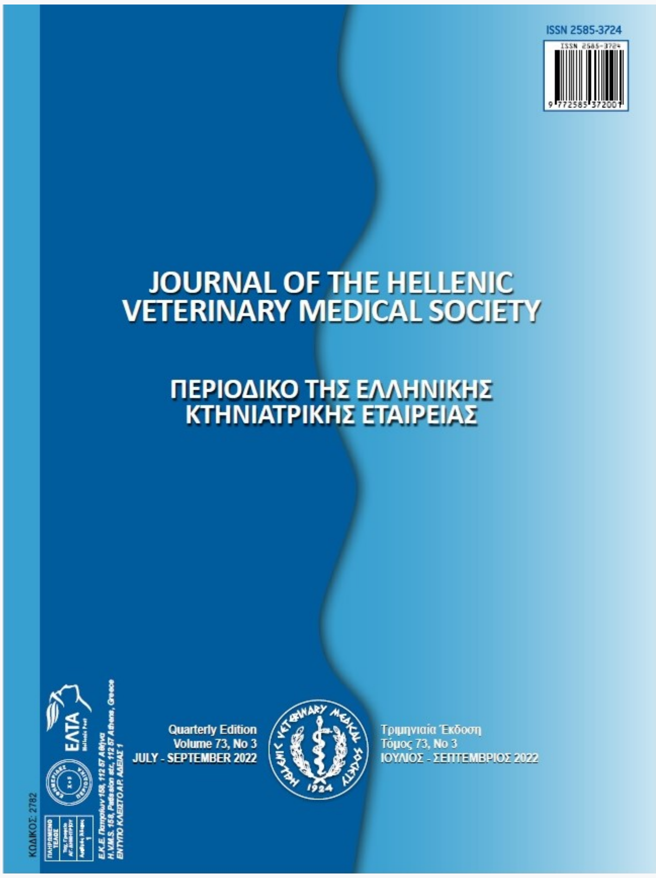
Journal of the Hellenic Veterinary Medical Society

Vol 73, No 3 (2022)



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doi: [10.12681/jhvms.27636](https://doi.org/10.12681/jhvms.27636)

J HELLENIC VET MED SOC 2022, 73 (3): 4511-4516
ΠΕΚΕ 2022, 73 (3): 4511-4516

Research article
Ερευνητικό άρθρο

Use of *Spirulina platensis* in Japanese quail diets in fattening period and responses of performance, meat quality, and immunity

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ABSTRACT: This study was carried out to determine the effects of different levels of Spirulina platensis (SP)in Japanese quails (Coturnix coturnix japonica) diet on performance, meat quality, and immune response. A total of 250 1-d old Japanese quails were randomly distributed to five dietary groups with five replicates. The powder form of SP was used, and experimental diets were formulated according to inclusion levels as; The negative control group (NC)(basal diet 0% SP no vaccine),the control group (C) (basal diet 0%SP vaccinated) the SP1 group (basal diet 2.5% SP vaccinated), the SP2 group (basal diet 5% SP vaccinated) and the SP3 group (basal diet 7.5% SP vaccinated). During five weeks experiment, growth performance parameters were determined and at the end of the trial a total of 75 quails (15 quails from each group) were slaughtered and antibody titers (ABT) against the Newcastle Disease (ND), meat colorvalues, meat quality parameters were detected, and some visceral organs were weighted. Results showed that the inclusion of SP to quail diets at the level of 2.5% increased the final body weight (FBW) and total body weight gain (TBWG) significantly (P<0.05) compared to the other groups. The highest feed intake (FI) was detected in the SP3 group, and the best feed conversion ratio was found in the SP1 group (P<0.05). Dietary SP did not affect carcass yield, liver, and bursa weights (P>0.05) however the carcass weight was affected by SP in the diet, and the highest value was found in the SP1 group(P<0.05). Colour values of breast meat changed as depend on the level of the dietary SP and a* and b* values increased by the addition of 2.5%SP to diet. The highest values of breast meat pH, cook loss (CL), and water holding capacity (WHC) were determined in the control group (P<0.05). ABT reading against the ND was similar between all vaccinated groups independently from the level of dietary SP but significantly low in the NC group (P<0.05). In a conclusion, the use of SP in quail diets at the level of 2.5% may positively influence performance, carcass weight breast meat quality parameters, colour values.

