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Dietary effects of duckweed on performance, carcass characteristics, hematology, immunity, sensory traits, and fatty acid profile of meat in broilers

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Abstract

We have evaluated the effects of duckweed (*Lemna minor*) diets on production performance, carcass characteristics, biochemical parameters and blood antioxidant status, immune system, sensory and taste traits and breast fatty acid profile in broiler chickens during three periods (starter, grower, and finisher) and the entire rearing period, using 150 broiler chickens of the Ross 308 strain. This included 3 levels of chickweed at 0, 3, and 4% with 5 replications and 10 birds in each replication, in a completely randomized design. The results showed that the duckweed did not have any negative effect on production performance, and the weight of the broilers in the waterweed groups was similar to that of the control group (P>0.05). The results of the immune system trait analysis showed that for all traits except Fabricius weight, there was no significant difference between the treatment groups. However, some significant differences were observed between the waterweed groups and control group: the smell of the meat was affected after duckweed feeding, increasing with increasing duckweed percentage (P<0.05). Overall, we have demonstrated that duckweed can help improve blood and antioxidant parameters while maintaining performance, although it affected the smell of the meat. However, we conclude that it has the potential to be used in functional diets for broiler chickens.

Keywords: Blood parameters and antioxidants, Broiler chickens, Duckweed, Meat smell

Introduction

The scarcity and increasing cost of poultry feed ingredients, especially high-quality protein sources, feed energy sources, and natural and synthetic additives, have increased the interest of poultry nutritionists in using new complementary and alternative sources that are high-quality, accessible, and economically viable in diet formulation[1-3]. The use of new edible plants that can meet the energy and protein requirements in poultry nutrition is always an important and strategic option in new studies and food security[4-5]. It is also estimated that about 30% of climate change is caused by the food industry, and about 70% of global freshwater withdrawals are used for agricultural and livestock production[3,6]. In addition the global food supply shortage will become more critical in the future as the world population increases and agricultural land becomes increasingly scarce[7-9]. Facing these challenges has led to increasing attention to new food plants, such as duckweed[10-12]. Duckweed will

grow in clean or very dirty water in all seasons of the year and has subsequently proven its amazing purification power for the environment, reducing sewage and heavy metal pollution in its cultivation environment. This is a potential and valuable advantage for helping the environment. Today, the unique characteristics of duckweed go beyond serving the Earth and human life and have been considered one of the most valuable options by the NASA Air and Space Agency for space life. This plant can be cultivated in a stagnant water environment in the place where astronauts dispose of metabolic waste and, while purifying sewage and turning it into washable water, it continuously and without interruption produces oxygen and protein plant food for them in the environment outside the earth's atmosphere. As a rich source of vegetable protein, but low in carbohydrates, duckweed is also acceptable in terms of amino acid profile, and its fatty acid profile and their ratios are also ideal, and its mineral, vitamin and bioactive compounds indicate its beneficial effects in nutrition[13-15]. Based on botanical studies, a type of specimen found in calm waters has characteristics that are: circular-elliptical or ovoid-shaped, symmetrical or slightly symmetrical, with flat or almost convex upper and lower surfaces, and with green, trivetted and a short, often persistent, blunt-pointed cap with a single curved ovule. Its fruit is elliptical with a compressed dorsa-ventral surface without wings and smooth seeds[16]. In their review, Xu et al.[15] suggested that exploring new agricultural and food systems for the duckweed plant has promising potential in human and livestock food, which could bring many benefits for food security and health, as duckweed species can contain protein content between 20 and 40% and provide all essential amino acids for the growth and development. In addition, duckweed contains moderate amounts of carbohydrates, starch, cellulose, hemicellulose, and pectin, and has been reported to be rich in minerals, vitamins, and valuable phytochemicals, especially lutein and beta-carotene. The chemical composition of this plant varies among species and can also be affected by cultivation conditions[15,17]. The high nutrient content of duckweed has been reported to vary widely, depending on the ammonium and nitrate levels of the culture medium and affects the percentage of crude protein, which can be up to 44% of the dry matter[1]and , the distribution of amino acids and their ratios are almost independent of environmental influences[19]. The development of a supply chain of duckweed as a new staple food is necessary and is on the agenda of many researchers around the world. Reviewing the scientific literature indicates the use of duckweed in poultry nutrition has been limited, and given its potential in many countries and the global challenges of climate change, the need for the use of this plant in poultry nutrition could become more important [20]. There have, however, been a few reports on supplementation with duckweek. Ahammad et al.[21], demonstrated that the best performance was observed at levels of 3 and 6% by replacing *Lemna minor* with sesame in the diet of broiler chickens. Zaffer et al.[22] observed the highest daily weight gain by supplementing duckweed diets with *Lemna minor*. Kabir et al.[23] in a study to investigate the effects of *Lemna minor* in broiler chickens, levels of 0, 4, 8 and 12% duckweed meal (DWM) for 42 days showed that body weight, feed intake, feed efficiency, protein efficiency, energy efficiency and profitability decreased linearly, and no significant superiority was observed compared to the control group. Baghban-Kanani et al.[24] observed an increase in egg yolk color, liver protection, and improved flock health by including *Lemna minor* in the diet of laying hens at levels of 7.5 and 15%, without having any detrimental effects on production performance, and they considered it an alternative to animal sources [25]. There has been, however, no comprehensive study about the effects of various doses of *Lemna minor* feeding to broilers, which

considers all aspects of meat production in terms of both quantity and quality. Therefore, we decided to evaluate the dietary effects of different levels of duckweed (*Lemna minor*) on production performance, carcass characteristics, immune system, biochemical parameters and blood antioxidant status, as well as fatty acid profile, and sensory and taste traits of breast meat in broiler chickens to provide more information about selecting the optimum levels of use of this dietary source in the formulation of functional diets.

Materials and Methods

All activities of the present research were carried out with the ethical code IR.IAU.RASHT.REC.1402.020 by implementing the welfare guidelines appropriate to the Laboratory Animal Ethics Committee of Islamic Azad University, Rasht Branch, Iran. Also, all methods were performed in accordance with the relevant guidelines and regulations. The study is reported in accordance with ARRIVE guidelines. All experimental protocols were approved by the Rasht Branch, Islamic Azad University.
Informed consent was obtained from all subjects and/or their legal guardians.

Management and Breeding

Using 150 Ross 308 broiler chickens (https://aviagen.com/eu/brands/ross/products/ross-308) with similar average weight (41±1 g) which included 3 treatments, 5 replicates and 10 birds per replicate, biological experiments were started in a completely randomized design during three periods, respectively, starter (1-11 d), grower (12-21 d) and finisher (22-42 d) in the Maaf Research and Development Farm belonging to Sepid Makian Company (Somehsara- Guilan- Iran). The levels examined included 0, 3, and 4% of the test ingredient. Each experimental unit for each replicate was in cages of dimensions 1.2□1.5□2 m. Vaccination, light, temperature, and ventilation management were based on the recommendations of the breeder's reference and in accordance with the guide catalog, and access to drinking water and food was ad libitum.

