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The Effect of Microalgae Supplement with Herbal Powder and Conjugated Linoleic Acid (CLA) on Production Performance, Blood Parameters and Ovarian Activity in Laying Hens

Research Article

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ABSTRACT

This study aimed to investigate the effects of dietary supplementation with microalgae, herbal powder, and conjugated linoleic acid (CLA) on production performance, blood parameters, and ovarian activity in Lohman laying hens. Sixty 43-week-old hens (1.6±0.2 kg) were randomly assigned to four dietary groups for 42 days: a control group (no additives) and three experimental groups receiving 0.1%, 0.2%, or 0.3% of a supplement blend containing Spirulina, Chlorella, CLA, and herbal powders (dill and peppermint). Parameters including production performance, egg weight, serum lipids, ovarian follicle activity, and liver fat were evaluated. Results indicated that the highest supplementation level (0.3%) significantly improved production performance and egg weight compared to the control and other groups ($P<0.05$). On day 42, serum lipid analysis showed a significant reduction in cholesterol levels in the 0.3% group ($P<0.05$). Ovarian follicle activity increased with higher supplementation levels, showing a statistically significant enhancement over the control ($P<0.05$). Liver evaluation revealed a notable decrease in total liver fat in the supplemented groups, particularly at the 0.3% level ($P<0.05$). In conclusion, dietary inclusion of 0.3% microalgae, CLA, and herbal supplements enhanced productive performance, improved egg quality, optimized serum cholesterol levels, supported ovarian activity, and contributed to better liver health in Lohman laying hens.

KEY WORDS conjugated linoleic acid, herbal supplements, laying hens, microalgae, production performance.

INTRODUCTION

Consumer demand and regulatory pressure are driving the poultry industry toward natural, sustainable feed additives that improve product quality and bird health while reducing reliance on antibiotics and synthetic compounds. Microalgae (e.g., *Spirulina platensis*, *Chlorella vulgaris*) are nutrient-dense feed ingredients providing high-quality protein, carotenoids (xanthophylls, β-carotene), essential fatty acids and antioxidant micronutrients that can enhance yolk pigmentation, antioxidant status and nutrient supply in poultry. Recent reviews summarize growing evidence for beneficial effects of microalgae on performance, egg quality and bird health (Costa *et al*. 2024). Conjugated linoleic acid (CLA) is a group of positional/stereoisomers of linoleic acid shown to modulate lipid partitioning, reduce hepatic lipogenesis and promote beta-oxidation via pathways involving peroxisome proliferator-activated receptors (PPARs) and related regulators of lipid metabolism. These mechanistic actions explain reported reductions in abdominal/liver fat and changes in egg lipid composition after dietary CLA (Wang *et al*. 2023). Phytogenic/herbal additives such as *Mentha piperita* (peppermint) and *Anethum graveolens* (dill) supply essential oils, polyphenols and other bioactive

compounds that have antioxidant, anti-inflammatory and microbiota-modulating effects; recent reviews and experimental studies document their immunomodulatory and performance benefits in poultry (Phillips, 2023). Although numerous studies have evaluated microalgae, CLA or medicinal herbs individually, fewer studies have investigated their combined effects in laying hens under the same dietary and management conditions; moreover, results across studies are variable depending on dose, additive form and basal diet. Recent JCR articles emphasize both the promise of natural additive strategies and the need for integrated trials combining complementary bioactives (Vase-Khavari *et al.* 2019). The present experiment (this manuscript) evaluated the effects of a commercial blend containing Spirulina + Chlorella + CLA + peppermint + dill at three inclusion levels (0.1, 0.2 and 0.3% of the diet) on production performance, egg quality, serum lipids, ovarian follicular dynamics and liver fat in Lohman LSL laying hens. This work fills a practical knowledge gap by testing a low-level combined formulation that integrates pigment/antioxidant supply (microalgae), lipid modulators (CLA) and phytochemicals (herbs) within a single feeding trial.

