated to meet the nutritional requirements presented by NRC (1994). Ingredients of the control and SOP-diets are shown in Table 1.

SIEVE OLIVE PULP PREPARATION

Olive pulp meal is the product that remains after oil extraction from the whole olive fruit. Due to its extremely high water content, it was air-dried then passing the milled fruits through a sieve after extracting the oil from the wild olive (2 mm-pore) to take out parts of the stones and add-

ing an antioxidant (HADOX-DRY) at a rate of 125 g/ton before being packaged and used to formulate the experimental diets.

The Econase® (Tex Biosciences, P, Ltd., India) is a commercial multi-enzymes product contains amylase (125.000 U/kg), Phytase (200.000 U/kg), cellulase (100.000.000 U/kg), lipase (10.000 U/kg), xylanase (1.500.000 U/kg), protease (15.000 U/kg), pectinase (30.000 U/kg), arabinase (7.000 U/kg), α -galactosidase (10.000 U/kg), and β -glucosidase (10.000 U/kg). The level of Econase used in this study was according to the recommended dose by the producer (100 g/ton of feed).

EXPERIMENTAL PROCEDURES

Change in body weight was calculated by recording the individual live weight at the beginning and end of the experimental period (from 28 to 40 weeks of age). During the first week of the experiment, all birds were acclimated, and over the following 12 weeks of the experiment, number and weight of eggs laid and feed intake by birds in each pen was daily recorded, then feed conversion ratio (g feed intake: g egg weight) and egg production rate per day were calculated.

EGG QUALITY TRAITS

External and internal measurements were taken to identify quality and composition of eggs. At 32, 33, and 34 weeks of age, 10 deposited fresh eggs/group were selected at random, then individually weighed and measured for the width and length to obtain the egg shape index. Albumin height, yolk height, and yolk diameter were measured after breaking on a smooth flat surface. The individual weight of eggshells and yolks was recorded, and eggshell thickness was determined. The yolk index was calculated as the yolk diameter divided by the yolk height multiplied by 100. The egg shape index was obtained by dividing the egg length by 100 of the egg width. Egg surface area (ESA) was determined as $ESA = 3.9782EW^{0.7056}$ according to Carter (1974; 1975). The Haugh unit score (HUS) for each egg was determined using the thick albumen height and egg weight using the equation of Larbier and Leclercq (1994). $HUS = 100 \log (H+7.57) - (1.7W^{0.37})$ where H is thick albumen height (mm) and W is egg weight (g).

EGG FERTILITY AND HATCHABILITY

A fixed volume of freshly collected semen from cockerels fed a diet containing 16% CP and 2750 kcal/kg diet was used for artificial insemination of the hens in each group. Three hatches at 35, 36, and 37 wk-old, were performed. The average weight of the healthy hatched chicks was computed, as well as hatchability and egg fertility percentages were determined.

BLOOD SAMPLING

Four birds from each group were taken at the end of experiment, blood samples were withdrawn from the wing veins and placed in test tube with heparin. In the whole blood samples, count of red blood cells and hemoglobin concentration were determined, then blood samples were centrifuged at 3000 rpm for 10 minutes to collect the blood plasma for determination of total protein, albumin, triglycerides, and cholesterol concentrations and the activity of alanine amino transferase (ALT) and aspartate amino transferase (AST) using commercial kits (Bio-Merieux). Globulin was calculated by subtracting albumin from total protein.

ECONOMIC EFFICIENCY (EE)

Economic efficiency of the experimental diets was calculated from total return (body weight gain and fertile eggs/hen) and total feed cost/hen. Price of various ingredients and treatments of each experimental diets based on the price of local market (2022). Economic feed efficiency = Net return / total feed cost \times 100.

STATISTICAL ANALYSIS

Statistical analysis was done by one-way analysis of variance, and the following model was used: $Y = \mu + T_i + E_{ij}$ Where Y = individual observation, μ = the overall mean, T_i = the impact of treatments, and E_{ij} the random experimental error. Duncan's multiple range test was used to distinguish differences among treatment means (Duncan, 1955) by using IBM SPSS analysis program (IBM Corp. Released, 2017 SPSS version 25).

