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RESEARCH ARTICLE

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The effects of replacing wheat and soyabean meal with duckweed (*Lemna minor*) and including enzymes in the diet of laying hens on the yield and quality of eggs, biochemical parameters, and their antioxidant status

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Abstract

Duckweed is a rapidly growing aquatic plant, which could be used in the diet of laying hens to enhance carbon capture and improve land use efficiency. Digestion may be improved by supplementation with exogenous enzymes. We replaced soyabean meal and wheat with duckweed in a 10-week study with 432, 60-week-old Hy-Line W-36 layers, divided into six isocaloric and isonitrogenous dietary treatments, each with eight replicates. Two factors were investigated: first, duckweed substituted for wheat gluten meal and soyabean meal at 0, 7.5 and 15% of the diet, and second, with and without a multi-enzyme supplement (500 mg/kg). Duckweed did not affect egg output or weight, but it improved yolk color ($P = 0.01$) and reduced the liver enzymes aspartate aminotransferase ($P = 0.04$) and alanine aminotransferase ($P = 0.02$) in serum, suggesting hepatoprotective effects. Enzyme addition did not alter the effects of including duckweed in the diet, but it increased feed intake ($P = 0.03$). It is concluded that, as well as offering the potential to increase land productivity, inclusion of duckweed in the diet of laying hens enhances egg yolk color and hepatoprotection, without detrimental effects on performance.

KEYWORDS
duckweed, exogenous enzymes, laying hen, performance, yolk color

1 | INTRODUCTION

Since 1990, world egg production has more than doubled (Stastia, 2023). The shortage of feed resources is encouraging poultry producers to investigate more efficient feeding methods. Soyabean meal (SBM) production has low sustainability, is relatively inefficient use of valuable land, and has largely been advanced by big multinational companies, with the aid of subsidies from, for example, the Brazilian government, which penalizes small farmers (Garrett & Rausch, 2016). There is a need to source efficiently produced local feed ingredients and then investigate their ability to supply nutrients to meet the requirements of different classes of poultry (Elamin, 2006). A search for alternative protein sources to replace SBM in animal diets has triggered renewed interest in the use of duckweed (Baek et al., 2021).

Duckweed (*Lemnaceae species)* is a small, floating aquatic plant that grows rapidly and has high protein content when grown on nutrient-rich water, making it an ideal candidate to be used as a protein source. The *Lemnaceae* family includes five genera, *Spirodela*, *Landoltia*, *Lemna*, *Wolffiella*, and *Wolffia*, and a total of 36 different species have been identified (Xu et al., 2022). *Spirodela polyrhiza*, *Lemna gibba*, *Lemna trisulca*, *Wolffia arrhiza*, and *Lemna minor* species have been identified in Iran (Mozaffarian, 1996). We studied the *L. minor* species because this species is available both in Iran and worldwide (Escobar & Escobar, 2017). The replication time for duckweed species can be as short as 1.2 days, and yields in optimum growth media are as high as 100 t dry matter (DM)/ha/year, almost 28 times more than conventional crops (Baek et al., 2021; Pagliuso et al., 2022). It is tolerant of a wide range of media conditions, including high temperatures (Baek et al., 2021). Duckweed can reduce ammonia concentrations in water (Baek et al., 2021), and its high rate of carbon capture can help to advance animal production towards carbon neutrality, by releasing land to be used for carbon capture, for example, by trees.

Being an aquatic plant, duckweed has little need for structural carbohydrates as a support system in the plant and the content of lignin and cellulose is much less than terrestrial plants (Pagliuso et al., 2022). It has a fiber content of just 9%–16% in the DM but is rich in starch (Chen et al., 2022). As a result of its rapid growth and high content of essential amino acids, duckweed is a potential source of protein for poultry (Sońta et al., 2019). It also contains many flavonoids, which contribute antioxidants to counteract reactive oxygen species and improve animals' health (Baek et al., 2021). This is especially important for laying hens in their second year. Including duckweed at up to 13% in the diet of laying hens has not had any harmful effects on production performance (Akter et al., 2011).

