Spirulina as a functional ingredient in broiler chicken diets

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Abstract

In recent years there has been increased interest in the production of novel functional foods by utilizing eco-friendly materials and methods. Therefore, the present study was undertaken to determine the effects of dietary spirulina *(Spirulina platensis)*, a blue-green microalga, on growth performance, meat oxidative stability and fatty acid profile of broiler chickens. One hundred and twenty one-day-old broiler chickens of mixed sex were weighed individually and assigned randomly to three treatment groups with four replications of 10 birds. All birds were housed in floor cages with litter, and conventional breeding and management procedures were applied throughout the 42-day trial period. The treatment groups were as follows: control: 0 g spirulina/kg feed; S05: 5 g spirulina/kg feed; S10: 10 g spirulina/kg feed. The birds were fed with maize and soybean meal-based commercial diets for the starter (1 to 14 days), grower (15 to 28 days) and finisher (29 to 42 days) periods. Feed and drinking water were offered to all birds ad libitum. The results of the experiment showed that bodyweight gain (at 21 d and 42 d), feed conversion ratio and mortality did not differ among the groups, nor did breast and thigh meat lipid oxidation differ among the groups. The fatty acid profile of the thigh meat was enriched in polyunsaturated fatty acids, especially eicosapentaenoic acid and docosahexaenoic acid after spirulina supplementation. Therefore, spirulina could be a promising functional ingredient in broiler chicken nutrition.

Keywords: Poultry, microalgae, performance, meat oxidative stability, fatty acid profile
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Introduction

Nowadays consumers demand natural, safe and eco-friendly products that can promote health benefits beyond their nutritional value, improve well-being and limit the risk of some chronic diseases. Such foods are named functional foods or nutraceuticals, a concept that was born in Japan early in 1980 (Plaza *et al*., 2009; Christaki *et al*., 2011; Borowitzka, 2013).

Natural environments could be a crucial biological source of functional ingredients, for example algae, which constitutes a promising approach to developing novel foods (Lordan *et al*., 2011; Draaisma *et al*., 2013). Among edible algae, spirulina or arthrospira – a blue green microalga – has served recently as an important source of valuable bioactive compounds. Dried spirulina is a good nutritional source with a high protein content (260 - 770 g/kg) and a significant lipid content (10 - 140 g/kg) (Ciferri, 1983; Ross & Dominy, 1990; Spolaore *et al*., 2006; Becker, 2007; Habib *et al*., 2008; Alvarenga *et al*., 2011; Zahroojian *et al*., 2013). Spirulina is high in unsaturated and polyunsaturated fatty acids in particular (25% - 60% of the total fatty acids), such as oleic acid, linoleic acid, gamma-linolenic acid and docosahexaenoic (DHA) (Hue *et al*., 2002; Yukino *et al*., 2005; Habib *et al*., 2008). It has also been reported that the amino acid pattern of these microalgae could be comparable with or superior to that of other vegetable foods and feeds, and that they have a high nutrient digestibility (Spolaore *et al*., 2006; Plaza *et al*., 2009; Alvarenga *et al*., 2011). In addition, spirulina contains substances such as pigments (for example carotenoids such as β-carotene and zeaxanthin) (Maoka, 2011), phycobiliproteins (for example phycocyanin, which is unique in the cyanobacteria (Eriksen, 2008), vitamins (Becker, 1994), macro and micro mineral elements (Becker, 1994; Spolaore *et al*., 2006) and antioxidants (Christaki *et al*., 2013). These compounds reveal potential biological properties such as antimicrobial, antioxidant, anti-cancer and anti-inflammatory or act as immune enhancers and colorants (Freitas *et al*., 2012; Batista *et al*., 2013; Christaki *et al*., 2013). However, the total amount of

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nutrients in spirulina could be greatly affected by available nutrients and environmental conditions during growth (Ciferri, 1983; Tonon *et al*., 2002; Tzovenis *et al*., 2003; Spolaore *et al*., 2006; Habib *et al*., 2008), by harvesting and drying techniques (Borowitzka, 1988; Spolaore *et al*., 2006; Alvarenga *et al*., 2011) and by methods of nutrient determination (Ciferri, 1983).