RESULTS AND DISCUSSION

PROXIMATE ANALYSIS OF SIEVED OLIVE PULP MEAL (SOP) AND THE TESTED DIETS

Results of proximal analysis of SOP presented in Table 2 showed that SOP contained moderate CP and higher CF percentage as well as acceptable cell wall contents. The obtained results in our study showed higher CP and ash contents, and lower CF, ADF, and NDF contents than the results of Abdallah et al. (2016) who found that olive cake meal contains 6.79% CP, 33.32% CF, 57% NDF, 41% ADF, and 10.92% ash. In comparable with the obtained results, olive cake meal had 7.68% CP, 27.11% CF, 9.20% EE, and 33.01% NFE (Walaa Salama et al., 2016), 90.7% DM, 85.5% OM, 12.65% CP, 16.76% EE, 30.68% CF, 25.41% NFE, and 5.2% ash (Bakr et al., 2019). The olive cake meal clearly has a lower ratio of CP (6.70%), a reasonable amount of NFE (47.51%), and 1900 kcal as DE/kg diet. Tannins, ether extract, and ash contents were 7.80, 5.90, and 8.30%, respectively. The differences among these results may be attributed to variations in olive type, maturity stage of the fruits, oil extraction method, and the

Table 1: The ingredients of the experimental diets.

Ingredient (%)	Control diet	15 % SOP-diet	20% SOP-diet
Sieved olive pulp (SOP)	-	15.0	20.0
Ground yellow corn	60.5	48.3	44.0
Soybean meal (44 % CP)	24.5	24.2	23.7
Wheat bran	04.1	01.7	01.5
Limestone ground	07.8	07.5	07.4
Di-calcium phosphate	01.1	01.3	01.3
Methionine	00.1	00.1	00.1
Sunflower oil	01.3	01.3	01.4
Vitamin and mineral premix*	00.3	00.3	00.3
Common salt	00.3	00.3	00.3
Total	100	100	100

* Each 1kg diet contains vit. A 12000 IU, vit. D₃ 3000 IU, vit. E 40mg, vit. K₃ 3mg, vit. B₁ 2.mg, vit. B₂ 6mg, vit. B₆ 5mg, vit. B₁₂ 0.02mg, pantothenic acid 12mg, niacin 45mg, biotin 0.075mg, folic acid 2mg, cobalt 0.1mg, iodine 1mg, Zn 600mg, Mn 30g, Fe 30mg, Cu 10mg, I 0.75g, Se 0.2mg, and Mg 100mg.

Table 2: Chemical analysis and cell wall contents of sieved olive cake meal (SOP).

Nutrient	DM	OM	CP	CF	EE	NFE	Ash	ADF	NDF	HC
%	91.42	80.12	9.77	31.50	12.50	34.93	11.30	38.16	49.62	11.46

DM: Dry matter, OM: Organic matter, CP: Crude protein, CF: Crude fiber, EE: Ether extract, NFE: Nitrogen free extract, ADF: Acid detergent fiber, NDF: Neutral detergent fiber, and HC: Hemicellulose. ADF% = 9.432 + 0.912 (CF%). NDF% = 28.924 + 0.657 (CF%).

HC% = NDF% - ADF%.

Table 3: The chemical composition of the experimental diets.

Item	Experimental diet		
	Control	15 % SOP	20% SOP
Crude protein (%)	16.06	16.09	16.00
Ether extract (%)	2.83	4.14	4.58
Crude fiber (%)	3.67	7.80	9.22
Calcium (%)	3.30	3.34	3.34
Available phosphorus (%)	0.38	0.39	0.38
Lysine (%)	0.89	0.86	0.84
Methionine (%)	0.39	0.36	0.34
Methionine+ cysteine (%)	0.56	0.50	0.48
Metabolizable energy (ME, kcal/kg)	2750	2751	2752

environmental conditions (Azazi et al., 2018; Seidavi et al., 2018), and may be attributed to the sieving process used in our study.

The chemical composition of the experimental diets in Table 3 indicated that all experimental diets were iso-nitrogenous (16% CP) and iso-energetics (2750 kcal ME/kg diet), regardless treatment. Also, contents of available phosphorus, methionine, and methionine+ cysteine were nearly similar in all tested diets.