Duckweed has a significant content of polysaccharides, whose digestion is restricted by lack of suitable enzymes in chickens (Khan et al., 2011). Supplementation with exogenous enzymes, such as phytase, is potentially beneficial (Baghban-Kanani et al., 2020; Kocher et al., 2002; Tabook et al., 2006). Some beneficial effects of multi-enzyme additives to the diet of poultry have been demonstrated (e.g., Liu & Kim, 2017), and no negative effects on egg quality and performance of hens have been reported (Baghban-Kanani et al., 2018; Rezaei & Hafezian, 2007). However, to the best of our knowledge, multienzyme additives have not been tested with diets containing duckweed before. Therefore, this experiment was conducted to evaluate the inclusion of duckweed and commercially available enzymes on performance, biochemical parameters, and antioxidant status of laying hens.

2 | MATERIALS AND METHODS

2.1 | Animals and diets

This experiment was conducted at the Department of Animal Science, Islamic Azad University, Rasht branch, Iran. All animal care and use procedures were approved by the Islamic Azad University, which is in compliance with international guidelines (FASS, 1999). Four hundred and thirty-two 60-week-old Hy-Line White Leghorn laying hens, variety W-36, were randomly allocated to six dietary treatments in a 3 × 2 factorial arrangement of a completely randomized design. These older birds were selected because laying birds in Iran are typically for two laying seasons, and it is in the second season that the birds' productivity is most likely to be reduced. Hens were randomly assigned to cages so that there were eight replications per treatment. Each replicate consisted of three adjacent cages with three hens per cage, to provide a total of nine hens per replicate. Cages were of dimensions 30 × 40 × 42 cm, which is normal for caged hens in Iran, where there are no legislative requirements for cage size. The small sizes of the cages was not expected to influence the birds' physiological stress levels (Davami et al., 1987). Mean temperature in the chicken house was 24°C and relative humidity 40%.

Before starting the experiment, egg production of hens was measured individually. Hens were placed in each replicate to equalize rate of egg production. Diets were formulated based on linear programming by using least cost rationing software (Pesti & Miller, 1993). The amino acid profile of duckweed was taken from Appenroth et al. (2017). Treatments consisted of three levels (0, 7.5, and 15%) of duckweed (0, Low Duckweed (LD) and High Duckweed (HD), respectively) and two levels of enzyme (E) (0 and 500 g/t). The composition of the diets is shown in Table 1. At the time of harvesting the duckweed from an experimental pilot pool for nutrition research, a representative sample was collected for analysis. This was desiccated for 48 h in the sun and then dried for 24 h in an oven (model Homuk-Langroud-Iran) at 75°C and transferred to the ViroMed Central Analytical Lab, in the Samaneh Payesh Salamat laboratory complex, Pardis Technology Park, Tehran, Iran, for analysis.

Feed was offered ad libitum as a mash together with ad libitum water during the experiment. Light was provided to the cages for 16 h per day during the experimental period. Duckweed (*L. minor*) powder was provided by Darvash Giah Khazar medicinal herbs complex company (Ltf) (Gilan, Rasht, Iran). This company used the experimental pool (30 × 10 × 1 m) located in the Kishestan Industrial District, Souma'eh Sara, Gilan, Iran, No. 180, in June 2022 under controlled conditions for the purpose of producing feed for the present research. The chemical composition of duckweed is presented in Table 2. The enzyme mixture used was a dried commercial multi-enzyme preparation, Kemin®WP (Kemin Industries, Inc., EMEA, Herentals, Belgium). This included six active ingredients: xylanase (20,000,000 units/kg), cellulase (5,000,000 units/

TABLE 1 The ingredients and chemical compositions of experimental diets with low duckweed (LD), high duckweed (HD), or a control, with and without exogenous enzymes (E).