Test Ingredient and Experimental Diets

The powdered entire plant of *Lemna minor* species from Darvash Giah Khazar medicinal herbs complex company (Ltf) (Rasht- Guilan- Iran) was used as the test ingredient in the present study, which is abbreviated as LM in this report. LM was prepared and ground according to the protocol of Ifie et al.[26] in a temporary pond located in the town of Kishestan, Somehsara, Guilan, Iran. Due to the specific physiological characteristics of the water duckweed, colonies are formed very quickly in short intervals (every 2 days). The crop was harvested during the growth stages of the plant and before full maturity, when the plant was green and bright in color, and the total plant organ (leaves, stem, and roots) was used according to the protocol mentioned in the present study.
Crude protein, ether extract, starch and sugar in LM were measured in Viromed laboratory (Rasht-Guilan- Iran) according to the Institute of Standards and Industrial Research of Iran (ISIR) method 10703-1, 10700, In House and 8986-2, respectively, and phytosterol and tocopherol compounds were measured according to the standard method 9760 in Tekno Azma laboratory (Tehran-Iran) (Table 1). Metabolizable energy corrected to zero nitrogen balance (AME_n) of the test ingredient was obtained

based on the equation proposed by the World Poultry Science Association (WPSA) as follows: AME_n (kcal/kg DM)= 15.51 (Crude Protein) + 34.31 (Ether Extract) + 16.51 (Strach) + 13.01 (Sugar). The mash diets of the experimental treatments were formulated based on corn and soybean meal using Amino Feed 5.0 software from Evonik (Table 2).

Table 1. Analysis of phytosterol, tocopherol, crude protein, crude fat, sugar, and starch compounds of *Lemna minor*

Items	**Values**
Total Sterols (mg/kg)	332.65
Alpha-Tocopherol (ppm)	1116.42
Gamma-Tocopherol (ppm)	119.91
Delta-Tocopherol (ppm)	63.29
Total Tocopherol (ppm)	1299.62
Campesterol (%)	3.34
Cholesterol (%)	1.50
Stigma Sterol (%)	22.46
Beta-Sitosterol (%)	60.19
Delta-5-Avena Sterol (%)	4.53
Delta-7- Avena Sterol (%)	5.65
Delta-7-Stigma Stanol (%)	2.60
Moisture and Volatile Matter (%)	3.83
Total Styrene (mg/kg)	0.04
Crude protein (g/kg)	104.70
Ether extract (g/kg)	12.50
Starch (g/kg)	9.60
Sugar (g/kg)	10.60

Table 2. Assay diets (AD) and calculation of nutrients of treatments

Items	**Starter (1-11 d)**			**Grower (12-21 d)**			**Finisher (22-42 d)**		
Ingredients (%)	**AD_1 (T_1)**	**AD_2 (T_2)**	**AD_3 (T_3)**	**AD_1 (T_1)**	**AD_2 (T_2)**	**AD_3 (T_3)**	**AD_1 (T_1)**	**AD_2 (T_2)**	**AD_3 (T_3)**
Corn	54.11	50.85	49.19	61.25	58.23	56.45	67.45	63.89	62.11
LM	0.00	3.00	4.00	0.00	3.00	4.00	0.00	3.00	4.00

Soybean meal 44%	40.45	40.75	40.93	33.49	33.80	33.99	27.48	27.82	28.02
Vegetable oil	1.48	2.56	3.12	1.38	2.46	3.06	1.89	3.08	3.67
Lysine hydrochloride	0.21	0.20	0.20	0.21	0.20	0.20	0.20	0.20	0.19
Methionine	0.35	0.34	0.34	0.28	0.27	0.27	0.23	0.22	0.21
Valine	0.04	0.04	0.04	0.02	0.03	0.03	0.01	0.01	0.01
Threonine	0.11	0.11	0.11	0.09	0.09	0.09	0.07	0.07	0.07
Calcium carbonate	1.19	0.06	0.00	1.07	0.00	0.00	0.92	0.00	0.00
Vit and Min Premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.01	0.05	0.05	0.07	0.07	0.07	0.07	0.07	0.07
Monocalcium phosphate	1.10	1.08	1.07	0.91	0.89	0.88	0.69	0.67	0.67
Sodium chloride	0.21	0.20	0.20	0.19	0.19	0.20	0.19	0.19	0.19
Sodium bicarbonate	0.24	0.25	0.25	0.27	0.26	0.26	0.27	0.27	0.26
Phytase	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Calculated nutrient compositions (%)									
ME (kcal/kg)	2857	2857	2857	2933	2933	2933	3038	3038	3038
Crude protein	22.72	22.92	22.98	20.08	20.27	20.33	17.76	17.95	18.01
Lysine	1.24	1.25	1.25	1.10	1.10	1.10	0.96	0.96	0.96
Met + Cys	0.93	0.93	0.93	0.81	0.81	0.81	0.71	0.71	0.71
Arginine	1.39	1.40	1.40	1.20	1.21	1.21	1.04	1.05	1.05
Threonine	0.83	0.83	0.83	0.72	0.72	0.72	0.65	0.65	0.65
Isoleucine	0.96	0.85	0.85	0.75	0.75	0.74	0.65	0.65	0.65
Valine	0.94	0.96	0.96	0.85	0.85	0.85	0.74	0.74	0.74
Tryptophan	0.25	0.24	0.25	0.21	0.21	0.21	0.18	0.18	0.18
Calcium	0.95	0.94	0.94	0.85	0.87	0.85	0.74	0.81	0.95
Sodium	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Potassium	1.00	1.00	1.00	0.88	0.88	0.88	0.78	0.78	0.78
Chlorine	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Av. Phosphorus	0.47	0.47	0.47	0.42	0.42	0.42	0.37	0.37	0.37
Choline (g/kg)	1.47	1.69	1.69	1.60	1.60	1.60	1.48	1.49	1.49
Linoleic acid	1.99	3.00	3.43	2.02	3.04	3.48	2.33	3.40	3.00
Ether extract	4.16	5.14	5.64	4.22	5.21	5.74	4.85	5.92	6.45
Ash	6.13	6.18	6.51	5.47	5.58	5.96	4.79	5.04	5.43
Starch	34.53	32.52	31.47	39.22	37.16	36.05	42.95	40.72	39.60
Crude fiber	3.10	3.37	3.45	2.94	3.23	3.31	2.81	3.08	3.17

NDF	9.65	10.57	10.83	9.70	10.62	10.87	9.70	10.59	10.84
ADF	4.18	4.90	5.13	3.98	4.70	4.93	3.79	4.51	4.73
DCAB (mEq/kg)	267	267	267	237	237	236	210	210	210

[1] LM: *Lemna minor*

[2] AD_1 (T_1): 0% LM or control group, AD_2 (T_2): 3% LM and AD_3 (T_3): 4% LM.