GROWTH PERFORMANCE

The effect of the experimental diet on final live body weight and total gain was not significant (Table 4). However, feed intake and feed conversion ratio (FCR, P<0.05) were affected significantly by treatment. Daily feed intake was significantly (P<0.05) increased by feeding hens on all SOP-diets as compared to the control diet. Hens provided diets inclusion SOP_{15%}+E only exhibited significant (P<0.05) improvement in FCR compared to those fed the control diet.

Table 4: Effect of the tested diets on body weight, weight gain, feed intake and feed conversion ratio of Mandarrah laying hens.

Tested Diet	Initial body weight (g)	Final body weight (g)	Total weight gain (g)	Feed intake (g/d)	Feed conversion ratio
Control	1462.00	1610.00	148.00	104.33 ^b	3.98 ^{ab}
SOP _{15%} +E	1460.67	1654.00	193.33	110.07 ^a	3.69 ^c
SOP _{15%} +DY	1459.33	1640.00	180.67	109.47 ^a	3.96 ^{ab}
SOP _{15%} +E+DY	1449.67	1637.33	187.67	110.00 ^a	4.12 ^a
SOP _{20%} +E	1459.00	1621.67	162.67	111.40 ^a	3.87 ^{abc}
SOP _{20%} +DY	1448.33	1604.00	155.67	111.73 ^a	3.81 ^{bc}
SOP _{20%} +E+DY	1444.33	1594.00	150.33	109.40 ^a	4.00 ^{ab}
SEM	40.75	41.52	16.14	0.73	0.09
P-Value (Sign.)	1.00 ^{ns}	0.952 ^{ns}	0.317 ^{ns}	0.001 ^{***}	0.023 [*]

^{a, b, c}: Means with different superscripts in the same column are significantly different at P<0.05.

^{ns}: Not significant. * P<0.05. *** P<0.001. SEM: Standard error of mean. E: Econase enzyme. DY= dry yeast.

Table 5: Effect of the tested diets on egg production traits of Mandarrah laying hens.

Tested Diet	Number of eggs/hen	Egg weight (g)	Daily egg mass (g)	Egg production rate/hen/day
Control	57.64 ^c	48.55	26.27 ^d	54.11 ^c
SOP _{15%} +E	63.64 ^a	49.65	30.09 ^a	60.61 ^a
SOP _{15%} +DY	58.91 ^{bc}	49.66	27.86 ^{bcd}	56.10 ^{bc}
SOP _{15%} +E+DY	56.82 ^c	49.49	26.78 ^d	54.11 ^c
SOP _{20%} +E	62.45 ^{ab}	48.59	28.90 ^{abc}	59.48 ^{ab}
SOP _{20%} +DY	62.45 ^{ab}	49.48	29.43 ^{ab}	59.48 ^{ab}
SOP _{20%} +E+DY	59.18 ^{bc}	48.70	27.45 ^{cd}	56.36 ^{bc}
SEM	1.29	0.35	0.60	1.23
P-Value (Sign.)	0.001 ^{***}	0.52 ^{ns}	0.000 ^{***}	0.001 ^{***}

^{a, b, ..., d}: Means within the same column with different superscripts differ significantly (P<0.05).

^{ns}: Not significant. *** P<0.001. SEM: Standard error of mean. E: Econase enzyme. DY= dry yeast.

Table 6: Effect of the tested diets on egg quality characteristics of Mandarrah laying hens.