Recently, the effects of spirulina supplementation on animal performance and quality of animal products have been examined in the diets of dairy cows (Simkus *et al*., 2007; Christaki *et al*., 2012), fattening lambs (EL-Sabagh *et al*., 2014), rabbits (Colla *et al*., 2008; Peiretti & Meineri, 2008; Gerencser *et al*., 2014), common carp (Abdulrahman & Hamad Ameed, 2014), pigs (Grinsteal *et al*., 2000), laying hens (Carrillo *et al*., 2008; Maries *et al*., 2012; Zahroojian *et al*., 2013) and broilers (Ross & Dominy, 1990; Toyomizu *et al*., 2001; Alvarenga *et al*., 2011; Bellof & Alarcon, 2013). Nevertheless, published data could not be found on the effects of dietary spirulina on chicken meat oxidative stability and fatty acid profile.

The rapid increase of poultry meat consumption in the last few decades is related to consumer beliefs that poultry meat is 'healthy' and costs less than red meat. The absence of cultural and religious restraints to poultry meat consumption is also important (Cavani *et al*., 2010; Petracci *et al*., 2013). The inclusion of spirulina in broiler nutrition, besides having possible favourable effects on health and performance (Ravi *et al*., 2010; Kharde *et al*., 2012; Holman & Malau-Aduli, 2013; Shanmugapriya & Saravana Babu, 2014), might be a simple and convenient strategy to introduce its bioactive compounds into the meat and produce functional products according to consumer demand for healthy natural foodstuffs (Jimenez-Colmenero *et al*., 2001).

Therefore, the present study was undertaken to determine the effects of dietary spirulina, as a natural functional ingredient, on the growth performance of broiler chickens, and the oxidative stability and fatty acid profile of their meat.

Material and Methods

The experiment was conducted at the School of Agriculture Technology, Food Technology and Nutrition, Department of Agricultural Technology, Technological Educational Institution of Western Macedonia, Florina, Greece, according to the ethical guidelines of the Regional Directorate of Agriculture and Veterinary Medicine of Greece.

One hundred and twenty one-day-old broiler chickens of mixed sex (as hatched) were assigned randomly to three treatment groups, with four replications of 10 birds per group. Initial bodyweight did not differ between the groups (average 49 ± 0.7 g per chick). Each replication was housed for 42 days in floor cages with litter. Conventional breeding and management procedures were applied throughout the trial, according to the principles of the Greek Directorate General of Veterinary Services for the care of animals in experimentation.

The birds of the control group were fed with maize and soybean meal commercial diets: starter (1 - 14 days), grower (15 - 28 days) and finisher (29 - 42 days), based on the guidelines of NRC (1994). The birds of S05 were offered the same feeds with the addition of 5 g spirulina powder/kg, whereas S10 were offered these feeds with the addition of 10 g spirulina powder/kg. The dried spirulina in this trial was produced in the area of Serres, Greece. Feed and drinking water were offered to all birds ad libitum.

Table 1 presents the ingredients and the proximate chemical analysis, namely dry matter, crude protein, crude fat, crude fibre and ash (AOAC, 2005), of the three diets. Moreover, the calcium, total phosphorus, lysine, methionine plus cystine and metabolizable energy content were calculated from the composition of the feed ingredients, based on Novus (1992) and NRC (1994).

Throughout the trial, feed consumption and mortality were recorded daily. All birds were individually weighed weekly. At the end of the experiment, bodyweight gain and feed conversion ratio were calculated.

