Acta Scientiarum

http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v46i1.63040

NONRUMINANT NUTRITION

Spirulina in diets of Japanese quail: Productive performance, digestibility, and egg quality

Maria Cristina de Oliveira[1*], Matheus Andrade Dias Oliveira[1], Nathália Rodrigues Gonçalves[2], Pauliny do Carmo Ferreira[1], Diego Silva Lima[1] and Uilcimar Martins Arantes[1]

[1]Fazenda Fontes do Saber, Universidade de Rio Verde, Campus Universitário, Cx Postal 104, 75901-970, Rio Verde, Goias, Brazil. [2]Universidade Federal de Goiás, Goiânia, Goiás, Brazil. *Author for correspondence. E-mail: mcorv@yahoo.com.br

ABSTRACT. The present study evaluated the effects of dietary inclusion of spirulina on productive performance, nutrient utilization, and egg quality of Japanese quails. One hundred twenty quails were included in a completely randomized design with four treatments and five replicates. Treatments consisted of a control diet and three diets containing 0.3, 0.6, or 0.9% of spirulina powder. Spirulina inclusion had no effect ($p > 0.05$) on the productive performance, digestibility coefficients of dry matter and ether extract, calcium and phosphorus retention, weight and pH of the eggs, yolk index, albumen quality, and eggshell thickness when compared with the control diet. However, crude protein consumption was higher ($p < 0.05$) and the digestibility coefficient of crude protein was lower ($p < 0.05$) in birds in the 0.9% and 0.3% spirulina inclusion groups, respectively. The weights of the yolk and eggshell were improved ($p < 0.007$) by the inclusion of 0.6% and 0.9% and 0.9% spirulina, respectively. Quails fed diets containing 0.3% and 0.6% spirulina exhibited lower specific gravity of eggs ($p < 0.019$) than those fed diets containing 0.9% spirulina. Therefore, spirulina inclusion at 0.9% improved the eggshell quality and reduced the total lipid levels in the yolks.

Keywords: *Arthrospira platensis*; nutrient utilization; quail nutrition.

Received on March 25 2022.
Accepted on January 17 2023.

Introduction

Concerns regarding food safety and the use of antibiotic growth promoters (AGPs) in animal feed have promoted a demand for products originating from animals raised without AGP (Ritter et al., 2019). Many studies have investigated alternatives to AGP for animal production, one of which is *Spirulina platensis*.

Spirulina refers to a large number of bacteria in the phylum Cyanobacteria. It comprises 35 identified species, the most common being *S. maxima, S. platensis,* and *S. fusiformis*. They were previously known as blue-green algae; however, it is now known that they have no phylogenetic relation to the algae group (Oliveira, Campos, Ribeiro, Oliveira, & Nascimento, 2013). The name "spirulina" is based on the spiral or helicoidal nature of their filaments (Karkos, Leong, Karkos, Sivaji, & Assimakopoulos, 2011).

These bacteria show anti-inflammatory and antiviral activities *in vitro,* as well as hypocholesterolemic, antitoxic (Karkos et al., 2011; Martínez-Galeroet al., 2016), antiproliferative (Abd El-Hack et al., 2019), antioxidant (Asghari, Fazilati, Latifi, Salavati, & Choopani, 2016; Gabr, El-Sayed, & Hikal, 2020), immunomodulatory (Wu et al., 2016), and hypoglycemic effects (Bitam & Aissaoui, 2020).

The chemical composition of *S. platensis* consists of 37.55-76.65% crude protein, 2.45-8.03% ether extract, 8.40-14.56% ash, 0.46-1.50% calcium, 0.96-1.06% total phosphorus, and 2560 kcal kg^{-1} metabolizable energy (Alvarenga et al., 2011; Gutiérrez-Salmeán, Fabila-Castillo, Chamorro-Cevallosl, 2015; Moor et al., 2016; Marrez, Naguib, Sultan, Daw, & Higazy, 2014; Seghiri, Kharbach, & Essamri, 2019), in addition to vitamins, other minerals and zeaxanthin, beta-carotene, γ-linolenic acid, superoxide dismutase enzymes, chlorophyll and phycocyanin (Gutiérrez-Salmeán et al., 2015), and several phenolic compounds (Moor et al., 2016; Seghiri et al., 2019). Hence, spirulina is a good source of nutrients for both birds and humans.