Tested diet	Shape index (%)	Shell (%)	Yolk (%)	Albumen (%)	Yolk index	Haugh Unit	SHT (mm)	ESA (mm ²)
Control	72.17 ^c	15.38 ^{ab}	32.47	58.26	43.71	87.84 ^c	31.40	61.08 ^b
SOP _{15%} +E	75.71 ^{bc}	13.71 ^{bc}	32.53	53.76	45.71	98.70 ^a	31.40	65.20 ^a
SOP _{15%} +DY	76.81 ^{abc}	14.42 ^{bc}	31.51	54.07	47.05	91.48 ^{bc}	32.00	66.32 ^a
SOP _{15%} +E+DY	76.06 ^{abc}	15.15 ^{ab}	32.11	52.73	46.05	94.80 ^{ab}	32.00	63.14 ^{ab}
SOP _{20%} +E	77.24 ^{ab}	12.96 ^c	32.84	54.19	43.99	90.12 ^{bc}	31.40	64.33 ^{ab}
SOP _{20%} +DY	77.38 ^{ab}	14.52 ^{bc}	32.80	52.69	46.81	90.33 ^{bc}	31.20	64.94 ^a
SOP _{20%} +E+DY	80.85 ^a	16.89 ^a	35.10	52.38	49.54	94.56 ^{ab}	31.00	62.76 ^{ab}
SEM	1.47	0.61	0.97	1.55	1.39	1.78	0.51	1.08
P-Value (Sign.)	0.03 [*]	0.01 ^{**}	0.4 ^{ns}	0.34 ^{ns}	0.18 ^{ns}	0.01 ^{**}	0.8 ^{ns}	0.04 [*]

^{a, b, c}: Means with different superscripts in the same column are significantly different at P<0.05.

^{ns}: Not significant. * P<0.05. ** P<0.01. SEM: Standard error of mean. E: Econase enzyme. DY= dry yeast. ESA: egg surface area. SHT: Shell thickness.

The present results indicated that treatment of SOP_{15%} diet with Econase showed the best FCR in comparison with control significantly and other treatments non-significantly. In agreement with the present results, Abd El-Galil et

al. (2017) reported that increasing the level of olive cake (OC) up to 16% in the diets had no effects on final body weight and body weight gain of laying hens, but tended to significantly increase feed intake. Similarly, El-dorie (2005)

found that feeding Gimmizah laying hens on diets containing up to 16% olive pulp meal had no significant effect on live body weight or body weight gain. Increasing feed consumption of SOP-diets may have made the feed more palatable or that the birds had the ability to regulate their energy needs by consuming more feed when given low-nutrient density diets (Attia et al., 2001). The significant increase in feed intake from SOP-diets (above 8%) was led to a decrease in FCR compared to the control group. Also, Al-Harhi (2015) referred that diet with 10 or 20% OP did not significantly affect the change in body weight even though the feed intake of laying hens fed diets containing 20% olive cake was significantly higher than those fed the control diet. Improving FCR of hens fed SOP_{15%}-E was mainly attributed to increasing egg production because other SOP-diets significantly increased their feed intakes as compared to control. Concerning the impact of DY treatment, Edrees et al. (2017) found an improvement in feed efficiency of laying hens fed diet supplemented with DY. Recently, it was reported that enzyme treatment resulted in significant improvement in feed conversion ratio (Gupta et al., 2020) and feed efficiency (Hoeck et al., 2021) of laying hens. The observed increase in daily feed intake of hens fed diet treated with enzymes was reported by Abreu et al. (2018), who found that hens that received a ration enriched with an enzyme complex at a rate of 100g/ton ration consumed more feed than hens that received a ration without of the enzyme. Contrary, Habib et al. (2021) found that enzyme supplementation had no impact at the lower levels of inclusion (250 gm phytase enzyme/ton) but significantly reduced feed intake at the highest level of inclusion (750 gm phytase enzyme/ton). In similar to our results, Yalçin et al. (2008) concluded that addition yeast (*Saccharomyces cerevisiae*) to the diet increased body weight gain and egg weight, despite other studies indicated no effects of yeast on dietary intake and feed efficiency of laying hens (Özsoy et al., 2018).

EGG PRODUCTION

Data in Table 5 showed that Mandarah laying hens fed SOP_{15%}+E diet showed the highest ($P<0.05$) egg production in terms of increasing number of eggs (NE), daily egg mass (DEM) and egg production rate/hen/day in comparison with controls. On the other hand, hens fed SOP_{20%} with Econase or DY diets showed higher ($P<0.05$) DEM and egg production rate than the controls, but both did not differ significantly from those fed SOP_{15%}-E diet. However, egg weight was not affected significantly by the tested diets.