At day 42, two birds from each replication (1 male, 1 female) were randomly selected, and were slaughtered under commercial conditions. Skinless breast *(m. pectoralis superficialis)* and thigh *(m. biceps femoris)* samples were prepared to determine lipid oxidation and fatty acid composition. Skinless samples were used as they are more homogenous than muscles with their skin on and represent the type of poultry meat that is preferentially consumed in Europe (Rymer & Givens, 2006). Samples were vacuum-packaged and placed at −45 ºC for further analysis. Prior to analyses, the samples were thawed at 4 ºC overnight.

Determination of the lipid oxidation of the samples was performed using a modified version of the method of Vyncke (1975), as described by Kasapidou *et al.* (2014). The previously frozen samples were placed in refrigeration (4 ºC) for five days. On the second and the fifth day of refrigerated storage, each sample of breast muscles *(m. pectoralis superficialis)* and thigh muscles *(m. biceps femoris)* was separated from the bones and skin, and was trimmed of external/adjacent fat and connective tissue, and blended in a food processor. Subsamples (5 g) were homogenized in 25 mL 7.5% trichloroacetic acid (w/v) containing 0.1% (w/v) of both n-propyl gallate and ethylenediaminetetraacetic acid disodium salt, using a Polytron (Kinematica AG, Littau, Switzerland model PT-MR 3000). Samples were left for approximately 15 to 20 min

to allow extraction of the thiobarbituric acid reacting substances (TBARS). The resulting residue was filtered, and 5 mL of the filtrate was mixed with 5 mL 0.02 M thiobarbituric acid. A blank sample containing 5 mL of the trichloroacetic acid solution and 5 mL of the thiobarbituric acid solution was prepared. All samples were left in the dark overnight and on the following day absorbance was read at 532 nm against the blank sample, using an UV–VIS spectrophotometer (U-2800 Double Beam Spectrophotometer, Hitachi, Tokyo, Japan). TBARS were calculated using 1,1,3,3 tetraethoxypropane (5-20 nM) as standard and expressed as mg of malondialdehyde (MDA) per kg muscle. Each sample was analysed twice and the average value was used.

Table 1 Ingredients and chemical analysis of the experimental diets (as-fed basis)

Ingredients (g/kg)	Diets		
	Starter 1 d – 14 d	**Grower 15 d – 28 d**	**Finisher 29 d – 42 d**
Maize	509.1	560.0	637.3
Soybean meal	339.0	342.0	283.0
Herring meal	46.5	-	-
Soybean oil	68.0	63.0	52.0
Dicalcium phosphate	15.8	20.0	21.0
Sodium bicarbonate	12.1	8.0	0.7
Methionine	3.5	1.0	-
Salt	3.0	3.0	3.0
Vitamin and mineral premix *	3.0	3.0	3.0
Total	1000	1000	1000
Chemical analysis (as fed basis)			
Dry matter	931.6	906.1	907.7
Crude protein	261.5	182.1	181.9
Crude fibre	32.4	36.6	35.3
Crude fat	65.6	63.3	31.5
Ash	63.0	42.2	45.2
Calculated analysis			
Metabolizable energy (MJ/kg)	13.3	13.3	13.3
Lysine	12.6	10.3	8.9
Methionine + cystine	10.5	7.2	5.7
Calcium	9.9	7.3	7.0
Phosphorus (total)	8.0	8.0	8.0

* Supplying per kg feed: 13 000 IU vitamin A; 5 000 IU vitamin D_3; 30 mg vitamin E; 3 mg vitamin K_3; 1 mg thiamine; 5 mg riboflavin; 3 mg pyridoxine; 0.02 mg vitamin B_{12}; 10 mg niacin; 15 mg pantothenic acid; 0.8 mg folic acid; 0.05 mg biotin; 10 mg vitamin C; 480 mg choline chloride; 100 mg Zn; 120 mg Mn; 20 mg Fe; 15 mg Cu; 0.2 mg Co; 1 mg I; 0.4 mg Se.