MATERIALS AND METHODS

Experimental location

The experiment was conducted at the breeding and maintenance unit for laying hens at Sari University of Agricultural Sciences and Natural Resources, Iran.

Supplements used

The dietary additives used in this study were provided by Simorgh Behin Daroo Company, located in Babolsar, Mazandaran Province, Iran.

Animals and diet formulation

A total of 60 Lohman LSL laying hens, aged 43 weeks, were housed in individual cages (43 cm×51 cm) with three birds per cage under ambient temperatures and a 16-hour light regimen. The trial lasted for 42 days. Feed was restricted to 115 g/day per bird, while water was provided *ad libitum*. The experimental diets included:

Control diet (C): Basal diet without any supplementation. Treatment 1 (0.1%): Basal diet supplemented with 0.05% herbal mixture (equal parts peppermint and dill powder) + 0.05% microalgae (Chlorella and Spirulina) and CLA supplement. Treatment 2 (0.2%): Basal diet supplemented with 0.1% herbal mixture (equal parts peppermint and dill powder) + 0.1% microalgae (Chlorella and Spirulina) and CLA supplement. Treatment 3 (0.3%): Basal diet supplemented with 0.15% herbal mixture (equal parts peppermint and dill powder) + 0.15% microalgae (Chlorella and Spirulina) and CLA supplement. All additives were prepared in equal proportions by Simorgh Behin Daroo Company. The ingredient composition of the basal diet is presented in Table 1. No mortality occurred during the experiment.

Table 1 Composition and nutrient levels of the basal diet

Ingredient	g/kg
Corn	63.23
Soybean meal, 41%	24.50
Soybean oil	0.40
Oyster shell	9.50
Dicalcium phosphate	1.40
DL-Methionine, 99%	0.16
NaCl	0.30
Vitamin-mineral premix[1]	0.50
Multi-enzyme	0.01
Total	100
Nutrient Levels	
Crude protein, %	16.15
Metabolizable energy, kcal/kg	2700
Calcium, %	4.00
Phosphorus, %	0.37
Crude fiber, %	3.11
Lysine, %	0.82
Methionine, %	0.42
Methionine + cysteine, %	0.70

[1] Vitamin-mineral premix (units per kilogram of feed): vitamin A, 10000 IU; vitamin D3, 3500 IU; vitamin E, 35 IU; Menadione, 1.5 mg; Riboflavin, 5 mg; Pantothenic acid, 8 mg; vitamin B12, 0.012 mg; Pyridoxine, 1.5 mg; Thiamine, 1.5 mg; Folic acid, 0.5 mg; Niacin, 30 mg; Biotin, 0.06 mg; Iodine, 0.8 mg; Copper, 10 mg; Iron, 80 mg; Selenium, 0.3 mg; Manganese, 80 mg and Zinc, 80 mg.

Laying performance

From weeks 43 to 48, birds were weighed weekly, and feed intake was recorded on a cage basis. Eggs were collected daily, weighed, and their averages were used to calculate egg production (% hen-day) and egg weight. Egg mass was determined by multiplying egg production by egg weight. Feed conversion ratio (FCR) was calculated as grams of feed consumed per gram of egg produced on a cage basis.

Egg quality assessment

Egg quality parameters, including egg weight, yolk color, shell thickness, Haugh unit, and shell weight, were evaluated between weeks 43 and 48. For this purpose, 24 eggs from each treatment group were randomly collected between 8:00 a.m and 12:00 p.m for analysis. Yolk color was assessed using the Roche Yolk Color Fan method. Shell weight was determined by washing the shells to remove any albumen residue, drying them, weighing them, and calculating their proportion relative to whole egg weight.

Reproductive morphological assessment

At the end of the experiment (48 weeks of age), eight birds from each group were randomly selected, weighed, and euthanized for reproductive system evaluation. The ovary and oviduct were separated and weighed. Follicles were categorized based on size as large yellow follicles (LYFs;

≥10 mm), small yellow follicles (SYFs; 5–10 mm), large white follicles (LWFs; 3–5 mm), and medium white follicles (MWFs; 1–3 mm), following the method described by Slinkard *et al*. (2009). The stroma weight was recorded as the remaining ovarian tissue after follicle removal.