Treatment effects	Control[1]	Control + E[2]	LD[3]	LD + E[4]	HD[5]	HD + E[6]
Ingredients (%)						
Corn	50.00	55.94	50.00	51.40	50.00	50.00
Wheat	11.59	15.00	6.67	15.00	1.79	11.53
Gluten meal (67% CP)	2.00	2.00	2.00	2.00	2.00	2.00
SBM[7]	19.27	15.44	16.60	12.06	13.93	9.18
Duckweed	-	0.00	7.50	7.50	15.00	15.00
Oyster mineral	10.32	7.71	10.07	7.42	9.74	7.12
Soy oil	3.75	0.74	3.84	1.13	3.93	1.37
Dicalcium phosphate	1.89	1.89	1.96	1.97	2.03	2.05
Vitamin premix[8]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[9]	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.32	0.33	0.33	0.33	0.34	0.34
Dl-methionine	0.15	0.12	0.17	0.15	0.19	0.17
Lysine	0.13	0.21	0.24	0.35	0.37	0.48
Threonine	0.08	0.07	0.12	0.14	0.18	0.21
Enzyme[10]	0.00	0.05	0.00	0.05	0.00	0.05
Total	100	100	100	100	100	100
Calculated composition (%)						
AME_n (kcal/kg)	2850	2850	2850	2850	2850	2850
Crude protein	16.00	16.00	16.00	16.00	16.00	16.00
Crude fiber	2.81	2.73	2.73	3.02	3.43	3.32
Calcium	4.40	4.40	4.40	4.40	4.40	4.40
Available phosphorus	0.45	0.45	0.45	0.45	0.45	0.45
Sodium	0.17	0.17	0.17	0.17	0.17	0.17
DL-methionine	0.40	0.40	0.40	0.40	0.40	0.40
DL-methionine + cysteine	0.75	0.75	0.75	0.75	0.75	0.75
Threonine	0.61	0.61	0.61	0.61	0.61	0.61
Lysine	0.81	0.81	0.81	0.81	0.81	0.81

[1]Basal diet.
[2]Basal diet with 500 g/t exogenous enzymes.
[3]7.5% duckweed without enzyme.
[4]7.5% duckweed with 500 g/t exogenous enzymes.
[5]15% duckweed without enzyme.
[6]15% duckweed with 500 g/t exogenous enzymes.
[7]Soybean meal (44% CP).
[8]Vitamin supplement provides per kilogram of diet: vitamin A, 8000 IU; vitamin E, 20 IU; menadione, 3.0 mg; vitamin D3, 2000 IU; riboflavin, 4.0 mg; pantothenate, 12 mg; nicotinic acid, 50 mg; choline 300 mg; vitamin B12, 15 mcg; vitamin B6, 0.12 mg; thiamine, 1.5 mg; folic acid, 1.00 mg; d-biotin, 0.10 mg.
[9]Mineral supplement provides per kilogram of diet: Trace mineral (milligrams per kilogram of diet): Mn, 100; Zn, 70; Fe 50; Cu 10; Iodine 1; Se, 0.30; antioxidant 50.
[10]Exogenous enzyme.

kg), and β-glucanase (3,000,000 units/kg) from *Trichoderma reesei*, *Trichoderma viride*, and *Aspergillus aculeatus*. It also contains protease (3,000,000 units/kg) and phytase (1,000,000 units/kg) from *Bacillus subtilis* and *Aspergillus oryzae*, and α-amylase (2,000,000 units/kg) from *Bacillus amyloliquefaciens*. Birds were fed a balanced commercial layer diet for 2 weeks prior to commencement of the study to allow them to adapt and reach a standard level of egg production. Mean egg production prior to the start of the experiment, which lasted 10 weeks, was 67.1%.

2.2 | Measurements

During the experiment, feed intake, egg mass, egg production, feed conversion ratio, and mortality were measured. The body weight of hens was recorded at the beginning and at the end of the experiment. Feed intake was measured weekly by subtracting the left-over feed from the quantity originally supplied to the animals. Eggs from individual layers were collected daily and weighed. The egg production and

TABLE 2 Chemical composition of duckweed.