The fatty acid composition of the breast and the thigh muscles samples was determined by gas chromatography. The protocol described by O'Fallon *et al*. (2007) was used to obtain fatty acid methyl esters from the frozen samples. Afterwards, the separation and quantification of the methyl esters were carried out with a gas chromatographic system (TraceGC model K07332, ThermoFinnigan, ThermoQuest, Milan, Italy) equipped with a flame ionization detector, a model CSW 1.7 chromatography station (CSW, DataApex Ltd, Prague, Czech Republic) and a fused silica capillary column, 30 m x 0.25 mm i.d., coated with cyanopropyl polysiloxane (phase type SP-2380) with a film thickness of 0.20 µm (Supelco, Bellefonte, Pa, USA). The chromatographic conditions were as follows: carrier: N_2; flow: 1 mL/min; oven: temperature 70 ºC for 0.5 min, increase 30 ºC/min to 180 ºC for 10 min, increase 5 ºC/min to 225 ºC for 15 min; inlet temperature: 250 ºC; detector temperature: 250 ºC; injection: 1 µL with split 1/20. Fatty acid methyl ester retention times and

elusion order were identified using as reference standards Supelco F.A.M.E Mix C8-C24 (C.N. 18918-1AMP), Supelco 37 Component FAME Mix (47885-U), Supelco linoleic acid methyl ester cis/trans isomers (4-7791) and Sigma Tridecanoic acid (T0502-5G), and accompanying Supelco reference material for the procedure. Fatty acids were quantified by peak area measurement and the results were expressed as percentage (%) of the total peak areas for all quantified acids.

Each individual replication (cage) was used as an experimental unit. The statistical analysis was performed with IBM SPSS Statistics 20 statistical package (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was performed, using the groups as fixed factors. Post-hoc analysis was undertaken using Tukey's test at P <0.050 (Hsu, 1996). The homogeneity of the measurements was examined with Levene's test (Levene, 1960).

Results and Discussion

The effects of spirulina supplementation in broiler performance parameters are presented in Table 2. The live weight of the birds of the control and treated groups did not differ (P >0.05) during the feeding trial, nor did the feed conversion ratio differ (P >0.05) among groups. Mortality was very low (only one bird in the control group died) and therefore did not differ (P >0.05) among groups. These results are in agreement with those of previous researchers (Ross & Dominy, 1990; Venkataraman *et al*., 1994; Qureshi *et al*., 1996; Gongnet *et al*., 2001; Toyomizu *et al*., 2001), who recorded nonsignificant effects of dietary spirulina supplementation on performance parameters, although it has been reported that spirulina inclusion over 100 g/kg could depress the birds' growth (Ross & Dominy, 1990). In contrast, other researchers (Kharde *et al*., 2012; Shanmugapriya & Saravana Babu, 2014) reported that dietary spirulina significantly improved weight gain and feed efficiency of chickens compared with the control groups. Furthermore, Bellof & Alarcon (2013) reported that under organic farming, dietary spirulina supplementation improved growth and carcass performance parameters of broilers significantly. Contradictory results are possibly due to the different spirulina inclusion levels and quality in the present trials. In addition, secondary parameters, such as feed composition, housing conditions and production systems, might be reasons for the variation in the results of the present study.

Table 2 Effects of dietary spirulina on broiler performance parameters

	Group *			SEM	*P*
	Control	S05	S10		
Live weight at 21 d (kg)	0.774	0.733	0.740	0.013	NS
Final live weight at 42 d (kg)	2.458	2.328	2.381	0.047	NS
Feed conversion ratio	2.070	2.132	2.103	0.023	NS
Mortality (%)	2.5	0	0	0.833	NS

* Four replications per group.
Control: 0 g spirulina/kg feed; S05: 5 g spirulina/kg feed; S10: 10 g spirulina/kg feed.
NS: Not significant (P >0.05).