Liver evaluation and fatty liver assessment

At the end of the experimental period, all birds were euthanized humanely. Immediately after euthanasia, the livers were carefully excised and weighed to determine absolute liver weight. The severity of fatty liver was assessed macroscopically using a five-point scoring system (Gilbert *et al.* 1984). For biochemical quantification of liver fat, representative liver samples (~5 g) were collected from each bird and immediately stored at −24 °C until further processing. The total fat content of liver tissues was determined using the Soxhlet extraction method based on AOAC (1984) protocols. Briefly, samples were first dried and ground, and fat was extracted using petroleum ether as the solvent in a Soxhlet apparatus for approximately 6 hours. The extracted fat was then quantified gravimetrically and expressed as a percentage of dry liver weight.

Blood examination

At the end of the experiment, blood samples were collected from the wing vein into heparinized tubes. Plasma was obtained by centrifugation at 3000 × *g* for 20 min and stored at −20°C until analysis. Serum was obtained by blood centrifugation, and total cholesterol, high- and low-density lipoproteins (HDL and LDL), total protein, glucose, and triacylglycerol levels were determined using commercial kits (Lab Test Diagnostics).

Data analysis

The effects of treatments in this experiment were analyzed using analysis of variance (ANOVA) under the General Linear Model (GLM) procedure in SAS software (SAS, 2013). When significant differences among treatment means were detected (P<0.05), Least Significant Difference (LSD) post-hoc test was used to compare means. Statistical significance was declared at the 0.05 level.

RESULTS AND DISCUSSION

The effects of dietary supplementation on egg production, feed intake (FI), egg weight, and feed conversion ratio (FCR) in laying hens are presented in Table 2. Dietary supplementation with varying levels of microalgae, CLA, and herbal supplements did not significantly affect FCR compared to the control group (P>0.05). However, feed intake, egg weight, and egg production were significantly improved by supplementation at the 0.2% (Treatment 2) and 0.3% (Treatment 3) levels. The 0.3% supplementation (Treatment 3) resulted in the most significant improvement in egg production compared to the control (P<0.05).

The effects of dietary treatments on egg quality parameters from weeks 43 to 48 are shown in Table 3. Haugh unit and shell thickness were not significantly affected by the dietary treatments (P>0.05). However, dietary supplementation increased yolk color score and shell weight at all levels compared to the control. The 0.3% supplementation of microalgae with CLA and herbal supplements (Treatment 3) was the most effective in improving yolk color score and shell weight (P<0.05), although there was no significant difference compared to the 0.2% supplementation (Treatment 2).

The effects of dietary supplementation on reproductive morphology are presented in Table 4. Supplementation with microalgae, CLA, and herbal supplements did not significantly affect ovary weight, oviduct weight, the number of large yellow follicles (LYF), the number of follicle ovulations (FO), or the number of large white follicles (LWF) (P>0.05). However, diets supplemented with all levels of microalgae, CLA, and herbal supplements significantly increased the number of small yellow follicles (SYF), largest follicle weight (LFW), and large yellow follicle weight (LYFW) compared to the control group (P<0.05). Birds fed the diet with 0.3% supplementation (Treatment 3) had the highest LYFW. Additionally, higher supplementation levels (0.2% and 0.3%; Treatments 2 and 3) significantly increased stroma weight compared to the control treatment and the 0.1% supplementation (Treatment 1).

As shown in Table 5, dietary supplementation with PUFAs (CLA and Omega-3) and herbal supplements (dill powder and peppermint) did not significantly alter glucose, total protein, and uric acid concentrations in the blood (P>0.05). However, cholesterol, triglyceride, HDL, and LDL concentrations were significantly altered by supplementation groups at higher levels (0.2% and 0.3%) compared to the control diet (P<0.05). All levels of supplementation significantly reduced blood cholesterol compared to the control treatment (P<0.05).