[3] Vit and Min Premix: The values of vitamins and minerals per kg in the assay diets: Vitamin A, 9000 IU; vitamin E, 18 IU; vitamin D_3, 3000 IU; vitamin K_3, 3 mg; vitamin B_1 (thiamine), 1.8 mg; vitamin B_2 (riboflavin), 6 mg; vitamin B_3 (niacin), 30 mg; vitamin B_6 (pyridoxine), 3 mg; vitamin B_5 (pantothenic acid), 10 mg; vitamin B_9 (folic acid), 1 mg; vitamin B_{12} (cobalamin), 0.012 mg; vitamin H_3, 0.24mg; 500 mg; Choline,100 mg; Mn, 100 mg; Zinc, 80 mg; Iron, 10 mg; Cu, 1 mg.

[4] ME: Metabolizable Energy

[5] Met + Cys: Methionine + Cysteine

[6] Av. phosphorus: Available Phosphorus

[7] NDF: Neutral-Detergent Fibre

[8] ADF: Acid Detergent Fibre

[9] DCAB: Dietary Cation-Anion Balance

Sampling and Experiments

During the three periods of starter, grower, and finisher, as well as the entire period, feed intake (FI), body weight (BW), and feed conversion ratio (FCR) were measured to evaluate the performance of the treatments. At the end of the period after four hours of starvation, two birds from each replicate with an average weight close to the experimental unit chickens were randomly selected, weighed and slaughtered and the weights of the defeathered body, eviscerated carcass, breast, thigh, abdominal fat, gizzard, heart, crop, liver, pancreas, spleen and bursa of Fabricius were measured with a scale having an accuracy of 0.001. Then, from each replicate, three birds were selected, and blood samples were taken from the wing vein using sterile 5 cc syringes. After centrifugation at 3000 rpm, the samples were transported to Viromed Laboratory (Rasht- Guilan- Iran) in commercial kits from Pars Azmoun Company (made in Iran) for evaluation of biochemical and antioxidant parameters of the plasma. The following items were measured using the colorimetric method: glucose, triglycerides, total cholesterol, HDL, LDL, total protein, albumin, uric acid, calcium, iron, TAC, and MDA. The atherogenic index was calculated as a health index based on dividing LDL by HDL values. AST and ALT items were measured using the Enzymatic method, and the phosphorus (P) item was measured using the Photometric method[27-29]. On days 28 and 36 of rearing, sheep red blood cells (SRBC) were injected in an amount of 0.2 cc with a 5% dilution into the breast muscle area of the chickens. Seven days later, on days 35 and 42, wing vein sampling was performed from two birds in each replicate with 3 cc sterile syringes, and the level of antibodies in the samples against SRBC was measured using the hemagglutination method to evaluate Newcastle (NDV) and influenza (AIV) titers, and parameters related to the immune system were evaluated[30]. By sampling breast meat from each replicate and cooking without spices to assess aroma, flavor, odor, crispness, color, and overall desirability, six panels of evaluators (food testers) evaluated the sensory and taste attributes of the meat using a questionnaire with a score from 1 to 10 [31-32]. The fatty acid profile of the meat was determined by selecting a whole chicken breast (without skin) from each treatment and homogenizing it with 100 ml of methanol: chloroform (2:1) solution for

four hours using standard methods. The samples were filtered and mixed again with 25 ml of saturated sodium chloride solution in a decanter funnel. In the next step, the chloroform phase, the fat was filtered using filter paper soaked in anhydrous potassium sulfate. The filtered samples were dried by a rotary evaporator under vacuum, and ten milligrams of extracted fat were mixed with two milliliters of potassium hydroxide, two milliliters of normal methanol, and 7 milliliters of n-hexane and then centrifuged for ten minutes. The samples were left at rest for 5 minutes until the supernatant was separated, and then about one microliter of the supernatant was injected into a gas chromatography device, and the fatty acid values were reported. Based on formulas (1-4), health-promoting indices, including the ratio of omega-6 to omega-3, atherogenic index (AI), thrombogenic index (TI), hypocholesterolemic index (HI), and hypocholesterolemic to hypercholesterolemic ratio, were reported[33].

Formula (1): $AI = (4 \times C14:0) + C16:0/(\Sigma MUFA + \Sigma PUFA - \omega\text{-}6 + \Sigma PUFA - \omega\text{-}3)$

Formula (2): $TI = (C14:0 + C16:0 + C18:0)/0.5 \times \Sigma MUFA + 0.5 \times \Sigma(\omega\text{-}6) + 3 \times \Sigma(\omega\text{-}3) + \Sigma(\omega\text{-}3)/\Sigma(\omega\text{-}6)$

Formula (3): $HI = (C18:1 + C18:2 + C18:3 + C20:3 + C20:4 + C20:5 + C22:4 + C22:6)/(C14:0 + C16)$

Formula (4): $\text{Hypocholesterolemic/Hypercholesterolemic index} = [(C18:1\ \omega\text{-}9 + C18:1\ \omega\text{-}7 + C18:2\ \omega\text{-}6 + C18:3\ \omega\text{-}6 + C18:3\ \omega\text{-}3 + C20:3\ \omega\text{-}6 + C20:4\ \omega\text{-}6 + C20:5\ \omega\text{-}3 + C22:4\ \omega\text{-}6 + C22:5\ \omega\text{-}3 + C22:6\ \omega\text{-}3)/(C14:0 + C16:0)]$

Data were collected in Excel, and the results were analyzed with statistical software (SAS 9.3). Comparisons of treatment means were reported with Duncan's multiple range test. Linear and nonlinear equations were obtained from quadratic, linear, and orthogonal equations, and the turning point of quadratic equations was reported in the "Solver" add-in of Excel.

Results

The results of growth performance up to 11 days of age (feed intake, body weight, and FCR) in Table 3 showed that there was no significant difference between the performance of the control group and the treated groups (different LM levels). Comparison of the control group with the accumulation of different levels of duckweed (Control Vs LM) as well as the linear and quadratic relationship in 11-day performance, was not observed.

Table 3. Performance results of LM-fed treatments in broiler chickens (1-11 d)

Treatments	Feed consumption (g)	Body weight gain (g) at 22-42 d	FCR (g/g)
T_1: 0%	275.20	281.40	0.977
T_2: 3%	272.20	267.60	1.017
T_3: 4%	265.80	264.80	1.004
SEM	7.255	9.197	0.019
P-value	0.655	0.420	0.272
Control Vs. LM	0.499	0.202	0.135

Linear	0.378	0.226	0.295
Quadratic	0.852	0.634	0.216

LM: *Lemna minor*

The results of growth performance from 12 to 21 days of age (for feed intake, body weight, and FCR) in Table 4 showed that there was no significant difference between the performance of the control group and the groups treated with different levels of LM. The comparison of the control group with the different levels of duckweed (Control Vs LM) in the performance from 12 to 21 days of age for feed intake and body weight was significant ($P<0.05$). The linear equation showed that there was a negative and decreasing linear relationship with increasing LM levels for body weight and feed intake parameters. The high coefficient of determination of these equations indicates that the performance of feed intake and body weight is strongly affected by the type of treatment.