These results are consistent with those obtained by Gupta et al. (2020), who observed that diet supplemented with enzymes significantly improved DEM, egg production, without significant effect on egg weight. Dietary supple-

mentation of enzymes “xylanase” significantly improved laying hen performance in terms of DEM as compared to the control diet (Hoeck et al., 2021). The beneficial impacts of SOP_{20%} with enzyme or DY treatment was indicated by some authors who found that diet containing up to 20% OC had no impacts on egg production rate, egg weight, or egg mass of laying hens (Al-Harhi, 2015). Also, egg production rate, and egg mass were not significantly affected by the inclusion of SOP in the diet (Afsari et al., 2014).

The cell walls of olive pulp contain non-starch polysaccharides such as xyloglucan and xylan-xyloglucan complexes (Coimbra et al., 1995) and glucuronoxylans with a xylose/glucose ratio of 1:7 (Reis et al., 2002), which are anti-nutritive effect on monogastric animals like chickens (Coimbra et al., 1995). Poultry can't be digested NSPs in the gastrointestinal tract because of their structural nature and their resistance to hydrolysis in the digestive system (Aftab and Bedford, 2018). The anti-nutritive effect of SOP can be removed by addition of enzymes to poultry diets. Enzymes break-down NSPs by decreasing intestinal viscosity via enhancing nutritional digestibility through improving gut performance.

Regarding the positive impact of DY on egg production, Edrees et al. (2017) found an increases in egg production of laying hens fed yeast-supplemented diets. Also, Desoky and Kamel (2018) reported that egg number and egg weight were increased due to 2% yeast supplementation to the diet. The yeast additive increased egg mass and egg production (Khochamit et al., 2021). This may be due to the prebiotic properties of the dried yeast's mannan oligosaccharide and fructooligosaccharide which may help the beneficial microbes that naturally colonies the chicken gut grow (Attia et al., 2020; Zhang et al., 2020). Also, Park et al. (2020) found that adding brewer's yeast hydrolysate to the diet had a positive impact on egg production and egg mass. Moreover, Araujo et al. (2018) reported that adding hydrolyzed yeast to the breeder hens' diet increased the rate of eggs produced. In contrast, some studies indicated no effects of yeast on egg production (yield and size) of laying hens (Özsoy et al. 2018). The most of the improvement in productive performance by including SOP in the diet is due to the dietary treatment of laying hens with enzymes or yeasts.

EGG QUALITY PARAMETERS

The effect of the tested diet on egg quality characteristics is presented in Table 6. In comparison with the control diet, the egg shape index (ESI) was significantly ($P<0.05$) higher by all SOP_{20%} diets, being the highest for SOP_{20%}+E+DY; the percentage weight of the shell was the lowest ($P<0.05$) by SOP_{20%}+E diet; Haugh unit (HU) was significantly ($P<0.05$) improved by SOP_{15%}+E, SOP_{15%}+E+DY, and

SOP_{20%}+E+DY diets, respectively; egg surface area was significantly ($P<0.05$) increased by SOP_{15%} with E or DY, and SOP_{20%}+DY diets. However, the effect of the tested diets on the percentage of yolk and albumen, yolk index, and shell thickness was not significant.

The obtained results coincide with the results of [Abd El-Moneim and Sabic \(2019\)](#), who reported that extending the feeding with OP may improve the quality of the eggs in terms of albumen, yolk, and shell percentages, ESI, yolk index, and HU score. Also, [Zarei et al. \(2011\)](#) and [Al-Harhi and Attia \(2015\)](#) obtained a greater impact on the HU score and yolk index of laying hens giving diets with 10 or 20% OP, while only 20% OC significantly decreased the ESI. In accordance with the present results, [Hameed et al. \(2019\)](#) found that adding baker's yeast at a level of 0.15-0.2% was enough to improve the layer performance significantly in terms of yolk weight, albumin height, HU, and grading value. In contrast, [Alagawany and Attia \(2015\)](#) found that the addition of Avizyme had no significant influence on the exterior or internal egg quality of laying Japanese quails during any of the experimental periods, except the shell percentage between 16 - 20 weeks of age. Diets supplemented with Avizyme showed significantly lower shell percentage values when compared to birds receiving the control diet.