Spirulina can be used as an additive to meet nutritional requirements, activate the endocrine system, and increase growth rate and intermediary nutrient metabolism (Yusuf, El Nabtiti, & Cui, 2016). Spirulina has several positive effects on the productive performance of and nutrient utilization by poultry. Japanese quails fed diets containing 1 to 8% spirulina during the rearing period showed better weight gain, feed consumption, and feed: weight gain ratio (Cheong, Kasim, Sazili, Omar, & Teoh, 2016; Kanagaraju & Omprakash, 2016; Abouelezz, 2017).

Data from the literature regarding the effects of spirulina on nutrient utilization by quails are scarce. Dalle-Zotte, Sartori, Bohatir, Rémignon, and Ricci (2013) reported that only lipid digestibility is reduced upon inclusion of 0.3% spirulina in the diets of rabbits. Park, Lee, and Kim (2018) observed a linear improvement in the digestibility of dry matter and nitrogen when *S. platensis* levels were increased from 0.25 to 1% in the diets of broilers.

Omri et al. (2019) demonstrated positive effects on the egg quality of laying hens fed diets containing 2.5% of spirulina. The birds produced heavier eggs with higher albumen weight and height, thicker eggshells, and better Haugh unit values.

In studies with quails, Hajati and Zaghari (2019) evaluated diets with 0.1, 0.3, and 0.5% spirulina and reported a decline in yolk and eggshell weight, eggshell thickness, Haugh unit value, and albumen and yolk heights; however, cholesterol levels were lower in the yolk (7.74 and 6.56 mg g^{-1}, respectively, for the control and diet containing 0.3% spirulina). Zahroojian, Moravej, and Shivazad (2013) did not observe any differences in the yolk index, Haugh unit, or eggshell upon inclusion of 1.5 to 2.5% spirulina in the diet of hens.

The present study evaluated the effects of inclusion of *S. platensis* in the diets of Japanese quails on productive performance, nutrient utilization, and egg quality.

Material and methods

The research was approved by the institutional committee on animal use (case number 03/19, approved on May 7, 2019) of the University of Rio Verde.

A total of 120 Japanese quails (50 days old; initial body weight of 131.8 ± 2.3 g) were included in this study, which lasted a total of 84 days. Birds were randomly divided into four treatment groups with five replicates each, and there were six birds per replicate. All quails in group 1 (the control) were fed a basal diet (Table 1) without spirulina, while those in groups 2 to 4 were fed the same basal diet supplemented with 0.3, 0.6, and 0.9% of spirulina powder, respectively.

Table 1. Composition and chemical analysis of the basal diet offered to Japanese quail.

	Spirulina levels (%)			
Ingredient	0.0	0.3	0.6	0.9
Yellow corn	53.11	53.11	53.11	53.11
Soybean meal	32.50	32.50	32.50	32.50
Soybean oil	3.20	3.20	3.20	3.20
Limestone	7.40	7.40	7.40	7.40
Dicalcium phosphate	1.20	1.20	1.20	1.20
NaCl	0.40	0.40	0.40	0.40
DL-methionine, 99%	0.20	0.20	0.20	0.20
L-lysine, 78.8%	0.29	0.29	0.29	0.29
Premix[1]	0.80	0.80	0.80	0.80
Spirulina[2]	0.00	0.30	0.60	0.90
Inert	0.90	0.60	0.30	0.00
Total	100	100	100	100
Calculated nutritional composition				
Crude protein, %	19.32	19.48	19.64	19.80
Metabolizable energy, kcal kg^{-1}	2876	2876	2876	2876
Calcium, %	3.17	3.17	3.17	3.17
Available phosphorus, %	0.33	0.33	0.33	0.33
Sodium, %	0.17	0.17	0.17	0.17
Total lysine, %	1.29	1.29	1.29	1.29
Total methionine, %	0.78	0.78	0.78	0.78
Total methionine + cystine, %	1.08	1.08	1.08	1.08