Keywords: Spirulina, meat color, meat quality, immune response

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Date of initial submission: 27-07-2021
Date of acceptance: 09-12-2021

INTRODUCTION

Microorganisms, that can synthesize nutrients from simple organic and inorganic substances, have great potential to solve the problems of food deficiency in the world. Especially, high protein and essential amino acid content products that formed because of microbial activity have promised hope to provide protein sources for human and animal nutrition. Moreover, single-cell organisms such as algae use wastes as substrates, can turn them into economically valuable products, and reduce environmental pollution. Edible blue-green algae, including Nostoc, Spirulina, and Aphanizomenon species have been used for food for thousands of years(Vonshak 1997). Spirulina (*Arthrospira sp*.) is an edible, filamentous, spiral-shaped cyanobacterium, formally classified as a blue-green microalga(Becker 2007; Gupta et al. 2008; Sousa et al. 2008). Spirulina may be found naturally in the alkaline lakes of Mexico and Africa (Belay et al. 1996; Shimamatsu 2004). It shows a high nutritional content characterized by a 70% protein content and by the presence of minerals, vitamins, amino acids, essential fatty acids,etc.(Campanella et al. 1999). Commercially, Spirulinais produced in nutrient-rich liquid media(Shimamatsu 2004) and, can be efficiently reproduced in fattening media enriched with desalinated wastewater and animal feces(Volkmann et al. 2008). When compared with other feed ingredients including wheat, corn, barley, and soybean its protein output per land unit is higher. Furthermore, it has been reported that spirulina produced using pig and bovine feces can be used safely in animal nutrition(Mitchell and Richmond 1988; Boiago et al. 2019)Previous knowledge demonstrated that spirulina has the potential to be effectively recycled as waste materials, human or animal food(Saxena et al. 1983).The most expensive component of the feed is the protein in livestock diets. Apart from many valuable nutrients, spirulina contains a high amount of protein. The high amount of protein it contains can be extremely beneficial, especially if it can be used in poultry feeding. Therefore, this study was conducted to determine the effects of gradually increasing levels of dietary SP on the growth performance, breast meat color values, meat quality, and the immune responses of quails.

MATERIALS AND METHODS

The animal experiment was conducted at the application farm facility of Selçuk University Agriculture Faculty. The animal experiment was carried out according to the local ethics committee directives of Selçuk University. In this study, a total of two hundred and fifty 1-day old quail (*Coturnix coturnix Japonica*) chicks were used. Quail chicks were randomly allocated to one of the five dietary groups and each treatment group had 50 quails with 5 replicates. In the experiment, feed and water were given *ad-libitum*, and a "23 h light-1 h dark" lighting program was used for 5 weeks growth period. The quails were reared in 33 x 40 x 28 cm size cages and under the semi-controlled environment terms (ventilation controlling system) and every compartment of cages had a water nipple, manger, and heater.

To determine the effects of different levels of dietary SP on serum HI-antibody titers of quails, the Newcastle Disease vaccine was applied to quails in the experimental groups with the eye drop method on the 7th and 21st days of the experiment. The experimental group diets were formulated and named according to SP inclusion levels and vaccination status as; The negative control group (NC) (Basal diet, 0% SP, no vaccine), the control group (C) (Basal diet, 0%SP, vaccinated), the SP1 group (basal diet, 2.5% SP, vaccinated), the SP2 group, (basal diet 5% SP vaccinated) and the SP3 group (basal diet 7.5 % SP vaccinated).