EGG FERTILITY AND HATCHABILITY

Results in [Table 7](#) revealed the tested diets had no significant effects on fertility, hatchability, and hatch-chick weight of Mandarrah eggs. The present results are in harmony with [El-dorie \(2005\)](#), who found insignificant effect of dietary SOP in the diet (up to 16%) on egg fertility, hatchability, and hatch-chick weight of eggs of Gimmizah laying hens. Also, [Ramos \(2011\)](#) reported that adding an enzyme mixture to nutritionally marginal broiler breeder meals did not enhance characteristics related to fertility or hatchability. On the other hand, [El-Faham et al. \(2016\)](#) demonstrated that birds fed diets supplemented with 200 g phytobex plus per ton had higher rates of fertility and hatchability. [Hassanien et al. \(2015\)](#) found that groups of Hubbard breeders given diets supplemented with a 500 g/ton of enzyme mixture had higher hatchability and fertility rates.

PLASMA BIOCHEMICAL PARAMETERS

The effect of the tested diets on some blood biochemicals of Mandarrah laying hens is shown in [Table 8](#). Concentration of total protein, albumin, and globulin in blood plasma were not affected significantly by the tested diets. Concentration of plasma total cholesterol and triglycerides significantly ($P<0.05$) decreased in hens fed SOP_{20%} diets with Econase, DY, or their combination. Only SOP_{15%}+E+DY diet significantly ($P<0.05$) decreased cholesterol level as

compared to control diet. [Zanaty et al. \(2019\)](#) showed that adding Polyzyme to a low-protein diet had no significant effects on total protein. [Zangeneh and Torki \(2011\)](#) demonstrated that dietary OP inclusion up to 9% had no significant impact on the blood parameters.

[Abd El-Moneim and Sabic \(2019\)](#) observed that feeding quails on a OP_{10%}-diet had no significant impact on concentration of blood total protein and albumin. [Hewida et al. \(2011\)](#) showed that total protein, albumin, and globulin did not change by yeast culture supplementation.

Concerning the lipid profile, triglycerides and total cholesterol concentrations were reduced by feeding a OP_{10%}-diet compared to the control group [Abd El-Moneim and Sabic \(2019\)](#). Also, adding Polyzyme to a low-protein diet had reduced serum total cholesterol compared to control treatment. Levels of cholesterol and V-LDL were low by incorporation of 5 and 10% OC in the diet ([Al-Harhi, 2017](#)).

Previous studies demonstrated that adding yeast autolysate to the diet at doses of 2, 3, and 4 g/kg decreased blood triglycerides and cholesterol levels ([Özsoy et al., 2018](#)). Similarly, [El-Kaiaty et al. \(2019\)](#) mentioned that adding yeast to laying hen diets significantly decreased serum levels of total lipids, cholesterol, and triglycerides when compared to the control group receiving no supplements. Yeast supplementation significantly decreased blood serum triglycerides and cholesterol levels ([Yaşın et al., 2014](#)). The hypolipidemic and hypocholesterolemic effect of olive cake was attributed to its crude fiber content, as fiber plays a role in cholesterol metabolism by decreasing cholesterol absorption, binding with bile salts in the intestinal tract, shorten intestinal transit time, and increasing fecal sterol excretion ([Boka et al., 2014](#)). Moreover, antioxidants in yeast may contribute to fat catabolism and thus reduce the amount of fat and cholesterol present ([Issa and Abo Omar, 2012](#)).

HEMATOLOGY AND LIVER FUNCTION

The effect of the tested diets on the count of RBCs, Hb concentration, and AST and ALT activities in blood plasma was not significant ([Table 9](#)). These results indicated that all SOP diets used in our study showed an average effect on the erythrocytic index and liver function of laying hens.

In accordance with our results, [Abd El-Moneim and Sabic \(2019\)](#) observed that feeding quails on a OP_{10%}-diet had no significant impact on the activities of the enzymes AST and ALT. Also, [Alharhi \(2015\)](#) and [Al-Harhi and Attia \(2016\)](#) found that commercial bird diets containing up to 20% OP, does not affect the activity of ALT and AST enzymes or the physiological maintenance of liver function.