Chemical composition (% on dry weight basis, unless otherwise stated)	
Moisture	9.2
Crude protein (CP)	22.55
Ether extract (EE)	2.85
Crude fiber (CF)	8.31
Neutral detergent fiber (NDF)	22.41
Acid detergent fiber (ADF)	17.98
Gross energy (GE) (kcal/kg DM)	2957.7
Ash	19.58
Nitrogen free extract (NFE)[1]	46.76
Non fibrous carbohydrates (NFC)[2]	24.35
Mineral concentrations (%)	
Calcium (Ca)	1.42
Phosphorus (P)	0.55
Magnesium (Mg)	0.48
Potassium (K)	4.57
Contents of carotenoid compounds (μg/g)	
Total carotenoids	100.68
Total xanthophylls	89.54

[1]Nitrogen free extract (NFE) = 100 − (CP + EE + CF + Ash).
[2]Non-fibrous carbohydrates (NFC) = 100 − (CP + EE + NDF + Ash).

feed efficiency were calculated as rate of production per hen per day and feed intake/egg mass, respectively. Before egg quality measurements were taken in the laboratory, the eggs were stored overnight at room temperature. Egg quality parameters (shell thickness, shell strength, yolk index, Haugh unit, and yolk color) were assessed 24 h after egg collection. Shell strength was measured by using a Digital Egg Shell Force Gauge (Wagner Instruments, USA). Shell thickness was measured at three locations (air cell, equator, and sharp end) using a digital micrometer (Mitutoyo, 0.01 mm, Japan). The yolk height (YH) was measured by a tripod micrometer (Mitutoyo, 0.01 mm, Japan) and the yolk diameter (D) by dividers. The yolk index was calculated by the formula [Yolk index = (YH/D) × 100]. Haugh units were calculated with the following formula, where AH is albumen height and EW is egg weight (Haugh unit = 100 log AH + 7.57–1.7 $EW^{0.37}$). Yolk color was evaluated with a Roche Yolk Color Fan (Vuilleumier, 1969), which included 15 colorimetric grades that had varying intensity of yellow. The colors of yolk were scored and expressed in grades. Thirty-six eggs from each treatment were randomly taken at the end of the experiment for egg yolk cholesterol analyses. One gram of each egg yolk was homogenized with 15 mL of chloroform-methanol (2:1 by volume), sonicated, and filtered as described (Baghban-Kanani et al., 2018). At the end of the experiment, blood samples (five hens randomly selected from each replicate) were taken from a wing vein into additive-free blood tubes. Serum was obtained following centrifugation at 4000×*g* for 10 min at 20°C. Serum was separated to determine antioxidant capacity, concentrations of malondialdehyde (MDA), triglyceride, and cholesterol. Liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using a commercial diagnostic kit (using the enzyme method). Then the obtained samples were stored at −80°C until further analysis.

2.3 | Antioxidant status parameters

Antioxidant capacity, including total antioxidant capacity (TAC), total superoxide dismutase (TSOD) and glutathione peroxidase (GSH-Px) activities were determined in serum samples using RANDOX kits (Germany) according to the manufacturer's instruction. Serum TSOD activity was assayed by the xanthine oxidase method (Winterbourn et al., 1975), which monitors the degree of inhibition of nitroblue tetrazolium reduction by O_2 generated by xanthine and xanthine oxidase. The absorbance was read at 550 nm using a spectrophotometer (UV-1201; Shimadzu, Japan). Serum lipid peroxidation was determined using the method of Kei (1978) and Yagi (1984) but with 1,1,3,3-tetraethoxypropane as the standard. This method is based on the reaction between MDA (an aldehyde lipid peroxidation product) and thiobarbituric acid (TBA). MDA forms a pink-colored complex with TBA. The absorbance of solution containing the complex was measured at 532 nm using a spectrophotometer (UV-1201; Shimadzu, Japan). The serum lipoperoxide values were expressed in terms of MDA as nmol/mL plasma (Baghban-Kanani et al., 2019; Feshanghchi et al., 2022).

2.4 | Statistical analysis

The data from this experiment were subjected to two-factor analysis of variance with six treatments and eight replicates and nine hens in each replicate. The General Linear Model (GLM) procedure of SAS software was used for this purpose. Individual means were compared by Tukey's test, with significance assumed at a 5% probability or less.

