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Algae usage in producing functional food: A case study on *Spirulina platensis* for enrichment quail egg

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Abstract

In this study, crude and enriched *spirulina platensis* with iron and zinc were used in two ways to improve the nutritional value of quail's eggs as a functional food. The number of 126 one-day old Japanese quail, in 7 treatments with 3 replications and 3 diets including a basal diet (as the control case), basal diet with enriched algae, and basal diets with crude algae (*Spirulina* consumed at three levels of 2.5, 5, and 7.5%) were considered. According to the results, the uptakes of iron and zinc in *spirulina* in the logarithmic phase were recorded about 1.7 and 2.8 times, respectively compared to the control group, and this difference in *spirulina* in the logarithmic phase in comparison with the *spirulina* in the maximum growth stage increased by more than 1 and 3 times, respectively. Furthermore, according to the statistical results, the minimum iron and zinc absorption in the treatments were 5.8% and 24.9%, accordingly, which was significantly different from the control group ($p < 0.05$). Yolk color and a* (redness index) in the enriched treatment of 7.5% showed the highest difference (16.4% and 38.1%, individually) compared to the other treatments ($P < 0.05$).

Keywords: Enrichment; Bioabsorption; *Spirulina platensis*; Quail eggs.

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1. Introduction

Currently, scientists are shifting from focusing on identifying nutrients and the needed amount to prevent eating disorders to focusing on improvement, health, and quality of life. Iron deficiency anemia is the most common nutritional disorder in the world (Paudel *et al*., 2018; Jamatia *et al*., 2019). Zinc deficiency also affects about 17-19% of the world's population. Enriching foods with minerals is done to correct the nutrient levels lost during processing or to enrich food with that are quantitatively or qualitatively low in nutrients (McClung, 2019; Garcia-Oliveira *et al*., 2018). In fact, functional foods are consumed as part of a regular diet and in addition to their nutritional efficiency, have beneficial physiological effects or reduce the risk of chronic diseases (Adefegha, 2018; Ovando *et al*., 2018). Microalgae are one of the most interesting food resources and functional foods (Benelhadj *et al*., 2016; Priyadarshani and Rath, 2012). Arthrospira (*spirulina*) are microscopic filamentous prokaryotes (Vaz *et al*., 2016). These cyanobacteria contain essential fatty acids, volatile compounds (food flavors), phytosterols (sterol removers), essential amino acids, pigments, vitamins, and phenolic compounds. *Spirulina platensis* is one of the most important microalgae that are widely used as a dietary supplement. This is due to its production of unsaturated fatty acids, especially long-chain polyunsaturated fatty acids such as omega 3 and omega 6 (Andrade, 2018). As the world's population grows, human demand for protein is increasing day by day, and studies show that quail eggs contain nutrients with therapeutic and preventive properties in diseases such as cardiovascular disease. Quail eggs are one of the nutrients in which nutritional value is three to four times higher than chicken eggs and a good source of nutrients for human health (Jeke *et al*., 2018). Numerous studies have been done on quail feed enrichment by *Spirulina platensis* (Boiago *et al*., 2019), lycopene (Sahin *et al*., 2008), fish oil (Kamely *et al*., 2016), vegetation such as licorice (Doğan *et al*., 2018) and rice bran (Gopinger *et al*., 2016) to enrich its eggs (Wang *et al*., 2007). There is an increasing prevalence of iron deficiency anemia and zinc deficiency in the population, especially in children and pregnant women, as well as the destructive effects of iron and zinc deficiency pills in the community. The natural enrichment of foods with iron and zinc by algae, as well as the productivity of phytobiotics in the food industry to achieve high-quality foods to prevent disease is essential to the goals of this study.

2. Materials and methods

2.1. Enrichment of Spirulina platensis algae

Two methods were used to enrich *Spirulina platensis* algae with iron and zinc, then iron and zinc absorption were evaluated for both methods and the best treatment with the highest iron and zinc absorption was applied to the quail feed in the experimental groups.