Table 4. Performance results of LM-fed treatments in broiler chickens (12-21 d)

Treatments	Feed consumption (g)	Body weight gain (g) at 12-21 d	FCR (g/g)
T_1: 0%	715.30	503.80	1.422
T_2: 3%	681.70	463.40	1.475
T_3: 4%	664.00	453.60	1.465
SEM	14.522	16.243	0.024
P-value	0.076	0.109	0.344
Control Vs. LM	0.034	0.042	0.159
Linear	0.028	0.050	0.270
Quadratic	0.665	0.458	0.337

LM: *Lemna minor*

The results of growth performance from 22 to 42 days of age for feed intake, body weight, and FCR in Table 5 showed that there was no significant difference between the performance of the control group and the treated groups (different LM levels). Also, the contrast effect of the control group with different LM levels, linear and quadratic equations, was not significant.

Table 5. Performance results of LM-fed treatments in broiler chickens (22-42 d)

Treatments	Feed consumption (g)	Body weight gain (g) at 22-42 d	FCR (g/g)

T_1: 0%	3037.40	1973.20	1.540
T_2: 3%	3024.10	1966.90	1.538
T_3: 4%	2918.60	1859.60	1.570
SEM	43.25	38.20	0.01
P-value	0.147	0.101	0.173
Control Vs. LM	0.237	0.224	0.333
Linear	0.076	0.057	0.106
Quadratic	0.401	0.302	0.333

LM: *Lemna minor*

The performance results from 1 to 42 days of age for feed intake, body weight, and FCR in Table 6 showed that there was no significant difference between the performance of the control group and the treated groups (different LM levels) (it was close to significant for feed intake and body weight). The results of the contrast of the control group with different LM levels and also the quadratic equation, showed no significant difference.

The linear equation showed that there was a significant and decreasing linear relationship for the traits of body weight and feed intake with increasing LM levels. The high coefficient of determination of these equations indicates that the performance of feed intake and body weight is strongly affected by the type of treatment.

Feed Intake = -36.306x + 3765.1 (R^2 = 0.740)

Body Weight = -35.077x + 2488.7 (R^2 = 0.749)

Table 6. Performance results of LM-fed treatments in broiler chickens (1-42 d)

Treatments	Feed consumption (g)	Final Body Weight (g)	FCR (g/g)
T_1: 0%	4027.86	2758.40	1.461
T_2: 3%	3978.02	2697.98	1.475
T_3: 4%	3848.40	2578.05	1.492
SEM	51.39	47.17	0.01
P-value	0.082	0.067	0.165
Control Vs. LM	0.107	0.083	0.159
Linear	0.036	0.029	0.075
Quadratic	0.551	0.546	0.721

LM: *Lemna minor*

The results of the carcass weight performance analysis in Table 7 showed that there was no significant difference between the performance of the studied treatments.

Table 7. Results of carcass weight traits of L- fed treatments in broiler chickens

Treatments	Live body (g)	Defeather body (g)	Eviscerated carcass (g)	Breast (g)	Thigh (g)	Abdominal fat (g)	Gizzard (g)	Heart (g)	Crop (g)	Wing (g)
T_1: 0%	2558.0	2134.0	1794.0	658.0	474.0	24.94	38.28	10.82	9.56	136.0
T_2: 3%	2408.0	2012.0	1696.0	648.0	448.0	26.02	35.94	10.76	6.80	132.0
T_3: 4%	2432.0	2026.0	1716.0	650.0	464.0	32.40	38.76	9.10	8.40	134.0
SEM	68.0	55.5	56.7	29.7	11.8	3.547	3.054	0.886	1.458	3.464
P-value	0.283	0.274	0.458	0.969	0.324	0.310	0.787	0.331	0.431	0.723
Control Vs. LM	0.123	0.117	0.229	0.809	0.236	0.345	0.808	0.428	0.294	0.493
Linear	0.214	0.194	0.350	0.852	0.559	0.163	0.913	0.195	0.584	0.690
Quadratic	0.316	0.337	0.412	0.872	0.171	0.553	0.504	0.475	0.246	0.493

LM: *Lemna minor*

The performance results of carcass relative weight traits in Table 8 showed that there was no significant difference between the treatments. The comparison of the control group with the LM groups and the linear and quadratic models for all traits was not significant. However, a relatively significant and upward linear relationship with increasing LM level was observed for the relative weight of the abdominal fat (%) trait ($P<0.01$).

Table 8. Results of the relative weight percentage of carcass traits of treatments fed with LM in broiler chickens

The results of the blood parameter analysis in Table 9 showed that there was a significant difference between the tested treatments in terms of blood parameters in all traits (P<0.01). Also, a significant

Defeather body (%)	Eviscerated carcass (%)	Breast (%)	Thigh (%)	Abdominal fat (%)	Gizzard (%)	Heart (%)	Crop (%)	Wing (%)
83.46	70.12	25.71	18.56	0.978	1.505	0.422	0.369	5.33
83.54	70.34	26.86	18.61	1.093	1.515	0.447	0.219	5.50
83.31	70.57	26.74	19.08	1.329	1.590	0.374	0.274	5.51
0.405	0.668	0.781	0.303	0.143	0.144	0.033	0.074	0.165
0.920	0.892	0.538	0.437	0.250	0.901	0.321	0.375	0.704
0.949	0.686	0.278	0.462	0.209	0.791	0.794	0.199	0.412
0.800	0.640	0.372	0.252	0.109	0.682	0.332	0.380	0.464
0.756	0.998	0.518	0.580	0.737	0.857	0.247	0.277	0.706

difference was observed between the control group with the contrast of LM effects (zero group compared to two levels of 3 and 4 percent LM). In general, the linear and quadratic models was significant between the blood parameters among the tested treatments. For the parameters, glucose, cholesterol, and LDL, the highest to lowest values were observed in the control group, the 4 percent LM group, and the 3 percent LM group, respectively, so that the control group showed a significant difference with both LM groups. Also, the numerical value of these three parameters for the 4% LM group was significantly higher than the 3% LM receiving group (P<0.01). The contrast comparison of these three parameters for the control group was significantly higher than the LM receiving groups. Both the linear and quadratic equations of these traits were significant. The results of statistical analysis for the traits HDL, triglycerides, total protein, and phosphorus showed that the highest to the lowest were related to the 4%, 3% LM, and control treatment, respectively. The 4% LM group

showed a significant difference from both other groups. Also, the numerical value of these three parameters for the 3% LM group was significantly higher than the control group (P<0.01). Also, the contrast comparison of these 4 parameters for the control group was significantly lower than the sum of the groups receiving LM. Both linear and quadratic equations of these traits were significant. For the atherogenic index and MDA parameters, the control treatment showed a numerical value that was significantly higher than each of the LM groups (P<0.01). For uric acid and albumin traits, although there were no significant differences between the LM groups, each of the different LM levels had significantly higher values than the control group (P<0.01), which resulted in a significant contrast between the control group and the additive effect of the LM groups. For iron and TAC parameters, the performance of the 3% LM group was significant compared to the other treatments (P<0.01). The 4% LM group showed a significantly higher amount of iron and TAC (P<0.01). For ALT and AST parameters, the control group showed a higher performance than the LM groups, so that with increasing LM levels, the ALT and AST values showed a significant decreasing trend. Also, blood calcium for the 4% LM group had a significant difference from the other treatment.