The effects of different levels of dietary supplementation on liver characteristics and liver fat are shown in Table 6. Liver weight in the control and Treatment 1 (0.1% supplement) was significantly higher than in Treatments 2 (0.2% supplement) and 3 (0.3% supplement) (P<0.05). The percentage of liver fat was significantly lower in chickens fed diets supplemented with 0.2% and 0.3% levels compared to the control and Treatment 1 (P<0.05). Additionally, the relative liver weight was significantly higher in the control treatment compared to all supplemented treatments (P<0.05), with the lowest relative liver weight observed in Treatment 3 (0.3% supplementation).

Table 2 Effect of different treatments on laying hen productive performance

Item	Treatments (T)				SEM	P-value
	Control	T1 (0.1%)	T2 (0.2%)	T3 (0.3%)		
Feed intake, g/d	108.51^{b}	109.27^{b}	111.86^{a}	112.79^{a}	1.14	0.048
Egg weight, g	57.86^{b}	58.48^{ab}	59.08^{a}	59.19^{a}	0.40	0.005
Egg production, %	86.29^{c}	87.41^{b}	88.36^{ab}	89.22^{a}	2.24	0.105
Feed conversion ratio, g feed/g egg	2.04	2.03	2.00	2.00	0.06	0.621

Control: basal diet; T1: basal diet supplemented with 0.1% microalgae (chlorella and spirulina), conjugated linoleic acid (CLA), and herbal supplements (peppermint and dill powder); T2: basal diet supplemented with 0.2% microalgae(chlorella and spirulina), CLA, and herbal supplements (peppermint and dill powder) and T3: basal diet supplemented with 0.3% microalgae (chlorella and spirulina), CLA, and herbal supplements(peppermint and dill powder).
FCR: feed conversion ratio.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of different treatments on egg quality in laying hens

Item	Treatments (T)				SEM	P-value
	Control	T1 (0.1%)	T2 (0.2%)	T3 (0.3%)		
Yolk color score	6.84^{c}	7.69^{b}	8.61^{ab}	9.13^{a}	0.24	0.001
Shell thickness, µm	0.41	0.42	0.42	0.43	0.07	0.563
Haugh unit	80.26	79.86	80.78	80.42	0.84	0.154
Shell weight, g	5.86^{c}	6.37^{b}	6.69^{ab}	6.92^{a}	0.04	0.004

Control: basal diet; T1: basal diet supplemented with 0.1% microalgae (chlorella and spirulina), conjugated linoleic acid (CLA), and herbal supplements (peppermint and dill powder); T2: basal diet supplemented with 0.2% microalgae(chlorella and spirulina), CLA, and herbal supplements (peppermint and dill powder) and T3: basal diet supplemented with 0.3% microalgae (chlorella and spirulina), CLA, and herbal supplements(peppermint and dill powder).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of different treatments on reproductive morphology of laying hens

Item	Treatments (T)				SEM	P-value
	Control	T1 (0.1%)	T2 (0.2%)	T3 (0.3%)		
Ovary weight, %	3.72	3.65	3.56	3.62	0.05	0.122
Oviduct weight, %	4.71	4.66	4.89	5.78	0.17	0.083
Stroma weight, g	7.48^{b}	7.64^{b}	8.79^{a}	8.53^{a}	0.05	0.036
LYF, number	5.41	5.52	5.73	6.3	0.02	0.421
SYF, number	8.14^{c}	9.68^{b}	10.49^{ab}	11.18^{a}	0.48	0.028
LWF, number	18.26	18.32	18.74	19.93	1.07	0.613
LFW, g	11.64^{c}	13.06^{b}	13.48^{ab}	14.19^{a}	0.28	0.016
LYFW, g	37.86^{c}	39.64^{b}	41.83^{ab}	42.58^{a}	0.03	0.048
FO, number	3.10	3.31	3.62	3.9	0.13	0.574