All experimental diets formulated nutrient requirements of quails in growth period according to (Council 1994) as to be isocaloric and isonitrogenous. The nutrient compositions of experimental diets are summarized in Table 1.Spirulina (*Spirulina platensis*) powder form (100% pure) we supplied from a commercial dealer (Sepe Natural®-Turkey) that contains 93% dry matter (DM) and 62% crude protein, 1.7 % crude fiber, 4.2 % lipids, and 1600 mg/kg total carotenoids of DM Table 1.Nutrient composition of experimental growth period diets.

On the first day of the experiment quail chicks were weighed and recorded as initial body weight (IBW). During five weeks trial, body weights (BW) and, feed intake (FI) of the quails were recorded by weighing the subgroups. Body weight gain (BWG) was calculated from differences of BW, feed conversion ratio (FCR) was calculated with FI/BWG ratio. The final body weight (FBW) of quails was weighed on the last day of the experiment and presented.

On the last day of the experiment 3 quails from each replicate, 15 from each treatment group, and a total of 75 quails were selected randomly and slaughtered. Using a vacuum tube blood samples were collected. The samples were centrifuged at 3000 rpm for 10 minutes at room temperature. HI-antibody titers were determined H-Agglutination test. H-Agglutina-

Table 1. The nutrient compositions of experimental diets

	Experimental Diets				
Ingredients (%)	NC	C	SP1	SP2	SP3
Corn	47.95	47.95	50.65	53.10	55.95
Soybean meal	44.50	44.50	40.20	36.10	31.80
Spirulina	0.00	0.00	2.50	5.00	7.50
Crude soybean oil	4.40	4.40	3.50	2.65	1.60
Limestone	1.20	1.20	1.20	1.30	1.40
DCP	1.00	1.00	1.00	0.90	0.80
Salt	0.30	0.30	0.30	0.30	0.30
Premiks[1]	0.25	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20
TOTAL	**100**	**100**	**100**	**100**	**100**
Calculated Nutrients					
Crude Protein, %	24.06	24.06	24.04	24.08	24.7
Metabolizable Energy, kcal/kg	2909	2909	2912	2916	2911
Calcium,%	0.87	0.87	0.86	0.86	0.86
Available phosphorus, %	0.36	0.36	0.39	0.39	0.40
Total Methionine, %	0.51	0.51	0.54	0.56	0.59
Methionine+Cysteine, %	0.83	0.83	0.81	0.80	0.79
Total Lysine, %	1.33	1.33	1.32	1.31	1.30

NG: Negative control, C: Control, SP1: 2.5% spirulina, SP2: 5%spirulina, SP3: 7.5% spirulina.
1Vitamin-mineral premix (per kilogram of diet): Vitamin A 15000 IU; Vitamin D3 1500 IU; Vitamin K 5 mg; Vitamin B1 3 mg; Vitamin B2 6 mg; Vitamin B6 5 mg; Vitamin B12 0.03 mg; Niacin 30 mg; Biotin 0.1 mg; calcium D-pantothenate 12.0 mg; folic acid 1.0 mg; choline chloride 400 mg; Manganese 80 mg; Iron 35 mg; Zinc 50 mg; Copper 5.0 mg; Iodine 2 mg; Cobalt 0.04 mg.

Table 2. Effects of different levels of dietary spirulinaon growth performance of quails

	NC	C	SP1	SP2	SP3	Pooled SE	P
IBW(g)	8.40	8.38	8.30	8.30	8.50	0.14	0.193
FBW(g)	171.73^{b}	171.06^{b}	181.62^{a}	175.04^{b}	173.87^{b}	2.62	0.001
TBWG(g)	163.33^{b}	162.68^{b}	173.32^{a}	166.74^{b}	165.37^{b}	2.60	0.001
FI(g)	450.76^{d}	449.16^{d}	462.48^{c}	472.48^{b}	488.96^{a}	5.23	0.001
FCR	2.76^{bc}	2.76^{bc}	2.66^{c}	2.83^{b}	2.95^{a}	0.05	0.001

NG: Negative control, C: Control, SP1: 2.5% spirulina, SP2: 5%spirulina, SP3: 7.5% spirulina.
IBW: initial body weight, FBW: final body weight, TBWG: total body weight gain,FI: feed intake, FCR: feed conversion ratio
a, b, c, d: The difference between averages that get different lowercase letters in the same line is significant ($P<0.05$).

tion tests were performed in U-bottomed 96-well microplates (Greiner) using 50 µL reagents from serum obtained from blood samples according to the method reported by Allan and Gough (1974) and Arda (1976). The HI titer was evaluated according to $\log_2$ base.