Table 3 describes the effect of dietary spirulina on broilers breast and thigh meat lipid oxidation after two and five days of refrigerated storage. There was no difference (P >0.05) on the measured TBARS among the three groups, although lipid oxidation was progressively higher in the second measurement both in breast and thigh meat. The increased TBARS values in thigh muscle are likely due to the high haem iron and myoglobin contents of these muscles (Alasnier *et al*., 2000; Min *et al*., 2008). Meat acceptability and rancidity can be affected by TBARS. Levels and values of TBARS over 0.8 mg/kg meat could be considered indicative of rancidity in poultry meat (O'Neil *et al*., 1998), giving an oxidised flavour to the meat and making it unacceptable to consumers. This threshold value of 0.8 mg TBARS/kg meat is remarkably higher than the TBARS values observed in this trial.

The effects of dietary spirulina on the meat fatty acid profile of the broiler breast are shown in Table 4. Group S10 had a higher (P <0.05) concentration of lauric acid (C12:0) compared with the control group. Group S10 had a higher (P <0.05) concentration of 6-*trans* linoleic acid (6-*trans*-C18:2) in comparison with group S05, but not compared with the control group. Moreover, no significant differences were noticed for

Table 3 Effect of dietary spirulina on breast and thigh muscle lipid oxidation (TBARS, mg malonaldehyde/kg muscle) after 2 and 5 days of refrigeration

Breast muscle	Group *			SEM	*P*
	Control	S5	S10		
2 d refrigeration	0.148	0.140	0.113	0.016	NS
5 d refrigeration	0.285	0.433	0.334	0.048	NS
Increase between 2 d and 5 d	0.137	0.311	0.221	0.042	NS
Thigh muscle					
2 d refrigeration	0.156	0.137	0.138	0.018	NS
5 d refrigeration	0.376	0.451	0.256	0.044	NS
Increase between 2 d and 5 d	0.220	0.314	0.118	0.044	NS

* Four replications per group.
Control: 0 g spirulina/kg feed; S05: 5 g spirulina/kg feed; S10: 10 g spirulina/kg feed.
NS: Not significant ($P > 0.05$).

the other fatty acids, and the total saturated, unsaturated and polyunsaturated fatty acids. The effects of dietary spirulina on broiler thigh meat fatty acid profile are presented in Table 5. Group S05 had higher ($P < 0.05$) concentrations of eicosatrienoic acid (C20:3n-3), arachidonic acid (C20:4n-6), eicosapentaenoic acid (C20:5n-3 EPA), lignoceric acid (C24:0), docosapentaenoic acid (C22:5n-3 DPA), docosahexaenoic acid (C22:6n-3 DHA), and lower ($P < 0.05$) concentrations of oleic acid (9-cis-C18:1), arachidic acid (C20:0), eicosenoic acid (C20:1n-9), compared with control group. Group S10 had higher ($P < 0.05$) concentrations of palmitoleic acid (C16:1), gamma-linolenic acid (C18:3n-6) and a lower ($P < 0.05$) concentration of eicosatrienoic acid (C20:3n-3) compared with group S05, while it had a lower ($P < 0.05$) concentration of eicosenoic acid (C20:1n-9) compared with control group. Overall, monounsaturated fatty acids were higher ($P < 0.05$) for Group S05 compared with the control group, and polyunsaturated fatty acids were higher ($P < 0.05$) for groups S05 and S10 than the control group, while saturated fatty acids did not differ ($P > 0.05$) among the three groups.

Fatty acid composition showed that dietary spirulina supplementation affected both the breast and thigh meat fatty acid profiles, but the effect was much more pronounced in the thigh meat. These findings cannot be compared with other research in broilers, since similar reports have not been found in recent literature, to the best of that authors' knowledge. This difference could be because raw chicken thigh meat contains an up to five times higher absolute amount of crude fat (about 40 - 140 g/kg) than raw chicken breast meat (about 10 - 30 g/kg), and the fatty acid composition can be different between these different muscle tissues possibly due to their different phospholipid contents (Botsoglou *et al.*, 2002; Cortinas *et al.*, 2004). Moreover, these differences in composition are affected by the combined result of endogenous fat synthesis and direct utilization of feed fat. Also, it is known (Cortinas *et al.*, 2004; Zelenka *et al.*, 2008) that different sources of fat in broiler diets directly affect the total amount and the percentages of individual fatty acids in the meat and subcutaneous fat. Thus it is possible to increase the percentage of polyunsaturated fatty acids.