[1]Enriched with: 10,000 IU vitamin A; 3,000 IU vitamin D_3; 25 IU vitamin E; 3 mg vitamin K_3; 1.96 mg vitamin B_1; 6.4 mg vitamin B_2; 2.94 mg vitamin B_6; 20 mg vitamin B_5; 35 mg vitamin B_3; 1.2 mg folic acid; 0.1 mg biotin; 0.363 g choline; 0.48 g lysine; 3 g methionine; 300 U phytase; 70 mg Mn; 60 mg Zn; 25 mg Fe; 12 mg Cu; 1.23 mg I; 0.33 mg Se; 30 mg halquinol; 3.6 mg antioxidant; and 0.12 g Mannan oligosaccharides . [2]Spirulina was included in the diet at the expense of the inert. [3]According to Rostagno et al. (2017).

The spirulina contained 9% moisture, 40% crude protein, 3.33% lipids, 0.22% calcium, 0.44% total phosphorus, and 3300 kcal kg^{-1} gross energy. Analysis of the spirulina was performed according to Silva and Queiroz (2002) and that of the lipids according to Bligh and Dyer (1959).

The birds were housed in galvanized wire cages (25 L × 15 H × 33 W cm) equipped with gutter-type feeders and drinkers and had free access to feed and water. Birds were maintained under 15h light for the first seven days, following which the light duration was gradually increased by 30 min. every seven days until it reached 17h, which was maintained until the end of the study period.

The excreta produced by 90-95-day-old birds were collected twice a day to evaluate digestibility. The rations were weighed at the beginning and end of the total excreta collection period to calculate average feed intake. The cages were lined with trays coated with properly identified plastics layers, which were removed and replaced after each collection (12-h interval), and the collected excreta were stored in freezers. Excreta were subsequently thawed, homogenized, weighed, dried in a forced-ventilation oven for 72h at 55°C, and ground for further analyses. The excreta and feed samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), gross energy (GE), calcium (Ca), and phosphorus (Pt) contents as described by Silva and Queiroz (2002) to estimate the metabolic coefficients of the nutrients and energy present in the diet. Apparent metabolizable energy corrected for nitrogen balance was also calculated (AMEn) as follows: AMEn (kcal kg^{-1}) = [GEingested − GEexcreted + 8.22 × NB]/DM intake, in which NB is the nitrogen balance (ingested nitrogen − excreted nitrogen).

The evaluated productivity parameters were feed intake (g/d), feed conversion (kg kg^{-1} eggs and kg dozen $eggs^{-1}$), laying rate (%), and egg mass (g $bird^{-1}$ d^{-1}). The number and weights of eggs were recorded daily during the study period, and egg mass was calculated by multiplying the number of laid eggs by their weight.

Six days before the end of the experimental period, 10 eggs from each replicate were collected for the analysis of DM, CP, EE, and ash. The egg content was placed in a recipient, homogenized and dried in a forced-ventilation oven for 7 days at 55°C, and ground for further analyses, as described by Silva and Queiroz (2002) and Bligh and Dyer (1959).

Two hundred normal eggs were randomly selected from the eggs laid during the final three days of the study (four groups × five replicates per group × ten eggs per replicate) to assess the following qualitative traits: weight (g), specific gravity (g cm^{-3}), pH, the Haugh unit of the egg, yolk and albumen weight (g) and index, and eggshell weight (g) and thickness (mm). The specific gravity of eggs was determined by immersing them in saline solutions of different densities (1.05-1.10 g cm^{-3}). The Haugh unit was calculated using the following formula: $HU = 100 \times \log (H - 1.7 \times W^{0.37} + 7.6)$, in which HU is the Haugh unit, H is the albumen height (mm), and W is the egg weight (g).