Table 9. Results of biochemical and antioxidant blood parameters of LM-fed treatments in broiler chickens

Items	Treatments			SEM	P-value	Control Vs. LM	Linear			Quadratic
	T_1	T_2	T_3				P value	Equal	R^2	
Glucose (mg/dl)	148.6^{a}	128.8^{c}	135.6^{b}	0.698	0.0001	0.0001	0.0001	y= -4.023x + 147	0.693	0.0001
Cholesterol (mg/dl)	144.2^{a}	131.6^{c}	139^{b}	1.169	0.0001	0.0001	0.0084	y= -1.969x + 142.7	0.419	0.0001
HDL (mg/dl)	33.14^{c}	38.28^{b}	40.44^{a}	0.307	0.0001	0.0001	0.0001	y= 1.799x + 33.1	0.99	0.0019
LDL (mg/dl)	84.72^{a}	67.34^{c}	71.92^{b}	1.115	0.0001	0.0001	0.0001	y= -3.798x + 83.5	0.77	0.0001
Atherogenic Index	2.557^{a}	1.759^{b}	1.779^{b}	0.032	0.0001	0.0001	0.0001	y= -0.211x + 2.52	0.93	0.0001
Triglycerides (mg/dl)	84.04^{c}	87.94^{b}	91.28^{a}	0.554	0.0001	0.0001	0.0001	y= 1.692x + 83.8	0.94	0.6869
Uric Acid (mg/dl)	4.916^{b}	5.422^{a}	5.534^{a}	0.039	0.0001	0.0001	0.0001	y= 0.157x + 4.9	0.99	0.0014
Albumin (g/dl)	1.944^{b}	2.14^{a}	2.182^{a}	0.018	0.0001	0.0001	0.0001	y= 0.061x + 1.95	0.99	0.0046

Total Protein (g/dl)	3.956^{c}	4.242^{b}	4.392^{a}	0.035	0.0001	0.0001	0.0001	y= 0.106x + 3.95	0.98	0.1352
Phosphorus (mg/dl)	5.232^{c}	5.892^{b}	6.086^{a}	0.043	0.0001	0.0001	0.0001	y= 0.215x + 5.24	0.99	0.0008
Fe (µg/dl)	124^{c}	136.6^{a}	128^{b}	0.698	0.0001	0.0001	0.0016	y= 1.738x + 125.5	0.32	0.0001
Ca (mg/dl)	10.91^{b}	11.01^{b}	11.19^{a}	0.034	0.000	0.0007	0.0001	y= 0.061x + 10.9	0.83	0.404
ALT (u/l)	18.94^{a}	14.98^{b}	11.98^{c}	0.489	0.0001	0.0001	0.0001	y= -1.643x + 19.1	0.93	0.4388
AST (u/l)	377.4^{a}	303.8^{b}	228.4^{c}	4.639	0.0001	0.0001	0.0001	y= -34.32x + 383.3	0.92	0.8768
MDA (nmol/ml)	20.68^{a}	18.36^{b}	17.98^{b}	0.192	0.0001	0.0001	0.0001	y= -0.698x + 20.6	0.98	0.0014
TAC (nmol/ml)	600.2^{c}	725.8^{a}	698.6^{b}	6.910	0.0001	0.0001	0.0001	y= 28.59x + 608.2	0.81	0.0001

Means with the same letter for each row are not significantly different

LM: *Lemna minor*

T_1: 0%, T_2: 3%, T_3: 4%.

HDL: High density lipoprotein, LDL: Low density lipoprotein, ALT: Alanine amino transferase, AST: Aspartate amino transferase, MDA: Malondialdehyde, Tac: Total antioxidant capacity

The results of the immune system trait analysis in Table 10 showed that for all traits except Fabricius weight, there was no significant difference between the treatment groups. Also, comparing the effect of the control treatment with the contrast of the effects of LM and the linear equation of Fabricius weight with the linear equation 0.2329x + 1.3483, and (R^2 = 0.510), and the quadratic was not significant between any of the traits. For Fabricius' weight, a significant difference was observed between the 4% LM treatment and the other treatments ($P<0.05$).

Table 10. Results of immunological parameters of LM-fed treatments in broiler chickens

Treatments	Antibody Titer (35 day-SRBC test)	Antibody Titer (42day-SRBC test)	Pancreas weight (g)	Relative weight of the pancreas (%)	Spleen weight (g)	Relative weight of the spleen (%)	Fabricius weight (g)	Relative weight of the Fabriciuss (%)

T_1: 0%	2.600	5.400	5.360	0.209	2.600	0.101	1.48^{b}	0.060
T_2: 3%	2.200	3.600	5.440	0.226	2.200	0.091	1.52^{b}	0.063
T_3: 4%	2.800	4.600	4.980	0.205	2.560	0.105	2.68^{a}	0.087
SEM	0.716	0.678	0.352	0.012	0.281	0.010	0.359	0.020
P-value	0.836	0.212	0.626	0.469	0.557	0.617	0.050	0.588
Control Vs. LM	0.911	0.144	0.734	0.714	0.534	0.843	0.185	0.543
Linear	0.847	0.421	0.460	0.783	0.921	0.758	0.036	0.353
Quadratic	0.579	0.118	0.543	0.239	0.291	0.360	0.228	0.681

Means with the same letter for each column are not significantly different.

LM: *Lemna minor*

The results of Table 11 showed that among the taste and sensory traits of meat, only meat smell was affected by the experimental treatments, such that a significant difference was observed between the meat smell of the 3% LM treatment and the control group ($P<0.05$). The results of the contrast of the control group compared to the effects of LM for the meat smell trait were significant. The linear equation for the meat taste parameter was significantly increasing and significant with increasing LM percentage. The above-mentioned significant equations are as follows:

Meat smell= $-0.367x^2 + 1.566x + 6.8$ (The predicted peak meat tenderness point is at 2.14.)