Polyunsaturated fatty acids cannot be synthesized by humans. For this reason they should be included in the normal daily diet (FAO, 2008). These fatty acids play an important role in the production and metabolism of substances such as prostaglandins, thromboxanes and leukotrienes (FAO, 2008; Simopoulos, 2008). Furthermore, in the present trial, nutritionally important n-3 fatty acids, such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), were increased in the thigh meat. These fatty acids are not consumed in adequate amounts in modern Western societies (Simopoulos, 2008), and it has been reported that their consumption could benefit foetus growth and protect from numerous chronic diseases, that is, infections, cancer, asthma, depression, heart and autoimmune diseases (Simopoulos, 2002; FAO, 2008; Swanson *et al.*, 2012). In addition, the increased consumption of unsaturated fatty acids, along with decreased consumption of saturated fatty acids, is considered beneficial, limiting the risk and the effects of cardiovascular and degenerative diseases (Cortinas *et al.*, 2004; FAO, 2008; Reiner *et al.*, 2011).

Table 4 Effects of dietary spirulina on broiler breast meat fatty acid profile (% fatty acid/total fatty acids)

Fatty acids (FA)	Common name	Groups*			SEM	*P*
		Control	S05	S10		
10:0	Capric	0.093	0.090	0.054	0.011	NS
12:0	Lauric	0.021 [a]	0.024 [ab]	0.038 [b]	0.002	0.021
14:0	Myristic	0.336	0.344	0.367	0.017	NS
14:1	Myristoleic	0.032	0.081	0.036	0.010	NS
16:0	Palmitic	16.708	17.232	16.521	0.432	NS
16:1	Palmitoleic	1.588	1.981	1.964	0.096	NS
18:0	Stearic	9.184	8.106	8.870	0.338	NS
9 trans-18:1	*trans*-Oleic	0.324	0.191	0.315	0.056	NS
9 cis-18:1	*cis*-Oleic	26.948	26.970	29.193	0.622	NS
6 trans-18:2	*trans*-Linoleic	0.044 [ab]	0.036 [a]	0.055 [b]	0.002	0.004
6 cis-18:2	*cis*-Linoleic	26.412	28.541	26.430	0.843	NS
18:3n-6	γ-Linolenic	0.160	0.216	0.181	0.009	NS
20:0	Arachidic	0.204	0.210	0.191	0.007	NS
18:3n-3	α-Linolenic	1.720	2.123	1.942	0.111	NS
20:1n-9	Eicosenoic	0.257	0.240	0.277	0.010	NS
20:2	Eicosadienoic	0.601	0.510	0.537	0.041	NS
20:3n-3	Eicosatrienoic	0.838	0.716	0.704	0.055	NS
20:4n-6	Arachidonic	5.573	4.814	4.711	0.515	NS
22:1n-9	Erucic	0.035	0.029	0.027	0.007	NS
20:5n-3 (EPA)	Eicosapentaenoic	0.232	0.215	0.222	0.019	NS
24:0	Lignoceric	1.237	1.022	1.039	0.111	NS
22:5n-3 (DPA)	Docosapentaenoic	0.874	0.770	0.782	0.079	NS
22:6n-3 (DHA)	Docosahexaenoic	0.859	0.784	0.717	0.078	NS
Σ SFA	Total saturated	28.418	27.551	27.582	0.622	NS
Σ MUFA	Total monounsaturated	29.775	29.942	32.373	0.634	NS
Σ PUFA	Total polyunsaturated	37.314	38.726	36.282	0.607	NS

* Four replications per group.
Control: 0 g spirulina/kg feed; S05: 5 g spirulina/kg feed; S10: 10 g spirulina/kg feed.
[ab] Values in the same row with no common superscript differ significantly at $P < 0.05$.
NS: not significant ($P > 0.05$).