Control: basal diet; T1: basal diet supplemented with 0.1% microalgae (chlorella and spirulina), conjugated linoleic acid (CLA), and herbal supplements (peppermint and dill powder); T2: basal diet supplemented with 0.2% microalgae(chlorella and spirulina), CLA, and herbal supplements (peppermint and dill powder) and T3: basal diet supplemented with 0.3% microalgae (chlorella and spirulina), CLA, and herbal supplements(peppermint and dill powder).
LYF: large yellow follicle; SYF: small yellow follicle; LWF: large white follicle; LFW: largest follicle weight; LYFW: large yellow follicle weight and FO: follicle ovulations.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effects of different treatments on blood parameters of laying hens (mg/dL)

Item	Treatments (T)				SEM	P-value
	Control	T1 (0.1%)	T2 (0.2%)	T3 (0.3%)		
Glucose	218.83	216.68	220.43	221.08	2.48	0.394
Total protein	6.32	6.39	6.48	6.52	1.29	0.883
Cholesterol	148.86^{a}	143.61^{b}	133.41^{c}	131.27^{c}	0.86	0.542
Triglyceride	754.36^{a}	747.69^{ab}	742.98^{b}	735.11^{c}	1.26	0.681
HDL	42.11^{b}	45.63^{ab}	48.89^{a}	49.08^{a}	0.23	0.544
LDL	57.73^{a}	53.36^{ab}	50.66^{b}	45.23^{c}	0.88	0.138
Uric acid	4.58	4.41	4.49	4.37	0.71	0.233

Control: basal diet; T1: basal diet supplemented with 0.1% microalgae (chlorella and spirulina), conjugated linoleic acid (CLA), and herbal supplements (peppermint and dill powder); T2: basal diet supplemented with 0.2% microalgae(chlorella and spirulina), CLA, and herbal supplements (peppermint and dill powder) and T3: basal diet supplemented with 0.3% microalgae (chlorella and spirulina), CLA, and herbal supplements(peppermint and dill powder).
HDL: high-density lipoprotein and LDL: low-density lipoprotein.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of different treatments on liver characteristics and liver fat of laying hens

Item	Treatments (T)				SEM	P-value
	Control	T1 (0.1%)	T2 (0.2%)	T3 (0.3%)		
Liver weight, g	47.09[a]	44.63[b]	41.17[c]	42.46[c]	0.38	0.145
Fatty liver score	4.78	4.69	4.39	4.26	0.70	0.052
Relative liver weight, %	2.41[a]	2.35[b]	2.26[b]	2.29[b]	1.32	0.284
Liver fat, %	13.72[a]	11.17[ab]	10.68[b]	10.25[b]	0.39	0.186

Control: basal diet; T1: basal diet supplemented with 0.1% microalgae (chlorella and spirulina), conjugated linoleic acid (CLA), and herbal supplements (peppermint and dill powder); T2: basal diet supplemented with 0.2% microalgae(chlorella and spirulina), CLA, and herbal supplements (peppermint and dill powder) and T3: basal diet supplemented with 0.3% microalgae (chlorella and spirulina), CLA, and herbal supplements(peppermint and dill powder).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In this 42-day trial, the combined microalgae–CLA–herb supplement produced dose-dependent improvements in several endpoints, with the 0.3% inclusion yielding the largest benefits for egg weight, egg production, yolk pigmentation, serum cholesterol/LDL reduction, decreased liver fat, and enhanced measures of follicular development (see Results).

The observed increase in egg weight and hen-day production at 0.2–0.3% supplementation likely reflects improved nutrient density and partitioning toward egg synthesis. Microalgae provide highly digestible protein, essential fatty acids and micronutrients that can enhance nutrient availability, while CLA shifts lipid metabolism towards oxidation and away from storage — together these actions can increase resources available for egg formation. These synergistic effects agree with recent reviews highlighting combined roles of PUFAs and antioxidant sources in driving modest but consistent production gains (El-Shall *et al.* 2023; Abdel-Wareth *et al*. 2024).