Meat taste= $0.2231x + 6.3462$ ($R^2 = 0.851$)

Table 11. Results of sensory and taste characteristics of breast meat from LM-fed treatments in broiler chickens

Treatments	Meat perfume	Meat taste	Meat smell	Meat crispy	Meat color	Desirability and acceptance of meat
T_1: 0%	7.40	6.40	6.80^{b}	6.80	7.80	8.20
T_2: 3%	8.00	6.80	8.20^{a}	7.60	7.60	8.20
T_3: 4%	6.80	7.40	7.20^{ab}	7.60	8.00	7.60
SEM	0.365	0.294	0.327	0.432	0.365	0.383
P-value	0.108	0.092	0.028	0.351	0.746	0.464
Control Vs. LM	0.990	0.076	0.044	0.156	0.990	0.535

Linear	0.268	0.033	0.404	0.215	0.705	0.290
Quadratic	0.067	0.786	0.011	0.464	0.515	0.535

Means with the same letter for each column are not significantly different.

LM: *Lemna minor*

The fatty acid profile of broiler breast meat is shown in Table 12, and health-related indices were reported in the laboratory for one case from each treatment, statistical analysis and Duncan's comparisons were not performed. Evaluation of indices for judging the health of meat products from future perspective of healthy nutrition can be one of the important goals of applied research in the poultry industry.

Table 12. Results of fatty acids profile and indices of breast meat in LM-fed treatments in broiler chickens

Items	Treatments		
	T_1: 0%	T_2: 3%	T_3: 4%
Caprylic acid (C8:0)	-	0.30	0.06
Capric acid (C10:0)	-	0.29	0.05
Lauric acid (C12:0)	0.15	3.09	0.33
Myristic acid (C14:0)	0.55	1.63	0.71
Pentadecanoic acid (C15:0)	-	0.17	0.12
Palmitic acid (C16:0)	24.35	25.11	24.16
Margaric acid (C17:0)	0.13	0.21	0.14
Stearic acid (C18:0)	6.91	8.98	6.76
Arachidic acid (C20:0)	0.20	-	0.19
Behenic acid (C22:0)	0.61	0.59	0.38
Total SFA	32.90	40.37	32.90
Myristoleic acid (C14:1)	0.55	1.63	0.71
Palmitoleic acid (C16:1)	3.61	1.86	3.56
Elaidic acid (C18:1t)	0.12	0.18	0.10
Oleic acid (C18:1c)	32.76	31.87	32.24
Gondoic acid (C20:1)	0.08	0.02	0.13
Erucic acid (C22:1)	0.05	-	-
Total MUFA	70.01	67.46	69.11
Linoleic acid (C18:2c)	25.38	20.30	26.54
Linolelaidic acid (C18:2c)	25.38	20.30	26.54
Cis-11,14-Eicosadienoic acid (C20:2)	0.26	0.34	0.53
Arachidonic acid (C20:4)	0.17	0.60	0.32
Cervonic acid (22:6)	0.31	-	-

Total PUFA, n-6	51.50	41.54	53.93
Dihomo-ɣ-linolenic acid (C20:3)	2.64	2.68	1.68
Linolenic acid (18:3)	1.84	1.21	1.99
Total PUFA, n-3	4.84	3.89	3.67
Total PUFA, n-6/Total PUFA, n-3	11.49	10.67	14.69
Other	0.11	0.18	0.10
UFA	121.62	109.18	123.14
UFA/SFA	3.69	2.70	3.74
Atherogenic index (AI)	2.39	6.74	3.03
Thrombogenic index (TI)	4493.31	4851.73	4409.94
Hypocholesterolemia index (HI)	2.53	2.11	2.52
Hypocholesterolemic / Hypercholesterolemic	4.88	4.08	4.95

LM: *Lemna minor*

PUFA: polyunsaturated fatty acids, MUFA: monounsaturated fatty acids, UFA: unsaturated fatty acids, SFA: Saturated fatty acids fatty acids

Discussion

We have found that different levels of duckweed supplementation had no negative effect on broiler performance, but had positive effects on vital blood and antioxidant parameters and meat quality, which was not unexpected given the valuable phytosterol and tocopherol active compounds reported in Table 2.

In the study by Zaffer et al.[22], feeding diets containing duckweed (*Lemna minor*) at levels of 5 and 10% with or without enzyme supplementation, their results showed that the blood levels of total protein, albumin, AST and ALT in the waterweed groups was similar to that of the control group, and an increase in glucose was observed in the groups fed duckweed with enzymes. Exogenous enzymes, by improving the digestibility of nutrients and energy, led to an increase in blood glucose. Th differences between the results of Zaffer etl and our own were probably due to the amounts of the duckweek supplement used.

Paguia et al.[34], in their study, examined the effects of feeding dried and fermented duckweed (*Lemna minor*) on free-range chickens at levels of 0 and 10%, and concluded that chickens fed fermented duckweed gained more weight. In contrast, the highest FCR and daily weight gain belonged to the group fed dried duckweed. No differences were , however, observed in blood parameters after feeding duckweed. In addition, Paguia et al. suggested that the superior status of essential amino acids of duckweed compared with most plant proteins and their similarity to animal protein, could improve the performance of chickens in isoenergetic diets containing water duckweed, and that no signs of disease and mortality were observed. The functional effects of their study were largely consistent with the results of our research , but the improvement of blood parameters we found was much better than theirs. . The reason for this difference was probably the processing method of the test ingredient and the animal model used.

Using laying hens, Baghban-Kanani et al.[24] concluded that feed intake increased in the groups fed with duckweed at levels of 0, 7.5, and 15%, but there was no negative effect on FCR. There was also a significant decrease in ALT and AST after duckweed feeding, and the MDA and TAC status remained stable. These researchers attributed this to the strong antioxidant properties of carotenoids present in duckweed and the role of this bioactive compound in the production of vitamin A as a precursor, and the role of apocarotenoids in modulating hepatic signaling and reducing the effects of hepatic pathology pathogenesis. The absence of negative effects on performance and positive effects on the antioxidant properties of bird blood after feeding duckweed in their study were consistent with the findings of our research, and the difference in details was probably due to the difference in the type of poultry used.

In the report of Haustetn et al.[35], the co-feeding of dried duckweed of the *Lemna gibba* species to laying hens at levels of 0, 15, 25 and 40% did not show any negative effects on production performance including FCR and feed consumption, and the use of duckweed at high levels could completely replace soybean and fish meal, although a large increase in the amount of feces was reported. Despite the difference in plant species, level, and animal model, the overall output of the functional results of the mentioned report was consistent with our results. In the study by Demann et al.[36], three species of duckweed, including *Lemna minor* (containing 17.5% protein), *Spirodela polyrhiza* (containing 24.6% protein), and *Lemna obscura* (containing 37% protein), were examined at levels of 50 and 75% on broiler chickens at the age of 21 to 27 days, there was a decrease in feed intake compared to the control group. They suggested that the reason for this was that at high levels of duckweed consumption, the low amino acid digestibility and low AME_n values in the diet hampered the supply of nutrients and subsequently led to a decrease in chicken performance. Given the large difference in the levels of duckweed consumed, the disagreement with the results in the present report was not unexpected. It seems that, unlike laying hens, the use of high levels of duckweed in broiler chickens has limitations that negatively affect performance, and the selection of the levels in the present study seems logical and acceptable. For bird health, especially blood parameters related to health at all levels (low to high), positive effects were promising in all studies, and no negative effects were reported.