Conclusion

The dietary supplementation of spirulina (5 g/kg or 10 g/kg) in broiler diets affected the fatty acid composition of the broiler meat, without any significant negative impact on performance parameters and meat oxidative stability. Of the two inclusion levels of spirulina, 5 g/kg increased the concentrations of certain vital and valuable polyunsaturated fatty acids, such as EPA, DPA, and DHA, in thigh meat. Therefore, spirulina could be a promising functional ingredient in broiler chicken nutrition. Nevertheless, more multidisciplinary research is required since this microalga fits the criteria for the development of potential functional ingredients in broiler nutrition.

Table 5 Effects of dietary spirulina on broiler thigh meat fatty acid profile (% fatty acid/total fatty acids)

Fatty acids	Common name	Groups*			SEM	*P*
		Control	S05	S10		
10:0	Capric	0.009	0.019	0.013	0.003	NS
12:0	Lauric	0.021	0.038	0.026	0.003	NS
14:0	Myristic	0.347	0.311	0.272	0.017	NS
14:1	Myristoleic	0.047	0.044	0.054	0.003	NS
16:0	Palmitic	17.229	16.852	16.701	0.190	NS
16:1	Palmitoleic	1.938^{ab}	1.183^{a}	1.993^{b}	0.128	0.030
18:0	Stearic	8.117	10.516	8.603	0.440	NS
9 trans-18:1	*trans*-Oleic	0.323	0.268	0.311	0.048	NS
9 cis-18:1	*cis*-Oleic	29.240^{b}	19.838^{a}	25.442^{ab}	0.992	0.003
6 trans-18:2	*trans*-Linoleic	0.048	0.057	0.068	0.005	NS
6 cis-18:2	*cis*-Linoleic	28.928	27.234	29.770	0.744	NS
18:3n-6	γ-Linolenic	0.248^{ab}	0.210^{a}	0.281^{b}	0.011	0.042
20:0	Arachidic	0.147^{b}	0.090^{a}	0.118^{ab}	0.008	0.028
18:3n-3	α-Linolenic	2.004	1.282	2.003	0.140	NS
20:1n-9	Eicosenoic	0.287 ^{b}	0.177 ^{a}	0.207 ^{a}	0.012	0.003
20:2	Eicosadienoic	0.523	0.909	0.616	0.067	NS
20:3n-3	Eicosatrienoic	0.729^{a}	1.354^{b}	0.779^{a}	0.083	0.009
20:4n-6	Arachidonic	3.515^{a}	7.804^{b}	4.950^{ab}	0.546	0.013
22:1n-9	Erucic	0.010	0.010	0.019	0.005	NS
20:5n-3 (EPA)	Eicosapentaenoic	0.126^{a}	0.234^{b}	0.177^{ab}	0.015	0.029
24:0	Lignoceric	0.795^{a}	1.624^{b}	0.956^{ab}	0.112	0.016
22:5n-3 (DPA)	Docosapentaenoic	0.633^{a}	1.382^{b}	0.849^{ab}	0.097	0.014
22:6n-3 (DHA)	Docosahexaenoic	0.692^{a}	1.435^{b}	0.813^{ab}	0.108	0.022
Σ SFA	Total saturated	27.159	30.154	27.165	0.666	NS
Σ MUFA	Total monounsaturated	32.242^{b}	22.433^{a}	28.526^{ab}	1.048	0.004
Σ PUFA	Total polyunsaturated	37.447^{a}	41.901^{b}	40.305^{b}	0.450	0.002

* Four replications per group.
Control: 0 g spirulina/kg feed; S05: 5 g spirulina/kg feed; S10: 10 g spirulina/kg feed.
^{ab} Values in the same row with no common superscript differ significantly at $P<0.05$.
NS: not significant ($P>0.05$).

Conflict of interest declaration

The authors declare that there was no conflict of interest.

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