Albumen weight (g) was calculated as the difference between the weight of the entire egg and the combined weight of the yolk and eggshell (g). Percentages of yolk, albumen, and eggshell were determined by the following formula: yolk (%) = [yolk weight (g)/egg weight (g)] × 100. Albumen and eggshell weights were substituted in the formula as necessary. Yolk and albumen indices were determined by dividing their heights (mm) by their respective diameters (mm), with height and diameter being measured using a digital caliper.

After the egg content was removed, eggshells were dried and weighed, and shell thickness (mm) was obtained by calculating the mean of three thickness measurements taken at three different points with a digital caliper (accuracy of 0.01 mm): at the two poles and in the lateral region of the egg.

Data were subjected to an analysis of variance using SNK tests at a 5% probability level with the Sisvar computer package version 5.6 (Ferreira, 2011).

Results and discussion

Spirulina had no significant effect ($p > 0.05$) on the productive performance of the birds, except that inclusion of 0.9% spirulina in the diets resulted in a higher ($p < 0.05$, 25% increase) daily protein consumption than that resulting from the control diet (Table 2). The diets supplemented with spirulina had increased levels of crude protein, which resulted in higher protein consumption even without an increase in ration consumption.

The results of this study are consistent with the findings of Omri et al. (2019) and Hajati, Zaghari, and Oliveira (2020) that included 0.1 to 2.5% spirulina in the diets of quail, resulting in no notable differences in the productive performance of the birds. However, Cheong et al. (2016) noted an increase in feed consumption in quail fed diets containing 1, 2, 4, and 8% spirulina than that fed the control diet. Selim, Hussein, and Abou-Elkhair (2018) observed better laying rates and egg mass in laying hens fed diets containing 0.1 to 0.3% spirulina. Hajati and Zaghari (2019) reported higher ration consumption in quail due to the dietary inclusion of 0.5 to 2% of spirulina.

Table 2. Productive performance of Japanese quail fed diets supplemented with spirulina.

Parameters	Spirulina levels (%)				SEM	P value
	0.0	0.3	0.6	0.9		
DRC (g d^{-1})	30.38	33.40	32.18	36.93	1.72	0.090
DPC (g d^{-1})	5.87 b	6.57 ab	6.37 ab	7.35 a	0.34	0.047
DEC (kcal d^{-1})	114	124	123	134	9	0.515
FC (kg kg^{-1})	3.05	3.32	3.28	3.55	0.24	0.555
FC (kg $dozen^{-1}$)	0.433	0.475	0.473	0.519	0.034	0.405
LR (%)	84.52	86.86	83.72	85.78	6.11	0.984
EM (g^{-1} $bird^{-1}$ d^{-1})	10.02	10.41	10.08	10.41	0.79	0.975

DRC – daily ration consumption; DPC – daily protein consumption; DEC – daily energy consumption; FC – feed conversion; LR – laying rate; EM – egg mass. SEM – standard error of the mean. [a, b] Means followed by different letters differ by the SNK test.

Treatments did not significantly affect ($p > 0.05$) the coefficient of digestibility of dry matter and ether extract, as well as the retentions of calcium and phosphorus; however, the digestibility of crude protein was lower ($p < 0.05$) in birds fed diets containing 0.3% spirulina than those fed the control diet (Table 3).

The cell wall of *Arthrospira platensis* is made up of an envelope composed of several layers of peptidoglycans and lipopolysaccharides (Coelho et al., 2019), which have low digestibility in monogastrics (Coelho et al., 2020) and may encapsulate nutrients, reducing their digestion and absorption (Madeira et al., 2017).

Table 3. Digestibility of nutrients and retention of minerals in quail fed diets containing spirulina.