In the first phase, after 7 days of initial cultivation, EDTA-FeNa.3H2O, Ferric Citrate, $ZnSO_4.7H_2O$, and $CuSO_4.5H_2O$ minerals were added 5 hours before harvesting the *Spirulina platensis* algae. These minerals were 13 mg L-1, 0.0396.0 mg L-1, 0.5994 mg L-1, and 0.1998 mg L-1 respectively, then harvested and dried. In the second method (Log p.) with the cultivate algae after entering the logarithmic phase, minerals were added in the same method to the medium as before. After 7 days the algae was harvested and dried (Montazeri Shahtoori, 2015).

2.2. Feeding and categorization of treatments

In this study, 126 one-day Japanese quail laying chickens were randomly divided into 7 treatments:

1) Basic meal diet BMD (control group),
2) BMD with 2.5% Enriched *Spirulina platensis* (SP Fe. Zn),
3) BMD treatment with 5%SP Fe. Zn,
4) BMD treatment with 7.5% SP Fe. Zn,
5) BMD treatment with 2.5% crude *Spirulina platensis* (SP),
6) BMD treatment with 5% SP and
7) BMD treatment with 7.5% SP.

Each treatment consisted of 3 replicates and 6 quail chicks per experimental unit were reared on the same environmental conditions (Foad, 2017). Experimental diets based on corn and soybean meal were adjusted for growth period and laying period (Table 1).

During the experiment (1 day to 92 days), the quails had free access to water and food and the rations were flour. The lighting was 24 hours until 35 days, but from 35 days the lighting was reduced to 22 hours, 2 hours of darkness, and 1 hour was added every night until it reached 8 hours of darkness and 16 hours of lighting.

2.3. Analysis of iron and zinc absorption in enriched and crude algae

To evaluate the iron and zinc absorption in algal samples, ICP-OES simultaneous Arcos EOP model was used. First, each treatment was digested and prepared separately (Sinaei *et al*., 2018). For this purpose, 1 gram of the sample was digested with nitric acid and oxygenated water in the microwave for two 10 minute steps at 200 °C and 800 W power and finally injected into the device. To improve the accuracy of the test, the blank sample (containing nitric

Table 1. Quail basic feed compounds during growth and laying period (Hajati and Zaghari, 2019)

Type	Growth period* Value based on grams	Laying period** Value based on grams
Corn	30	35
Soy	23	19
Oil	10	7
Phosphate	10	16
Carbonate	10	50
Mineral supplement	1	1.5
Vitamin supplements	1	1.5
Methionine	1	1.5
Leysin	0.5	0.6
Threonine	0.1	0.6
Acid fire	-	0.6
Anti-coccidiosis	0.1	-
Salt	1	1.5

* 0-35 days old
** 35 days old- end of the period

acid and oxygenated water without the original sample) was also injected into the device and finally, the concentration of heavy metals and elements was calculated using the following equation:

$$M(\mu g/g) = (C \times V)/W$$

where, M is the final concentration of the elements and heavy metals of the sample in µg/g, C is the concentration obtained from the device in µg/l, V is the final sample volume in l (0.025), W the primary sample weight for acid digestion in (g) (Saeid *et al*., 2013a).

2.4. Analysis of iron and zinc absorption in quail eggs

Iron and zinc absorption of quail eggs in the final period (last 15 days) were evaluated of iron and zinc absorption as in the algal samples.

2.5. Yolk color

The yolk color parameter (YC) was determined using 0-15 degrees Roche color fan (Ludke *et al*., 2018). To ensure the accuracy of the yolk color test Hunter Lab Ultra scan VIS model was used which was evaluated the yolk color in the final 15 days by Hunter Lab (Carson *et al*., 1994).

2.6. Statistical analysis

Using SPSS software version 22 and ANOVA (two-way analysis of variance), the difference between the mean of different samples and the factorial statistical test was included for the effect of values of each sample over a different period time, at 5% probability level. The data were first normalized to perform the statistical tests. Excel software was used to draw the charts.