3 | RESULTS

The effects of experimental diets on performance and egg parameters of laying hens are presented in Table 3. No significant differences in egg production, egg weight, egg mass/d and food conversion ratio (FCR) were observed between dietary treatments ($P > 0.05$). Hens fed the diets with enzymes had increased feed intake ($P < 0.05$).

Shell thickness, shell strength, shape index and Haugh units were not significantly affected by either inclusion of duckweed or enzymes (Table 4). However, the hens fed diets containing either 7.5% or 15% duckweed produced the highest yolk color score ($P = 0.01$). There were also trends for shell thickness ($P = 0.08$), shell strength ($P = 0.09$), and Haugh unit ($P = 0.09$) to be increased by inclusion of

TABLE 3 Effect of experimental diets (0%, 7.5%, and 15% duckweed and 0 or 500 g/t enzymes) on performance and egg parameters of laying hens.

Treatment effects	Feed consumption (g/d/bird)	Egg production (%)	Egg weight (g)	Egg mass (g/d/bird)	FCR (kg feed:kg egg)
Duckweed, %					
0	108.46	70.85	58.74	41.49	2.61
7.5	108.52	70.68	58.76	41.53	2.61
15	107.85	70.03	58.55	41.00	2.63
SEM	0.29	0.57	0.45	0.44	0.03
Enzymes, g/t					
0	107.92	70.10	58.68	41.08	2.62
500	108.64	70.94	58.65	41.61	2.61
SEM	0.23	0.47	0.36	0.36	0.02
Duckweed × enzymes					
0 × 0	108.12	70.71	58.74	41.52	2.60
0 × 500	108.80	71.00	58.42	41.46	2.63
7.5 × 0	107.94	69.86	58.61	40.95	2.63
7.5 × 500	109.11	71.49	58.91	42.12	2.59
15 × 0	107.69	69.72	58.46	40.77	2.64
15 × 500	108.00	70.33	58.63	41.23	2.62
SEM	0.41	0.81	0.63	0.62	0.04
P values					
Duckweed	0.20	0.56	0.93	0.64	0.90
Enzymes	0.03	0.21	0.92	0.31	0.72
Duckweed × enzymes	0.13	0.63	0.99	0.71	0.96

TABLE 4 Effect of experimental diets (0%, 7.5%, and 15% duckweed and 0 or 500 g/t enzymes) on egg quality.

Treatment effects	Shell thickness (mm)	Eggshell strength (kg/cm^2)	Shape index (%)	Haugh unit	Yolk color score
Duckweed, %					
0	0.41	3.60	62.38	79.98	8.56^b
7.5	0.42	3.65	62.42	80.52	10.06^a
15	0.42	3.66	62.74	80.22	11.43^a
SEM	0.004	0.03	0.21	0.37	0.47
Enzymes, g/t					
0	0.40	3.61	62.51	79.85	8.48
500	0.42	3.67	62.53	80.64	8.88
SEM	0.003	0.02	0.17	0.30	0.39
Duckweed × enzymes					
0 × 0	0.40	3.58	62.35	79.60	8.62
0 × 500	0.41	3.62	62.43	80.39	8.50
7.5 × 0	0.40	3.61	62.45	79.91	10.12
7.5 × 500	0.42	3.70	62.39	81.12	10.00
15 × 0	0.41	3.64	62.72	80.05	11.37
15 × 500	0.42	3.69	62.76	80.40	11.50
SEM	0.006	0.04	0.30	0.52	0.67
P values					
Duckweed	0.62	0.26	0.46	0.61	0.01
Enzymes	0.08	0.09	0.93	0.09	0.94
Duckweed × enzymes	0.09	0.30	0.89	0.43	0.07

Note: Means within a column with different superscripts differ significantly ($P < 0.05$).

enzymes in the diet. The effects of dietary treatments on egg weight, yolk weight, yolk cholesterol, and plasma cholesterol were not significant ($P > 0.05$) (Table 5). The plasma AST, ALT, and triglyceride of the laying hens are shown in Table 6. The birds fed 7.5% and 15% duckweed had significantly lower activity of AST ($P = 0.04$) and ALT ($P = 0.02$) in comparison to the control group. No significant difference in triglyceride was observed among treatment groups. There were no significant treatment effects on serum GSH-Px activity, MDA, TSOD, and total antioxidant capacity (T-AOC, $P > 0.05$) (Table 7).