A report by Kabir et al.[23] showed that body weight, feed intake, and feed efficiency decreased with increasing amounts of duckweed powder in the diet of Cobb broiler chickens from 8 to 42 days of age at 0, 4, 8, and 12 percent, and the reason for this was the presence of high fiber, tannin, unpleasant fish odor, and bulkiness of the source. In fact, the basis for the suggestions to use lower levels of duckweed in feeding chickens in our research was confirmed.

In the study of Islam et al.[37], feeding duckweed at levels of 3, 6, and 9 percent was associated with a decrease in feed intake and performance in chickens, which conflicted with the results of the present study, and the reason was the difference in the levels studied. It seems that duckweed can have a negative effect on feed intake and performance of chickens at high levels due to their fiber and binding capacity and the presence of water tannins. Therefore, choosing optimal levels of duckweed for

different ages is very important for the performance of broiler chickens, which, while improving the health of the bird, prevents side effects and negative effects on performance.

It should be noted that duckweed is one of the plant sources containing protein with very low phytate, which preserves the potential for phosphorus digestibility, and on the other hand, the richness of this source in terms of bioactive compounds can provide the conditions for using this food source to create consumer health. Given that duckweed contains vitamin E in the form of tocopherol and increasing the content of this valuable compound in functional poultry diets, especially in broilers, the important role of this vitamin as a strong, fat-soluble antioxidant in reducing heat stress and neutralizing free radicals through activating blood antioxidant systems and improving these parameters by this vitamin was clearly evident in the present report, and the approach of utilizing rich sources containing this vitamin in a natural form in diet formulation is acceptable in the poultry industry[38-39]. In general, a review of the scientific literature has also shown that the use of plant compounds to reduce lipid peroxidation, increase the antioxidant activity of serum and breast meat, and increase phenolic content is possible[40-41].

We used a single line (Ross 308) of broilers under controlled experimental conditions; therefore, results need to be validated under commercial farm conditions before broad recommendations are made. Economic implications suggest that inclusion of duckweed could improve feed conversion with limited additional cost, but on-farm trials are required to confirm cost-benefit under variable management systems.

Conclusions

The present study has demonstrated that supplementing poultry diets with duckweed and maintaining performance and will significantly improve health, is promising, although at high levels of consumption, attention should be paid to product quality, especially meat odor, and necessary quality control should be considered to avoid potential concerns in this regard. Feeding this feedstuff will reduce the pressure of consumption of corn and soybean meal and also help the health of the bird with a natural, non-synthetic, and non-antibiotic supplement which shows a promising prospect in poultry nutrition in the future.

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The study was approved by the Research Committee of the Rasht Branch, Islamic Azad University, Rasht, Iran.

Informed Consent to participate (include appropriate statements)
Informed consent was obtained from all subjects and/or their legal guardians.

Informed Consent for publication (include appropriate statements)
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Availability of data and material (data transparency)
Raw data is available from the corresponding author upon reasonable request.

Code availability (software application or custom code)
Not applicable.

Authors' contributions
SS, AS, and MN participated in preparing the manuscript equally, and all authors approved of the manuscript.

References

1. Sońta, M., Rekiel, A. & Batorska, M. Use of duckweed (*Lemna* L.) in sustainable livestock production and aquaculture-a review. Ann. Anim. Sci. **19**, 257-271 (2019).
2. Phillips, C. J. et al. Immunomodulatory effects of natural feed additives for meat chickens. Life **13**, 1287 (2023).
3. Hosseintabar-Ghasemabad, B. et al. The potential of amaranth grain for feeding to poultry. World's Poult. Sci. J. **80**, 481-509 (2024).
4. Seidavi, A. et al. Feed additives and future perspectives. In Sustainable use of feed additives in livestock: novel ways for animal production, 929-961 (Springer, 2023).
5. Biyatmoko, D., Sulaiman, A. & Amilia, P. Duckweed (*Lemna minor*) in feed to increase the carcass and giblet proportion of male alabio ducks. *J. Penelitian Pendidikan IPA*, **11**, 99-105 (2025).
6. Sosa, D. et al. *Lemna minor*: Unlocking the value of this duckweed for the food and feed industry. Foods. **13**, 1435 (2024).

7. Rusoff, L. L., Blakeney, E. W. & Culley, D. D. Duckweeds (Lemnaceae family): a potential source of protein and amino acids. J. Agric. Food Chem. **28**, 848-850 (1980).
8. Hasegawa, T., Sakurai, G., Fujimori, S., Takahashi, K., Hijioka, Y. & Masui, T. Extreme climate events increase risk of global food insecurity and adaptation needs. Nat. Food **2**, 587-595 (2021).
9. Rodríguez, J. H. V. et al. Chemical study of the macrophyte duckweed (*Lemna minor* L.). Rev. Fac. Agron. Univ. Zulia. **42** (2025).
10. Said, M. Z. M. et al. Effect of harvest rate, waste loading, and stocking density on the yield of duckweeds. Proc. World Maricult. Soc. **10**, 769-780 (1979).
11. Duan, P., Chang, Z., Xu, Y., Bai, X., Wang, F. & Zhang, L. Hydrothermal processing of duckweed: effect of reaction conditions on product distribution and composition. Bioresour. Technol. **135**, 710-719 (2013).
12. Vulpe, C. B. et al. A review on uses of *Lemna minor*, a beneficial plant for sustainable water treatments, in relation to bioeconomy aspects. Water. **17**, 1400 (2025).
13. Escobar, C. & Escobar, A. Duckweed: A tiny aquatic plant with enormous potential for bioregenerative life support systems. In 47th International Conference on Environmental Systems, **47**, 281-290 (2017).
14. Appenroth, K. J., Sree, K. S., Böhm, V., Hammann, S., Vetter, W., Leiterer, M., & Jahreis, G. Nutritional value of duckweeds (*Lemnaceae*) as human food. *Food Chem.* **217**, 266-273 (2017).
15. Xu, J. et al. Duckweed (Lemnaceae) for potentially nutritious human food: A review. Food Rev. Int. **39**, 3620-3634 (2023).
16. Mozaffarian, V. Flora of Gilan. Farhang Ilia Publishing, p. 592 (2018).
17. Appenroth, K. J., Sree, K. S., Bog, M., Ecker, J., Seeliger, C., Böhm, V. & Jahreis, G. Nutritional value of the duckweed species of the genus Wolffia (Lemnaceae) as human food. *Frontiers Chem.* **6**, 483 (2018).
18. Devlamynck, R., de Souza, M. F., Michels, E., Sigurnjak, I., Donoso, N., Coudron, C. & Meers, E. Agronomic and environmental performance of *Lemna minor* cultivated on agricultural wastewater Streams-A Practical Approach. *Sustainability*, **13**, 1570 (2021).
19. Rosyadi, R., Agusnimar, A. & Hadi, K. Utilization of chicken manure enriched with Lemna liquid organic fertilizer *Lemna minor* on cell density of Chlorella sp. J. Akuakultur Indones. **24**, 92-101 (2025).
20. Ahammad, M. U., Swapon, M. S. R., Yeasmin, T., Rahman, M. S., & Ali, M. S. Replacement of sesame oil cake by duckweed (*Lemna minor*) in broiler diet. *Pakistan J. Biol. Sci.* **6**, 1450-1453 (2003).
21. Zaffer, B. et al. Study on blood biochemistry and histomorphology of various organs in broiler chicken fed duckweed (*Lemna minor*) based diet with or without enzyme supplementation. Indian J. Anim. Health. **61**, 132-138 (2022).
22. Kabir, J., Islam, M. A., Ahammad, M. U. & Howlider, M. A. R. Use of duckweed (*Lemna minor*) in the diet of broiler. Indian J. Anim. Res. **39**, 31-35 (2005).
23. Baghban-Kanani, P., Oteri, M., Hosseintabar-Ghasemabad, B., Azimi-Youvalari, S., Di Rosa, A. R., Chiofalo, B., Seidavi, A. & Phillips, C. J. 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. *Anim. Sci. J.* **94**, e13888 (2023).

