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Original Article

Effects of Dietary Supplementation with Red Algae Powder (Chondrus crispus) on Growth Performance, Carcass Traits, Lymphoid Organ Weights and Intestinal pH in Broilers

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ABSTRACT

The aim of this study was to determine the effects of dietary supplementation with red algae powder (*Chondrus crispus*) on the growth performance, carcass traits, lymphoid organ weights and intestinal pH y in broiler chickens. A total of 300 1-day-old B34 line male broiler chickens were randomly allotted to three treatments, four replicates per treatment and 25 birds per replicate. The experimental treatments consisted of a basal diet (T0) and dietary supplementation of 0.30 (T2) and 0.40% (T2) red algae powder. Body weight at 1, 21 and 32 days did not show significant differences (p>0.05) among treatments. At 21 days, T1decreased (p<0.05) the feed intake and feed conversion ratio. However, from 22 to up to 32 days, these productive indicators increased (p<0.05) with the use of the natural product (red algae powder) tested. Meanwhile, T1 improved (p<0.05) the carcass and breast yields and decreased the abdominal fat yield, and T2increased (p<0.05) the relative weights of the bursa of Fabricius and the thymus. The relative weight of the spleen, the other edible parts and the intestinal pH did not change (p>0.05) with the red algae supplementation. The dietary supplementation of 0.30% red algae improved the growth performance (at 21-day-old) and some edible parts; also, the dietary supplementation of 0.40% increased the relative weight of the lymphoid organs, without changing the intestinal pH of broilers.

INTRODUCTION

More than 75% of the land is covered by water, where marine species comprise about half of the global biodiversity (Arnold *et al*., 2016). This wide diversity is a reservoir of potent bioactive molecules, which are produced by these organisms to survive in hostile environments (Krumhansl *et al*., 2015). Algae are single-celled organisms that contain chlorophyll and perform photosynthesis; they are grouped in colonies or as organisms with many cells. They are located in all parts of the earth: in the sea, rivers and lakes, in soil and walls and in animals and plants (Trentacoste *et al*., 2015).

Also, among marine organisms, algae have been identified as an under-exploited source. The explorations of these organisms for pharmaceutical purposes have revealed important chemical prototypes for the discovery of new agents, which has also allowed to stimulate the synthesis of compounds with biomedical applications (Radhika *et al*., 2012; Offret *et al*., 2016). In China and Japan, marine macroalgae have been used as drug preparations, especially for the treatment of Basedow's disease, Acquired Immunodeficiency Syndrome (AIDS), rheumatoid arthritis, hyperthyroidism and cancer, as a vermifuge, and as hypocholesterolemic and hypoglycemic agents (Kaleağasıoğlu *et al*., 2013; Astorga-España & Mansilla, 2014; Sakulpong *et al*., 2015; Anand *et al*., 2016; Paiva *et al*., 2016). Moreover, the incorporation of algae in other products improves or increases the nutritional value of foods; because these are rich in amino acids, vitamins, fatty acids, polyphenols, phytosterols, minerals, dietary fiber and antioxidant compounds (Farvinand & Jacobsen, 2013; Sakulpong *et al*., 2015).

Chondrus crispus or Ireland moss is a perennial red alga (*Rhodophyta phylum*), common in the intertidal and superficial subaltern communities in the American and Eurasian Continent. In these algae, important biologically active metabolites have been found with potentials such as anticoagulants, antioxidants, antibacterial, antiviral, antitumor, anti-inflammatory and analgesic agents (Liu *et al*., 2015).

Although these marine organisms have aroused interest in the international scientific community in recent years due to their pharmacological properties (Offret *et al*., 2016), research on their use as a nutraceutical in animal diets is insufficient, specially as an alternative to the indiscriminate use of antibiotic growth promoters (AGP) in farm animals, because these synthetic drugs have led to an increase in the number of resistant strains and the transfer of cross-resistance to other microorganisms (Ni *et al*., 2016). Therefore, the objective of the current study is to evaluate the effects of dietary supplementation of red algae powder (*Chondrus crispus*) on growth performance, carcass traits, lymphoid organs weight and intestinal pH of broilers.

MATERIALS AND METHODS

Location

The Committee on the Ethics of Animal Experiments of Institute of Animal Science, Mayabeque, approved the protocol for this study, and it was conducted out in an experimental farm in Mayabeque province, Cuba, according to the recommendations in the Guide for the Care and Use of Poultry of Institute of Animal Science (Instituto de Ciencia Animal). An average relative humidity of 78%, and average minimum and maximum temperatures of 18.5 °C and 27.6 °C, respectively, were recorded using a hygro-thermometer placed inside the experimental house.

Red algae powder

The red algae, *Chondrus crispus*, was selected for the investigation, based on reports of its pharmacological, preventive and nutraceutical uses. 15 kg of red algae (*Chondrus crispus*) were taken at random on the shores of Campechuela, Granma province, Cuba. This area is characterized by a mountainous topography and muddy brown soil. Following the collection, the samples were washed, which was performed three times with water with salt to avoid damaging the quality of the sample and to eliminate the greatest amount of impurities.