3. Results and Discussion

3.1. Spirulina platensis enriched and crude

Table 2 shows the amount of iron and zinc in the algae enriched with two ways, during the logarithmic phase (log p.) and maximum stationary phases (M.E.P).

The statistical results showed that the amount of iron and zinc in the three groups was significant ($p < 0.5$). According to the results, the most suitable method for the absorption of iron and zinc in this study with *Spirulina platensis* algae enrichment is bioabsorption

Table 2. Iron and zinc content in *Spirulina platensis* during the enrichment process

	algae sample		
Parameter	Logarithmic phase (Log P.)	Maximum stationary phases (M.E.P)	Crude
Iron (µg/g)	511162.30±25558.1[c]	458952.80±22947.64[b]	296594.00±14829.7[a]
Zinc (µg/g)	11832.50±591.625[b]	3528.50±176.425[a]	4161.10±208.055[a]

The same name letters above the numbers of each sample indicate no significant differences between the samples and the inconsistent letters indicate the significance of the samples with each other.

(Log p.). The algae enriched by the (Log p.) method showed a higher iron and zinc absorption than the (M.E.P) method (1 and 3 times, correspondingly) (p<0.5). Zinc showed higher absorption in (Log p.) alga, compared to the control group (2.8 times) (p<0.5). However, the iron absorption in the (Log p.) method was (1.7 times more) compared to the control group (p<0.5), which suggests that enrichment by the (Log p.) method is a suitable method for achieving greater absorption of elements (iron and zinc). Investigating the modification of absorption of other elements such as copper, chromium, etc. can be a future study. According to the research results of Saeid *et al*. (2013a), the best method for enriching *spirulina platensis* is bioabsorption, which is in consistent with the results of this study.

3.2. Iron and zinc Absorption of eggs

The effects of enriched *Spirulina platensis* (Log p.) and crude algae on iron and zinc quail eggs are shown in Table 3.

According to the present study, eggs of quail fed with enriched algae (Log p.) and the control group had significant differences in iron and zinc absorption (p <0.05). Using the *Spirulina platensis* (Log p.) and crude compared to the control group comprising eggs from quail fed with increased iron and zinc, it was found that feeding on crude *Spirulina platensis* 5 in quail did not increase with statistical significance, iron and zinc absorption in eggs compared to the control group. The investigation into the cause of this lack of significance for iron and zinc uptake by using crude *Spirulina platensis* in comparison with the control group at 5% of the *spirulina* level could be one of the future studies. In general, the higher zinc absorption than iron in enriched *spirulina platensis* (Log p.), zinc in *spirulina* fed (Log p.) experimental groups, especially the 5% and 7.5% treatments (76% and 73% increase, respectively), showed higher absorption than the experimental groups fed with crude *spirulina*. The crude treatment of 7.5% indicated the lowest absorption rate of 24.9%, and by increasing the iron absorption which the lowest absorption in the enriched 7.5% treatment was 5.8% in comparison with the control group. Enrichment quail eggs could be a suitable food to prevent and improve iron

Table 3. The amount of iron and zinc in eggs of crude and enrich quail fed with *spirulina platensis*

Nutrition treatments	Control	*Spirulina* 2.5%		*Spirulina* 5%		*Spirulina*7. 5%	
Element							
		enrichment	crude	enrichment	crude	enrichment	crude
Iron (μg/g)	11272.00±563.6^{b}	15848.00±792.4^{c}	15512.00±775.6^{c}	12136.00±606.7^{b}	9112.00±455.6^{a}	11976.00±598.7^{b}	17088.00±854.4^{d}
zinc (μg/g)	6768.00 ±338.4^{a}	11632.00±581.6^{c}	20264.00±1013.2^{d}	28312.00±1415.6^{f}	5984.00±299.2^{a}	25352.00±1267.6^{e}	9016.00 ±450.7^{b}