Table 7: Effect of the tested diets on egg fertility, hatchability and chick weight at hatch of Mandarah laying hens.

Tested Diet	Fertility rate	Hatchability rate ⁽¹⁾	Hatchability rate ⁽²⁾	Chick weight at hatch (g)
Control	87.78	87.39	76.67	31.65
SOP _{15%} +E	85.33	87.52	74.67	32.63
SOP _{15%} +DY	86.27	86.35	74.50	32.31
SOP _{15%} +E+DY	86.67	85.73	74.29	31.03
SOP _{20%} +E	85.86	86.40	74.16	31.31
SOP _{20%} +DY	87.25	86.52	75.49	31.49
SOP _{20%} +E+DY	87.72	86.01	75.44	32.70
SEM	11.77	6.26	10.17	3.32
P-Value (Sign.)	0.95 ^{ns}	0.85 ^{ns}	0.91 ^{ns}	0.14 ^{ns}

^{ns}: Not significant. SEM: Standard error of mean. E: Econase enzyme. DY= dry yeast.

(1): Hatchability of fertile eggs. (2): Hatchability of total eggs.

Table 8: Effect of the tested diets on some plasma biochemicals of Mandarah laying hens.

Tested Diet	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total cholesterol (mg / d)	Triglycerides (mg/d)
Control	5.50	2.37	3.13	139.33 ^a	125.00 ^a
SOP _{15%} +E	5.63	2.43	3.20	131.00 ^a	116.67 ^{abc}
SOP _{15%} +DY	5.68	2.42	3.26	133.00 ^a	121.67 ^{ab}
SOP _{15%} +E+DY	6.09	2.58	3.52	127.67 ^a	112.67 ^{bc}
SOP _{20%} +E	5.74	2.42	3.32	110.00 ^b	111.00 ^{bc}
SOP _{20%} +DY	5.54	2.46	3.08	103.00 ^b	110.00 ^c
SOP _{20%} +E+DY	6.16	2.48	3.68	110.00 ^b	112.00 ^{bc}
SEM	0.13	0.09	0.17	3.36	2.54
P-Value (Sign.)	0.06 ^{ns}	0.90 ^{ns}	0.32 ^{ns}	0.00 ^{**}	0.04 [*]

^{a, b, c}: Means with different superscripts in the same column are significantly different at P<0.05.

^{ns}: Not significant. * P<0.05. ** P<0.01. SEM: Standard error of mean. E: Econase enzyme. DY= dry yeast.

Table 9: Effect of the tested diets on hematological parameters and enzyme activity in blood plasma of Mandarah laying hens.

Tested diet	RBCs (10 ⁶ /mm ³)	Hb (g/dl)	AST (U/L)	ALT (U/L)
Control	2.38	12.33	31.67	12.67
SOP _{15%} +E	2.72	14.60	23.67	11.67
SOP _{15%} +DY	2.80	14.53	30.00	10.33
SOP _{15%} +E+DY	3.20	16.73	26.33	12.33
SOP _{20%} +E	2.55	13.50	29.17	10.67
SOP _{20%} +DY	2.45	14.13	24.33	11.00
SOP _{20%} +E+DY	2.47	13.27	27.33	12.33
SEM	0.14	0.75	2.80	0.81
P-Value (Sign.)	0.08 ^{ns}	0.08 ^{ns}	0.61 ^{ns}	0.41 ^{ns}

^{ns}: Not significant. * P<0.05. ** P<0.01. SEM: Standard error of mean. E: Econase enzyme. DY= dry yeast.

RBCs: Red blood cells. Hb: Hemoglobin. AST: Aspartate aminotransferase. ALT: Alanine aminotransferase.

Robins and Brooker (2005) and Rubanza et al. (2005) reported that hens given a 16% olive cake decreased RBCs count and Hb level due to the higher concentration of anti-nutritional components in olive cake, particularly phenols and condensed tannins, which have been known to

have an anti-nutritional impact. Zanyaty et al. (2019) who showed that adding Polyzyme to a low-protein diet had no significant effects on activity of blood AST or ALT. Based on our results treatment of SOP with Econase, DY, or their combination improved the nutritive values of SOP

Table 10: Economic feed efficiency of Mandarah laying hens fed different experimental diets.