There was no mortality.

4 | DISCUSSION

The addition of enzymes to the basal diet slightly increased feed consumption by hens over that of the negative control group. Poultry do not produce enzymes capable of digesting dietary non-starch polysaccharides (NSPs). Enzymes in the feed industry have mostly been used for poultry to neutralize the effects of non-starch polysaccharides in cereals and meals. These antinutritive carbohydrates are undesirable as they reduce digestion and absorption of all nutrients in the diet, especially fat and protein. Enzyme inclusion can improve the performance of hens by improving utilization of fibrous material (Khan et al., 2011; Olukosi et al., 2015). Adeola and Cowieson (2011) reported that a single enzyme or multiple enzymes effectively lowered the anti-nutritional factors present in the diet, leading to improved performance in laying hens. Commercial enzyme products may have more benefit in diets containing high concentrations of fiber (Kocher et al., 2002). Results from this study were consistent with some previous literature, which reported that the addition of a single enzyme or multiple enzymes increased feed consumption of laying hens (Lee et al., 2014). However, other studies have produced contradictory results—Torki et al. (2016) reported that the addition of β-glucanase and xylanase or β mannose-containing enzymes reduced feed consumption, and another study reported that the addition of protease to protein-restricted diets did not affect feed consumption (Joshua, 2016). We did not conduct a digestibility study, but the increase in feed intake was small, less than 1%, and therefore not reflected in increased egg production. As the enzymes probably do not have a particular taste, they are unlikely to increase the attractiveness of the feed to the birds. The discrepancy between research reports is more likely affected by either the type and inclusion rate of enzymes or factors that change the efficiency of the enzyme function,

TABLE 5 The effects of experimental diets (0%, 7.5%, and 15% duckweed and 0 or 500 g/t enzymes) on yolk weight, yolk cholesterol, and plasma cholesterol.

Treatment effects	Yolk weight (g)	Yolk cholesterol (mg/yolk)	Yolk cholesterol (mg/g of yolk)	Plasma cholesterol (mg/dL)
Duckweed %				
0	18.52	231.07	12.47	90.50
7.5	18.71	225.48	12.03	87.59
15	18.67	221.31	11.84	85.86
SEM	0.18	5.45	0.25	2.13
Enzymes[1] g/t				
0	18.60	223.95	12.03	88.66
500	18.68	227.96	12.20	87.31
SEM	0.14	4.45	0.20	1.74
Duckweed × enzymes				
0 × 0	18.59	232.97	12.53	93.71
0 × 500	18.46	229.18	12.41	87.30
7.5 × 0	18.58	221.77	11.91	86.54
7.5 × 500	18.85	229.20	12.15	88.63
15 × 0	18.62	217.12	11.66	85.72
15 × 500	18.73	225.52	12.03	86.00
SEM	0.25	7.71	0.36	3.06
P values				
Duckweed	0.74	0.45	0.21	0.13
Enzymes	0.70	0.52	0.58	0.58
Duckweed × enzymes	0.73	0.81	0.77	0.27

[1]Xylanase (20,000,000 units/kg), cellulase (5,000,000 units/kg), β-glucanase (3,000,000 units/kg), protease (3,000,000 units/kg), phytase (1,000,000 units/kg) and α-amylase (2,000,000 units/kg).

TABLE 6 Effect of experimental diets (0%, 7.5%, and 15% duckweed and 0 or 500 g/t enzymes) on aspartate aminotransferase (AST), alanine aminotransferase (ALT), and triglycerides of laying hens.