Parameters	Spirulina levels (%)				SEM	P value
	0.0	0.3	0.6	0.9		
CDDM (%)	74.41	65.80	69.80	71.29	2.26	0.124
CDCP (%)	89.54a	85.83b	89.12a	87.27a	0.98	0.046
CDEE (%)	94.20	95.37	96.85	93.07	1.71	0.450
RCa (%)	79.25	73.71	75.97	76.43	2.46	0.524
RP (%)	45.44	45.90	38.72	47.10	4.21	0.719

CDDM – coefficient of digestibility (CD) of dry matter; CDCP – CD of crude protein; CDEE – CD of ether extract; Ca – retention of calcium; RP – retention of phosphorus. SEM – standard error of the mean. [a, b] Means followed by different letters differ by the SNK test, at 5% probability.

Although the diets containing 0.6% and 0.9% of spirulina may have had an antinutritional factor related to the cell wall, there was a higher crude protein level available for absorption in these diets. Authors have reported that higher levels of crude protein in the rations result in higher digestibility of these nutrients in quails (Li et al., 2011; Dowarah & Sethi, 2014; Rabie & Abo El-Maaty 2015).

Literature on how diets containing spirulina affect digestibility in quails is scarce; nevertheless, Gerencsér et al. (2014) studied the influence of 5% spirulina inclusion in the diets of rabbit and verified higher digestibility of crude protein, lipids, and fiber, as well as higher retention of calcium and phosphorus. In a study with broilers, Park et al. (2018) observed a linear increase in the digestibility of nitrogen and dry matter owing to the inclusion of 0.25%-1% of spirulina in their diets.

Spirulina inclusion in the diets had no significant effect ($p > 0.05$) on the weight and pH of the eggs (Table 4). The obtained values were in agreement with the reference values for quail eggs (Yannakopoulos & Tserveni-Goussi, 1986; Narinc, Aygun, Karaman, & Aksoy, 2015; Hanusová, Hmcár, Hanus, & Orovcová, 2016; Hegab, & Hanafy, 2019), implying that spirulina supplementation was inefficient in improving the weight and pH of the eggs in this study.

Table 4. Egg quality in quails fed diets containing spirulina.

Parameters	Spirulina levels (%)				SEM	P value
	0.0	0.3	0.6	0.9		
Egg weight, g	11.85	11.95	12.04	12.14	0.11	0.360
pH	6.80	6.75	6.75	6.75	0.08	0.964

SEM – standard error of the mean.

Abouelezz (2017), Hajati and Zaghari (2019), and Boiago et al. (2019) noted that 1, 5 and 10, and 15% of dietary spirulina, respectively, had no impact on the weight and pH of quail eggs.

Spirulina levels did not significantly affect ($p > 0.05$) the yolk and albumen indexes, albumen weight, Haugh unit, and eggshell thickness; however, supplementation with 0.6% and 0.9% spirulina improved ($p < 0.001$) yolk weight, and supplementation with 0.9% spirulina improved ($p < 0.007$) eggshell weight and specific gravity (Table 5) compared to the other treatments.

Table 5. Quality of the yolk, albumen, and eggshells of eggs from quail fed diets containing spirulina.

Parameters	Spirulina levels (%)				SEM	P value
	0.0	0.3	0.6	0.9		
Yolk weight, g	3.76 b	3.95 b	4.74 a	4.33 a	0.16	0.001
Yolk index	0.454	0.460	0.499	0.531	0.051	0.667
Albumen weight, g	6.25	6.98	6.27	6.71	0.65	0.818
Albumen index	0.099	0.106	0.117	0.126	0.001	0.309
Haugh unit	89.58	93.98	89.96	90.18	8.08	0.454
Eggshell weight, g	1.00 b	1.01b	1.02b	1.10 a	0.02	0.007
Eggshell thickness, mm	0.233	0.235	0.241	0.246	0.007	0.594
Specific gravity, g cm^{-3}	1.080 ab	1.079 b	1.079 b	1.081 a	0.001	0.019

SEM – standard error of the mean. [a,b] Means followed by different letters differ by the SNK test, at 5% probability.