Tested diet	Price (L.E.) per kg	Feed intake (kg)	Feed cost (L.E.)	Price (L.E)		Return (L.E)		EE (%)	REE (%)
				Weight gain	Fertile eggs	Total	Net		
Control	6.03	8.76	52.82	5.92	124.69	130.61	77.79	147	100.0
SOP _{15%} +E	5.71	9.25	52.82	7.73	135.75	143.48	90.66	172	117.0
SOP _{15%} +DY	5.66	9.20	52.07	7.23	127.05	134.28	82.21	158	107.5
SOP _{15%} +E+DY	5.76	9.24	53.22	7.50	123.11	130.61	77.39	145	98.64
SOP _{20%} +E	5.52	9.36	51.67	6.51	134.06	140.57	88.91	172	117.0
SOP _{20%} +DY	5.47	9.39	51.36	6.23	136.23	142.46	91.10	177	120.4
SOP _{20%} +E+DY	5.57	9.19	51.19	6.01	129.79	135.80	84.61	165	112.2

E: Econase enzyme. DY= dry yeast. Based on the local market price in year 2022, price of kg weight gain = 40 L.E; price of kg weight gains = 40 L.E, price of kg diet = 15 L.E and price of fertile egg= 2.5 L.E

Number of fertile eggs/hen = number of produced eggs/hen x fertility rate.

Price of fertile eggs/hen = Number of fertile eggs x price of fertile egg.

Total return = price of fertile eggs + price of weight gain.

Net return = Total return - total feed cost.

Economic feed efficiency (EE%) = net return/total feed cost × 100

Relative economic efficiency (REE) = EE of each group/EE of the control x100.

and prevented the effect of anti-nutritional factors in SOP.

ECONOMIC FEED EFFICIENCY

Table 10 shows the economic efficiency of different experimental diets fed to Mandarah laying hens. Results showed that the price/kg of SOP-diet was reduced by increasing the level of SOP incorporation compared with control, being higher with Econase than with DY treatment. Despite of the observed increase in feed intake of all SOP diets, the total feed cost was lower for SOP diets than the control diet, except for the SOP_{15%}+E+DY. Generally, feeding SOP_{20%}+DY diet showed the highest economic efficiency relative to the control diet (REE, relative economic efficiency) due to the highest net return and the lowest feed cost. This superiority was followed by feeding SOP_{15%} or SOP_{20%} diets supplemented with Econase. On the other hand, hens fed SOP_{15%}+E+DY showed lower economic efficiency relative to those fed the control diet.

Our findings are in agreement with Taklimi et al. (1999) who fed laying hens on diets containing up to 20% OP. Similarly, Hashish and Abd El-Samee (2002) concluded that inclusion 5% olive cake to the diet of Lohman hens can improve the feeding's economic efficiency. According to Zanaty and Hussein (2021), the diet containing 0.5% *Saccharomyces cerevisia*, 0.5% *Aspergillus awamori*, and 0.5% lactic acid bacteria produced the maximum REE.

CONCLUSION

Based on the foregoing results, sieved olive pulp could be incorporated in the diets of Mandarah laying hens up to 15% with Econase or 20% with Econase or dry yeast to improve productive performance, egg production, and

economic feed efficiency. Further studies on the feeding of sieved olive pulp treated with different enzymes/compounds or at different levels to growing and laying hens are required in the future.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

NOVELTYS STATEMENT

This work indicated that to maximize the benefits of sieved olive pulp in poultry diet it can treat with enzymes, yeast and particularly their combination.

AUTHOR'S CONTRIBUTION

Alderey, A.A. was the designer of this experimental work. Nabila E.M. El-Kassas conducted the experimental procedures, collected data and conducted the statistical analyses. Eman A. Hussein performed the sample preparations for laboratory analysis. Eman S. El-Hadad and Manal H. Gomaa were contributed in drafting the manuscript and critically revised the manuscript. All authors were approved the final manuscript for publication.

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