After slaughtering of animals visceral removed from body and carcass weights were determined. Carcass yields were computed with carcass weight and FBW ratio. Liver and bursa fabricius were removed from the body, weighed, and recorded. L *, a * and b * values of breast meat determined by colorimeter(CR-400 Minolta Co, Osaka, Japan) (Hunt et al. 1991). The L*, a*, and b* parameters correspond to the lightness (-100/+100, dark/white), redness (-100/+100, green/red), and yellowness (-100/+100, blue/yellow), respectively and pH values(HI 8314) of breast meat samples(24 h postmortem) detected by portable pH-meter (WTW 2A20-1012 Waterproof pH-Meter). The water holding capacity (WHC) of the samples was determined according to the method of Wardlaw et al. (1973) and the cooking loss (CL) according to the method of Kondaiah et al. (1985).

The trial was designed as a complete randomized model and data were analyzed by using the ANOVA procedure with Minitab(Minitab 2000). Tukey test was used to determine the differences among treatments which were found to be significantly different ($P<0.05$).

RESULTS AND DISCUSSION

The effects of different levels of dietary SP on growth performance were given in Table 2. The FBW of the SP1 group was significantly higher than the other groups but the difference between the other groups was not significant.FI increased with the supplemental SP in diet and the highest FI was determined in the SP3 and results showed that FI raised with the increasing SP level of diet(P <0.05).

The FCR was found to be significantly higher in the SP3 group compared to the other groups with the addition of SP to the diet. Although the lowest value was in the SP1 group, the difference between the NC and C groups was not statistically significant. Abouelezz (2017) reported that adding SP to quail diets and drinking water during the growth period affected positively the performance. Hajati and Zaghari (2019) concluded that the use of SP in the diets of Japanese quails improved their growth performance. It was reported that adding 1 or 2 g / kg SP to quail diets improved the performance (Yusuf et al. 2016). Gongnet et al. (2001) investigated the effects of the addition of SP to broiler diets instead of soybean meal and at the level of 0, 50, 100, and 150 g / kg -feed and reported that FI decreased at 100 and 150 g / kg inclusion levels, BW was 80% less than the control group. Dogan et al. (2016) determined the effects of SP addition to laying quail diets on performance, egg quality, and serum parameters and, they reported that while feed efficiency was not affected by feed intake significantly, body weight was significantly affected (P <0.05). It was reported that adding spirulina at the level of 4% to the quail diet may be an appropriate dose to achieve the best performance (Cheong et al. 2016). Toyomizu et al. (2001) reported that performance was not affected by the addition of 0, 40, 80 g / kg levels of spirulina to broiler diets. Previous studies mentioned above confirmed that SP may be used in the growth period of poultry depending on the dietary level. Our results proved that dietary level of SP may be effective on growth performance and revealed that 2.5% supplementation of SP in diet may be appropriate for quail's growth. The data regarding the use of different levels of dietary SP on the slaughter parameters are presented in Table 3. SP additions to quail diets at different levels did not significantly affect the carcass yield, liver and bursa fabricius weights. However, carcass weight changed with inclusion of SP in diet and the highest carcass weight was determined in the SP1 group.

Studies regarding the effect of dietary SP on carcass features of poultry have been limited. However, some studies reported coherent results with the current study. Cheong et al. (2016) reported that adding 4% spirulina to quail diets caused the heaviest breast and thigh weights. Similarly, with our results, Yusuf et al. (2016) reported that the addition of SP to quail diets did not significantly affect the carcass yield. Hajati and Zaghari (2019)concluded that the carcass yield of quails was increased with the 2.5% SP addition to the diet. In this study, 2.5% SP addition to quail diet significantly increased the carcass weight compared to other groups. 2.5% SP in the diet as parallel with BW caused better carcass weight and improved meat production of quails.