The cell wall of *Arthrospira platensis* is less digestible in monogastric animals (Coelho et al., 2019), causing lower efficiency in the use of nutrients (Madeira et al., 2017); this was most likely the reason for the lower nutrient deposition on the yolk following treatment with 0.3% spirulina. In the treatments with 0.6 and 0.9% spirulina, the amount of nutrients, particularly crude protein, available for absorption was higher, thus improving the nutrient deposition in the yolk. Similar results were found by Mariey, Samak, and Ibrahem (2012).

The determination of specific gravity and eggshell weight are the most common techniques to evaluate eggshell quality owing to their speed, practicality, and low cost (Kibala, Rozempolska-Rucinska, Kasperek, Zieba, & Lukaszewicz, 2018). The higher eggshell weight in birds fed diets containing 0.9% spirulina compared to the other treatments is likely due to a higher calcium absorption in the birds receiving spirulina diet. Ekantari, Harmayani, Pranoto, and Marsono (2017) demonstrated that spirulina has the potential to supplement calcium, magnesium, and phosphorus in rats subjected to mineral deficiency, and, according to the authors, the calcium in spirulina showed higher bioavailability than other calcium sources in rats subjected to vitamin D deficiency.

Higher eggshell weight was noted by Dogan et al. (2016) and Selim et al. (2018) due to spirulina supplementation, compared to control group. Conversely, Boiago et al. (2019) evaluated the effects of supplementation with 5, 10, and 15% of dietary spirulina and did not observe any notable effects on specific gravity and eggshell thickness, but reported an increase in the yolk index owing to an improvement in the monounsaturated fatty acid levels in the yolks. The deterioration in the weight of albumen, yolk, and eggshell of quail eggs due to dietary spirulina was reported by Hajati and Zaghari (2019).

The dry matter content, minerals, and crude protein of the eggs were not affected ($p > 0.05$) by spirulina inclusion in the diets; however, the lipid levels were reduced ($p < 0.014$) as the spirulina levels were increased in the diets (Table 6).

Table 6. Nutritional levels of eggs from quail fed diets containing spirulina.

Parameters	Spirulina levels (%)				SEM	P value
	0.0	0.3	0.6	0.9		
Dry matter, %	27.57	27.58	27.46	27.30	0.58	0.985
Minerals, %	1.97	1.78	1.59	1.32	0.17	0.093
Total lipids, %	10.62 a	9.19 ab	7.65 bc	6.62 c	0.87	0.014
Crude protein, %	14.57	14.62	15.09	14.95	0.27	0.481

SEM – standard error of the mean. [a,b] Means followed by different letters differ by the SNK test, at 5% probability.

Quail eggs contain, on average, 26-29% dry matter, 13-13.3% protein, 11-12% total lipids, and 1.07-1.1% minerals (Tserveni-Goussi & Fortomaris, 2011; Thomas, Jagatheesan, Reetha, & Rajendran, 2016). Total lipids are concentrated in the yolk, and a small amount are strongly associated with the vitelline membranes (Réhault-Godbert, Guyot, & Nys, 2019).

Mariey et al. (2012), Dogan et al. (2016), Selim et al. (2018), and Hajati and Zaghari (2019) noted a reduction in the cholesterol content of the yolk due to spirulina inclusion in the diets of hens and quails. However, Zahroojian et al. (2013) and Boiago et al. (2019) did not verify the effects of dietary spirulina on cholesterol and total lipids in hen and quail egg yolks. The reduction in the lipids of the egg may be related to lower plasmatic levels of total lipids (Abouelezz, 2017; Selim et al., 2018) due to the antioxidant, hypocholesterolemic, and hypolipidemic properties of the spirulina, which promote inhibition of the jejunal absorption of both cholesterol and biliary acids (Hernández-Lepe et al., 2015), and/or due to the fiber levels of the spirulina (Selim et al., 2018).

The variation in the results may be due to the use of birds of different species and ages, differing concentrations of spirulina included in the diets, and differing types of ingredients in the diets.

Conclusion

The inclusion of 0.9% dietary spirulina improves eggshell quality and reduces the total lipids in the yolk, making the eggs easier to handle and more nutritious healthier for consumption.

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