Treatment effects	AST (U/L)	ALT (U/L)	Triglyceride (mg/dL)
Duckweed %			
0	193.14[a]	5.54[a]	103.95
7.5	190.72[b]	4.85[b]	101.91
15	190.51[b]	4.78[b]	101.19
SEM	0.81	0.21	1.75
Enzymes[1] g/t			
0	192.07	5.18	102.47
500	190.84	4.94	102.23
SEM	0.66	0.17	1.43
Duckweed × enzymes			
0 × 0	194.32	5.83	105.04
0 × 500	191.96	5.26	102.87
7.5 × 0	191.07	4.90	101.39
7.5 × 500	190.37	4.80	102.44
15 × 0	190.82	4.82	100.99
15 × 500	190.19	4.75	101.39
SEM	1.14	0.30	2.47
P values			
Duckweed	0.04	0.02	0.51
Enzymes	0.19	0.32	0.90
Duckweed × enzymes	0.13	0.10	0.87

Note: Means within a column with different superscripts differ significantly ($P < 0.05$).
[1]Xylanase (20,000,000 units/kg), cellulase (5,000,000 units/kg), β-glucanase (3,000,000 units/kg), protease (3,000,000 units/kg), phytase (1,000,000 units/kg) and α-amylase (2,000,000 units/kg).

such as age of animals, dietary raw material types, or the environmental and climatic conditions used in the studies (Hosseintabar-Ghasemabad et al., 2020; Polat & Denli, 2019). Baghban-Kanani et al. (2018) found that the exogenous enzyme phytase increased food conversion efficiency and egg-shell strength, with the latter tending to be increased in this study, presumably reflecting the tendency for eggshell to be thicker when the enzymes were included in the diet. Another study found that the Haugh unit increased, but there were no effects on eggshell strength or thickness, when a non-starch polysaccharide degrading multi-enzyme was included in the diet of laying hens (Sun & Kim, 2019). Multi-enzyme additives need to be tailored to the diet composition. The increase in Haugh unit is speculated to be because of enhanced absorption of nutrients, following a reduction in the antinutritive effects of non-starch polysaccharides (Sun & Kim, 2019). Other studies found that supplementation of the diet of laying hens with xylanase and β-glucanase did not affect the bacterial phyla in the ileum or caecum (Munyaka et al., 2016), nor did it improve digestibility of the diet (Lei et al., 2018; Min et al., 2011). However, Liu and Kim (2017) found that xylanase inclusion increased Lactobacilli and reduced *Escherichia coli* in broilers. Our inclusion of xylanase, cellulase, and β-glucanase in the diet needs further testing, in relation to the rate of inclusion, formulation of the enzyme additive, and timing of provision to the birds.

Akter et al. (2011) used duckweed (*L. minor*) in laying hen diets at a level of 15% and reported that feed consumption was affected by duckweed, which is in contrast to the present experiment. This result might be because of difference in crude fiber (8.31 vs 4.26%), in addition to age and purpose of birds used for experimentation. The increased amount of fiber can lead to an increased amount of NSP (Kiarie et al., 2014). In our study, except for yolk color score, the other internal quality characteristics of eggs did not vary significantly. The color of egg yolk is very important for consumers' satisfaction, and consumers usually prefer yolk color ranging from golden yellow to orange (Hasin et al., 2006). Because of the importance of color, studies related to the pigmentation properties of different carotenoid sources are less focused on increasing the carotenoid content in egg yolk. Therefore, comparison of the results of the present study with previous studies is somewhat difficult, especially in the case of less studied plants. Egg yolk color is commonly determined by the color scale, with Iranian consumers preferring coloration between 10 and 14 (Jebelli Javan et al., 2021). In the present study, only eggs from duckweed-supplemented diets achieved values in the acceptable range for Iranian consumers. Significant improvement of yolk color with increasing level of duckweed in the diet indicates that duckweed contains sufficient carotenoids and xanthophyll pigment. Akter et al. (2011) reported increased yolk color score when duckweed (15%) was included in the diet. The higher the intake of diets containing carotenoids by birds, the greater the deposition of pigment in the egg yolk and the intensity of the coloration (Garcia et al., 2002). Carotenoids are efficiently deposited in the egg yolk when included in layer diets (Selim et al., 2018; Tufarelli et al., 2021, 2022; Zahroojian et al., 2013).