Table 3. The effect of different levels of dietary spirulina on slaughter parameters of quails

	NC	C	SP1	SP2	SP3	Pooled SE	P
Carcass(g)	125.39^{b}	127.45^{b}	136.13^{a}	126.39^{b}	127.45^{b}	4.31	0.006
Carcass yield (%)	73.01	74.47	74.96	72.21	73.30	2.05	0.249
Liver(g)	3.87	4.08	4.41	4.00	4.11	0.56	0.665
Liver (%)	2.25	2.38	2.42	2.29	2.36	0.32	0.916
Bursa(g)	0.23	0.27	0.26	0.26	0.28	0.06	0.784
Bursa (%)	0.13	0.15	0.14	0.15	0.16	0.03	0.785

NG: Negative control, C: Control, SP1: 2.5% spirulina, SP2: 5% spirulina, SP3: 7.5% spirulina. Percentage values of liver and bursa were given percent of body weight.
.a, b:The difference between averages that get different lowercase letters in the same line is significant (P<0.05).

Table 4. Effects of different levels of dietary SP on meat quality parameters and color values of quail breast meat

	NC	C	SP1	SP2	SP3	Pooled SE	P
L*	58.39	59.31	62.28	60.93	60.76	3.464	0.456
a*	6.32^{b}	6.48^{b}	7.07^{a}	6.58^{b}	6.42^{b}	0.244	0.001
b*	7.33^{b}	7.32^{b}	8.16^{a}	7.64^{b}	7.61^{b}	0.223	0.001
pH	5.77^{a}	5.79^{a}	5.73^{ab}	5.67^{bc}	5.61^{c}	0.044	0.001
CL (%)	21.67^{a}	21.94^{a}	20.24^{ab}	20.13^{ab}	18.78^{b}	1.195	0.003
WHC (%)	25.91^{ab}	28.35^{a}	23.30^{abc}	20.99^{bc}	17.10^{c}	3.362	0.001

NG: Negative control, C: Control, SP1: 2.5% spirulina, SP2: 5% spirulina, SP3: 7.5% spirulina.CL: Cook loss, WHC:Water holding capacity.
a, b, c:The difference between averages that get different lowercase letters in the same line is significant (P<0.05).

Dietary SP and its effects on breast meat color values L*, a*, b* are given in Table 4. Different levels of SP in quail diets, did not effective on L* value, however, the a* and b* values of breast meat changed by every level of dietary SP(P <0.05). Both, the highest a* and b* values were found in the SP1 group (P <0.05). Inclusion of SP to diet at the 5 and 7.5 % additional levels did not make a difference to control groups in terms of meat color values.

Toyomizu et al. (2001) reported that 40g/kg spirulina addition to broiler diets increased significantly yellowness and redness in the muscles. Venkataraman et al. (1994) concluded that the addition of spirulina in broiler diets increased the density of skin color. As a result of the experiment in which Zahroojian et al. (2011)investigated the effects of spirulina and synthetic color pigment addition to egg hen rations on egg yolk color. They found that the group to which spirulina was added at a level of 2.5% did not differ significantly from the group to which synthetic color pigment was added in terms of egg yolk color. It has been reported that adding 1% spirulina to broiler diets between the ages of 35-42 days would be more effective in the possibility of obtaining optimum skin and fat coloring to the consumer preference(Valdivié and Dieppa 2001). In their experiments conducted to determine the effects of spirulina addition in adult quail diets on egg yolk pigmentation. At the end of the trial, the group with 1% SP added had optimum egg yolk pigmentation(Anderson et al. 1991). Boiago et al. (2019)in their experiments to reported that Spirulina supplementation to diets increased egg yolk color pigmentation(P <0.05). Spirulina contains high levels of carotenoids and xanthophylls, as can be guessed from its color. It is a natural result that these color pigments affect meat color(Toyomizu et al. 2001). The highest redness (a) and yellowness (b) values were measured in the group with the 2.5% SP, and the difference between the other groups was statistically significant (P <0.05).