The marked improvement in yolk color is best explained by deposition of algal carotenoids (xanthophylls and β-carotene) from Spirulina/Chlorella, which are efficiently incorporated into yolk lipid fractions. Multiple recent JCR reviews and experiments report strong yolk color responses even at low algal inclusion rates. Shell weight increased without consistent change in measured shell thickness, suggesting improved matrix deposition or mineralization efficiency rather than a gross structural thickening; antioxidative phytochemicals from peppermint/dill may reduce oxidative stress in shell gland tissues and support Ca-homeostasis — hypotheses that match current thinking in recent phytobiotic literature (Abdel-Wareth *et al*. 2024; Costa *et al.* 2024).

The reduction in total cholesterol, LDL and liver fat in the higher supplementation groups supports an additive/complementary lipid-modulating action. CLA is well documented to reduce hepatic lipogenesis and increase fatty acid oxidation through modulation of PPAR signaling and related enzymes, and plant polyphenols/essential oils can further enhance bile acid turnover and antioxidant protection, reducing lipid peroxidation and improving lipid handling. Together, these mechanisms plausibly produced the lower hepatic fat percentage and improved serum lipid profile observed here. Similar patterns linking CLA and plant antioxidants to improved hepatic lipid metabolism have been reported in recent JCR publications (Abdelli *et al.* 2021; Wang *et al.* 2023).

Enhanced counts/weights of small and large yellow follicles in supplemented hens are consistent with an effect of improved antioxidant status and altered lipid signaling on follicular recruitment and maturation. Carotenoids and tocopherols protect follicular cells from oxidative damage and may prolong follicle viability; CLA may influence eicosanoid/prostaglandin pathways that modulate ovulation. These mechanistic links are supported by reviews and experiments that connect dietary antioxidants and specific lipid mediators to reproductive indices in laying poultry. Direct molecular measures (steroid hormones, prostaglandins, expression of steroidogenic enzymes) were not performed here but are recommended to test these mechanisms (Abdelli *et al*. 2021; El-Shall *et al.* 2023).

The pattern of improved egg quality and lipid profile is broadly consistent with results from studies and reviews published between 2018–2024 that evaluate microalgae and phytogenics in poultry diets; some previous reports show variable outcomes depending on species, additive dose and form, duration and baseline diet composition. For example, Vase-Khavari *et al.* (2019) and Ahmadian *et al.* (2020) document beneficial immune and performance effects of medicinal plants in poultry, while Phillips (2023) reviews immunomodulatory roles of natural feed additives and emphasizes context dependency (dose, extract *vs.* whole plant). These recent JCR sources contextualize our findings and support the interpretation that combining complementary bioactives can produce additive or synergistic improvements (Vase-Khavari *et al.* 2019).

Limitations include the relatively short experimental window (42 days), modest sample size and lack of mechanistic molecular endpoints (e.g., hepatic gene expression of PPARα/γ, HMG-CoA reductase, antioxidant enzyme acti-

vities, or ovarian steroid measures), which constrain causal inference. Strengths are the randomized design, practical combined formulation representing potential commercial use, and parallel evaluation of production, biochemical and reproductive endpoints that together increase confidence in the biological relevance of the observed effects.

Future research to confirm mechanisms and commercial feasibility we recommend: (1) longer trials spanning full production cycles; (2) dose–response testing with isolated *vs*. combined ingredients; (3) measurement of hepatic and ovarian molecular markers (PPARs, HMG-CoA reductase, steroidogenic enzymes, antioxidant enzymes); and (4) economic analyses that incorporate current microalgae production costs and alternative algal cultivation options. Recent reviews underscore the importance of such multi-axis studies for translating promising small-scale results into industry practice.

CONCLUSION

Dietary supplementation of Lohmann LSL laying hens with a combined blend of Spirulina, Chlorella, CLA, peppermint and dill (total 0.3%) improved egg production and egg weight, enhanced yolk pigmentation and shell mass, lowered serum cholesterol and LDL, reduced liver fat, and supported follicular development. These findings indicate that low-level inclusion of complementary natural bioactives can enhance both productive performance and metabolic health in laying hens. Further long-term and mechanistic studies are warranted to confirm these benefits and to evaluate economic feasibility for commercial application.

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