The layers receiving duckweed had significantly reduced serum AST and ALT activity. Rajput et al. (2013) reported that carotenoids were effective in preventing liver damage and produced a concomitant reduction in plasma AST activity. This beneficial effect is generally associated with the potent antioxidant properties of carotenoids, though other possible mechanisms of action exist, including the generation of vitamin A from pro-vitamin A carotenoids, and the emerging role of apocarotenoids in modulating hepatic signaling and the pathogenesis of liver abnormality. There are no extant published data on the effects of duckweed on laying hen serum AST and ALT activity.

One of the potential advantages of inclusion of duckweed in the diet of laying hens is its high growth rate, providing potential for land released to be used to further enhance carbon dioxide capture by trees. Assuming a yield of 3.3 t/ha/year (FAOSTAT, 2022) for wheat grains, and with wheat flour containing about 33% gluten (Kaushik et al., 2015), soyabean yields are less than this, usually about 2 t/ha/year (Ksiezak & Bojarszczuk, 2022). Compared with yields for duckweed ranging from 10 to 30 t/ha/year (Baek et al., 2021), it is clear that the potential exists for considerable amounts of land to be released from poultry production for other purposes. Changes in infrastructure associated with duckweed production would be required.

TABLE 7 Effect of experimental diets (0%, 7.5%, and 15% duckweed and 0 or 500 g/t enzymes) on serum glutathione peroxidase (GSH-Px) activity, malondialdehyde (MDA), total superoxide dismutase (TSOD), and total antioxidant capacity (T-AOC).

Treatment effects	GSH-Px (U/mL)	MDA (nmol/mL)	TSOD (U/mL)	TAC (U/mL)
Duckweed %				
0	842.24	8.03	186.2	9.32
7.5	845.01	7.57	190.3	9.94
15	844.99	7.55	189.9	9.97
SEM	1.65	0.19	1.74	0.12
Enzymes[1] g/t				
0	843.5	7.79	188.0	9.75
500	844.7	7.64	189.6	9.93
SEM	1.34	0.15	1.42	0.09
Duckweed × enzymes				
0 × 0	840.7	8.19	185.4	9.57
0 × 500	843.8	7.87	187.0	9.70
7.5 × 0	844.9	7.57	189.7	9.84
7.5 × 500	845.1	7.48	190.8	10.04
15 × 0	844.8	7.62	188.7	9.83
15 × 500	845.2	7.28	191.0	10.07
SEM	2.33	0.27	2.46	0.17
P values				
Duckweed	0.40	0.15	0.20	0.12
Enzymes	0.53	0.49	0.41	0.18
Duckweed × enzymes	0.74	0.46	0.55	0.30

[1]Xylanase (20,000,000 units/kg), cellulase (5,000,000 units/kg), β-glucanase (3,000,000 units/kg), protease (3,000,000 units/kg), phytase (1,000,000 units/kg) and α-amylase (2,000,000 units/kg).

5 | CONCLUSIONS

There were no detrimental effects of replacing wheat germ meal and soyabean meal with duckweed, at up to 15% of the diet, on feed conversion ratio, egg production or egg quality, or antioxidant status of the birds. There was evidence of beneficial effects on egg yolk color and liver enzyme concentrations. The use of exogenous enzymes in the diet did not enhance the benefits of including duckweed in the diet, but it increased feed intake. There are likely to be significant benefits of inclusion of duckweed in the diet of laying hens on egg output per hectare, because plant growth rate is high, and this study found no detrimental effects on production or product quality.

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CONFLICT OF INTEREST STATEMENT

The authors declare that Payam Baghban-Kanani is the CEO and scientist at the Noavaran Arka Tejarat Kabodan Company, and Babak Hosseintabar-Ghasemabad is affiliated to the Darvash Giah Khazar Medicinal Herbs Complex Company. Other authors declare no conflicts of interest.

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