The same name letters above the numbers of each sample indicate no significant differences between the samples and the inconsistent letters indicate the significance of the samples with each other.

and zinc deficiency diseases in humans. Iron depletion in the 7.5% enriched treatment and also the higher absorption of this element in the crude treatment could be due to the antagonistic effect of iron with copper, iron with zinc. There may also be incompatibilities in the inorganic forms which the elements were supplied. The results of Saeid *et al*. (2013b) study also referred the antagonistic effect of zinc with copper, zinc with iron, or their inappropriate complement form, the use of copper-enriched *spirulina* in pig feed, increases the absorption of elements such as iron, copper, chromium, selenium, manganese and zinc reduction, this is also true for the 2.5% enriched treatment.

3.3. Yolk color

The effects of *Spirulina platensis* on quail egg yolk color are shown in Table 4.

The effect of *Spirulina platensis* on YC evaluated by Roche color fan and the results showed a significant (p <0.05) effect between treatments. So that, all treatments, especially (log p.) treatments showed a significant difference with the control group in which the enriched treatment (7.5%) shows the highest increase of 16.4%. Also, the evaluation of yolk color by Hunter lab showed a significant (p <0.05) effect of *spirulina platensis* on the Lightness index (L*) and redness index (a*), respectively, with consumption of *spirulina* (Log p.) and crude in feed, (a*) increased and (L*) decreased and the highest redness was observed in the (log p.) especially enrich 7.5% treatments (38.1%). The highest transparency in the control group could be due to the presence of more iron in the enriched treatments and presence of pectin in *spirulina*. The yellowness index (b*) did not show a significant difference (p> 0.05) among the treatments, but the control group showed more b* than the other treatments. Hajati and Zaghari (2019) reports indicated an increase in yolk color, which is consistent with the results of this study. The colorimetric results of yolk are consistent with the reports of Omri *et al*. (2019), in which *Spirulina platensis* was used at a feeding level of 1.5% and 2.5% in 44-week-old chickens.

Table 4. Yolk color evaluation by Roche fan and Hunter lab

	Average treatment percentage						
Parameter	Crude 2.5%	Crude 5%	Crude 7.5%	Enrich 2.5%	Enrich 5%	Enrich 7.5%	Control
yolk color**	1.01±0.02[b]	1.02±0.02[bc]	1.03±0.01[cd]	1.03±0.01[bcd]	1.02±0.02[bc]	1.05±0.01[d]	.88±0.02[a]
L*	53.81±3.3[a]	52.92±2[a]	52.28±1.47[a]	51.62±0.97[a]	51.43±0.96[a]	51.17±3.3[a]	57.97±0.77[b]
a*	1.19±0.8[b]	1.29±0.8[c]	1.33±0.99[c]	1.33±1.74[c]	1.30±1.35[c]	1.34±0.9[c]	.8324±0.45[a]
b*	35.97±3.05[a]	39.72±2.63[a]	39.57±3[a]	39.36±0.8[a]	37.72±3.04[a]	39.33±4.15[a]	41.64±2.02[a]

The same name letters above the numbers of each sample indicate no significant differences between the samples and the inconsistent letters indicate the significance of the samples with each other, ** yolk color determined by using (0-15 degrees Roche color fan), L*=lightness, a*=redness, b*=yellowness.

Conclusion

Increased iron and zinc absorption, especially zinc in *spirulina* (Log p.), were 1.7 and 2.8 times higher respectively than those of crude *spirulina* raising the amount of these elements in experimental specimens, especially those fed with *spirulina* (Log p.) resulted in at least 5.8% iron and 24.9% zinc compared to the control data. Due to the role of diet and type of nutrition it resulted in better absorption of these microalgae as well as improving or preventing the deficiency of these two elements. Also, the yellow color and lack of color in quail egg yolk were particular factors in decreasing customer satisfaction. According to the results, yolk color in *Spirulina* treated (Log p.) was increased about 16.4% and also a* was increased about 38.1% over the control group, which can be an effective factor in increasing its marketability.

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