24. Ahmad, M., Qureshi, S., Akbar, M. H., Siddiqui, S. A., Gani, A., Mushtaq, M., Hassan, I. & Dhull, S. B. Plant-based meat alternatives: Compositional analysis, current development and challenges. *Appl. Food Res.* **2**, 100154 (2022).
25. Ifie, I., Olatunde, S., Ogbon, O. & Umukoro, J. E. Processing techniques on phytochemical content, proximate composition, and toxic components in duckweed. Int. J. Veg. Sci. **27**, 294-302 (2021).
26. Janmohammadi, H. et al. Effect of dietary amaranth (Amaranthus hybridus chlorostachys) supplemented with enzyme blend on egg quality, serum biochemistry and antioxidant status in laying hens. Antioxidants. **12**, 456 (2023).
27. Khatami, S. A., Shakouri, M. D. & Mojtahedin, A. Effect of milk thistle (Silybum marianum) seed on performance, nutrient digestibility and small intestine morphology of broiler chickens. Iran. J. Appl. Anim. Sci. **13**, 767-773 (2023).
28. Amjadian, T., Shahir, M. H., & Moeini, M. M. The effect of dietary antioxidants supplementations on growth performance, carcass traits, and immune response of heat-stressed broiler. *Iranian J. Appl. Anim. Sci.* **14**, 627-636 (2024).
29. Akbari, G., Zarei, A. & Eila, N. The Effect of *Echinacea purpurea* Extract on Performance, and Interleukin-2 Gene Expression in Broilers. *Iranian J. Appl. Anim. Sci.* **15**, 143-153 (2025).
30. Amjadian, T., Shahir, M. H., & Moeini, M. M. The effect of dietary antioxidants supplementations on growth performance, carcass traits, and immune response of heat-stressed broiler. *Iranian J. Appl. Anim. Sci.* **14**, 627-636 (2024).
31. Azizi, C. M., Bouyeh, M. & Seidavi, A. R. Effect of different levels of L-carnitine on growth performance, carcass characteristics, function of immune system, cecal microbial population, histology of the small intestine, and composition of fatty acids of broiler breast meat. *J Anim Scie Res,* **31**, 111-1127 (2021).
32. Samadian, F., Torshizi, M. A. & Eivakpour, A. Effects of dietary supplementation of fermented wheat bran on performance, immunity, meat quality, and tibia bone characteristics in two strains of broiler chicken. Iran. J. Appl. Anim. Sci. **14**, 615-626 (2024).
33. Attia, Y. A., Al-Harthi, M. A., Al-Sagan, A. A., Alqurashi, A. D., Korish, M. A., Abdulsalam, N. M., Olal, M. J. & Bovera, F. Dietary supplementation with different ω-6 to ω-3 fatty acid ratios affects the sustainability of performance, egg quality, fatty acid profile, immunity and egg health indices of laying hens. *Agriculture*, **12**, 1712 (2022).
34. Paguia, H. M. et al. Feeding value of fermented duckweed meal (*Lemna minor* Linn.) as a plant protein component in the formulated diets of Free-Range chicken (*Gallus gallus domesticus* Linn.). J. Adv. Agric. Technol. **10**, 16-20 (2023).
35. Haustetn, A. T., Gilman, R. H., Skillicorn, P. W., Vergara, V., Guevara, V. & Gastanaduy, A. Duckweed, a useful strategy for feeding chickens: performance of layers fed with sewage-grown Lemnacea species. Poult. Sci. **69**, 1835-1844 (1990).
36. Demann, J., Petersen, F., Dusel, G., Bog, M., Devlamynck, R., Ulbrich, A., Olfs, HW. & Westendarp, H. (2022). Nutritional value of duckweed as protein feed for broiler chickens-digestibility of crude protein, amino acids and phosphorus. *Animals*, *13*(1), 130.

37. Islam, K. M. S., Tareque, A. M. M. & Howlider, M. A. R. Complete replacement of dietary fish meal by duckweed and soybean meal on the performance of broilers. Asian-Australas. J. Anim. Sci. **10**, 629-634 (1997).
38. Vakili, R. & Rashidi, A. A. The effects of dietary fat, vitamin E and zinc supplementation on fatty acid composition and oxidative stability of muscle thigh in broilers under heat stress. Afr. J. Agric. Res. **6**, 2800-2806 (2011).
39. Vakili, R., Rashidi, A. A. & Sobhanirad, S. Effects of dietary fat, vitamin E and zinc supplementation on tibia breaking strength in female broilers under heat stress. Afr. J. Agric. Res. **5**, 3151-3156 (2010).
40. Moharreri, M., Vakili, R., Oskoueian, E. & Rajabzadeh, G. Effects of microencapsulated essential oils on growth performance and biomarkers of inflammation in broiler chickens challenged with salmonella enteritidis. J. Saudi Soc. Agric. Sci. **21**, 349-357 (2022).
41. Moharreri, M., Vakili, R., Oskoueian, E. & Rajabzadeh, G. Phytobiotic role of essential oil-loaded microcapsules in improving the health parameters in Clostridium perfringens-infected broiler chickens. Ital. J. Anim. Sci. **20**, 2075-2085 (2021).