Data regarding the effects of dietary SP on meat pH value, cooking loss, and water holding capacity are presented in Table 4. Spirulina addition to quail diets at different levels significantly affected all meat quality parameters (P< 0.05). Increasing SP amount in the diet decreased the pH value of meat and the lowest pH was observed with 7.5% SP inclusion to diet (P<0.05). CL and WHC of meat decreased with the addition of SP in the diet and the lowest values were found in the SP3 group (P<0.05).

It is well known that feed ingredients may affect meat color and quality and, meat color is an important factor that can influence consumers' behaviors. On the other hand, meat quality parameters such as pH and WHC have a relation with the shelf life of meat. Previous studies reported various results about relation dietary SP and meat quality. Yusuf et al. (2016) reported that 1 or 2 g additional SP in corn-soy-based quail diets did not affect meat quality parameters. But, Cheong et al. (2016) reported that 4% of supplemental spirulina in the quail diet has resulted in the best meat quality. The quality of meat is shaped by a complex interaction between the genotype of the animal from which it is obtained and the environment (Bihan-Duval 2004). Meat texture is affected by quality parameters such as CL, WHC. Low cooking loss and high water holding capacity positively affect the desired meat texture. And low pH indicates low water holding capacity. In this study SP incorporation in the diet significantly decreased meat pH, CL and WHC compared to the control group and improved meat quality. Results showed that different levels of supplemental SP caused better meat quality and additional SP in diet may be used to improve the quality of meat in quails.

The effects of different levels of dietary SP on HI-antibody immunity titers results against the Newcastle disease are summarized in Table 5. The effects of different levels of dietary SP between vaccinated groups on HI-antibody immunity titers were found insignificant. The ABT was found significantly lower in the NC group than vaccinated groups including the C group (P <0.05).

It was reported that the addition of 0, 10, 100, 1000, and 10000 ppm levels of spirulina to the diets of laying hens and broilers increased some immune responses, and especially the addition of 10000 ppm spirulina can increase the potential resistance against diseases (Qureshi et al. 1996). Al-Batshan et al. (2001) concluded that SP addition to broiler diets may be increased the functions of the mononuclear phagocytic system and thus may improve the disease-resistance potential in chickens. Previous studies in poultry showed that SP supplementation to the diets improved immune symptoms(Hayashi et al. 1998).

Table 5. Effect of different levels of SP supplementation in diet on HI-antibody immunity titers of quails against Newcastle disease

	NC	C	SP1	SP2	SP3	Pooled SE	P
Hi-antibody immunity titers	0.00^b	7.264^a	6.664^a	6.598^a	6.864^a	1.008	0.001

NG: Negative control, C: Control, SP1: 2.5% spirulina, SP2: 5% spirulina, SP3: 7.5% spirulina.
a, b:The difference between averages that get different lowercase letters in the same line is significant (P<0.05).

In the current study differences of HI-antibody titers between C and SP supplemented groups compared to the grouping NC groups (without vaccination) were significant. The HI-antibody titer of C was not statistically different from the SP addition groups. This result is not compatible with the literature and shows that the addition of SP to quail diets did not affect immunity against the Newcastle disease.

CONCLUSION

In conclusion results of this study showed that SP addition to the quail's diet in the fattening period, affected positively the performance, meat quality, and meat color. Also, outcomes revealed that employed levels of dietary SP are an effective factor and directly influence performance and meat quality. According to the data of the current study 2.5%, SP in quail's diet may use to improve performance and meat parameters. In a nutshell, SP seems a potential feed ingredient for poultry in the growth period, and clearly, further research isrequired to display its effects on poultry nutrition.

ACKNOWLEDGEMENT

This project was supported by a grant from the Scientific Project (BAP)(Project number: 19401018) coordinating Office of Selçuk University, Turkey

CONFLICT OF INTEREST

The author declares no conflict of interest.

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