Firstly, the samples were dried out naturally for five days at room temperature and then artificially with a stove (WSU 400, Germany) at a temperature of 60°C for 1 h. Then, the pulverization proceeded until a fine powder (1 mm) was obtained. After, the obtained productwas stored in airtight plastic bags at room temperature (Yin *et al*., 1993).

Birds, treatments and diet

A total of 300 1-day-old B34 line male broiler chickens were randomly allotted to three treatments, four replicates per treatment and 25 birds per replicate. The hybrid chicken B34 is a fast-growing chicken obtained from crossing the pure lines E1, H2, B4, E3 and was developed by the Poultry Science Institute of Cuba. The treatments consisted of a basal diet (T0), basal diet+0.30% (T1) of red algae powder and basal diet+0.40% (T2) of red algae powder. The experimental diet was formulated according to the requirements recommended by Rostagno *et al*. (2011); a three-phase feeding system was applied: pre-starter (1 to 7 days), starter (8 to 21 days), and grower (22 to 32 days). Table 1 shows the ingredients and nutritional

Table 1 – Dietary ingredients and nutrient levels in diets (as fed).

Ingredients (g/kg)	Pre-tarter	Starter	Grower
Corn meal	519.0	580.2	610.0
Soybean meal	402.1	349.6	320.4
Soybean oil	30.00	26.00	29.90
Salt	2.50	2.50	2.50
L-Lysine	2.60	2.10	1.70
DL-Methionine	1.40	1.00	1.00
L-threonine	1.70	1.30	0.90
Dicalcium phosphate	19.90	15.10	12.90
Calcium carbonate	10.40	11.80	10.30
Choline	0.40	0.40	0.40
Vitamin and trace element premix1	10.00	10.00	10.00
Calculated nutritional composition			
Metabolizable energy (kcal/kg)	2925	2980	3050
Crude protein(g/kg)	220.0	200.0	190.0
Calcium (g/kg)	9.20	8.60	7.50
Available Phosphorus (g/kg)	4.70	3.80	3.40
Methionine plus cystine (g/kg)	10.40	9.10	8.40
Lysine (g/kg)	14.40	12.60	11.50
Threonine (g/kg)	9.80	8.60	7.80
Tryptophan (g/kg)	2.90	2.60	2.40
Crude fiber (g/kg)	30.00	28.70	27.7

Each kg contains: vitamin A, 13,500 IU; vitamin D3, 3,375 IU; vitamin E, 34 mg; B2, 6 mg; panthotenic acid, 16 mg; nicotinic acid, 56 mg; Cu, 2,000 mg; folic acid, 1.13 mg; vitamin B12, 34 mg; Mn, 72 mg; Zn, 48 mg.

level of the basal diet. The results of Kulshreshtha *et al*. (2014) and Liu *et al*. (2015) were taken into account to select the levels of red algae supplementation.

Experimental conditions

Each replicate consisted of a pen with deep corn stover litter and 12 birds/m^2. The birds had free access to feed and water, in hopper type feeders and nipple waterers, respectively. Heating lamps until 14 days of age were used, from this stage the lighting system was increased up to 23 hours per day. At the hatchery, the birds were vaccinated against smallpox, infectious bronchitis, New Castle and Gumboro. No medications were used or veterinary care was offered during the experimental stage.

Growth Performance parameters

All the body weight (g) of the birds was weighed on days 1, 21 and 32 of the experiment. The feed was measured daily during the whole experimental period and feed intake (g/bird/day) (FI) was calculated as the difference between the amount of feed offered and feed residue. Feed conversion ratio (kg/kg) (FCR) was calculated as the amount of feed intake to gain one kg body weight. Mortality (%) was determined as the difference between the initial number of birds and recorded viability.

Carcass traits and lymphoid organ relative weights

At day 32, 8 birds per replicate was sacrificed by bleeding of the jugular vein after four hours of feed fasting (water was offered *ad libitum*) to collect samples. Carcass traits was determined by weighing the birds before slaughter. After which carcass, breast, thigh+leg, neck, heart, liver, gizzard and abdominal fat pad were weighed (Aguilar *et al*., 2011). Also, bursa of Fabricius, spleen and thymus as lymphoid organs were weighed (Aguilar *et al*., 2013). The relative weight of the edible portions and lymphoid organs was calculated by the formula: Relative weight = (Absolute weight x 100)/final body weight.

Intestinal pH

In the slaughter (32 days of age), samples of the small intestine and left cecum were taken and stored at -20°C and 24 hours later the samples were thawed to room temperature. Then 2 g samples were put in a porcelain mortar, 10 ml of distilled water was added and homogenized in a vortex for 2 minutes. The pH was determined by a digital potentiometer (300 A Bantex model), calibrated with buffer solutions pH 7 and 10 (Martínez *et al*., 2012).

Statistical analysis

Data were shown as means ± standard error of the mean (SEM) and analyzed with analysis of variance (ANOVA) for simple classification of completely randomized design. Prior to the analysis of variance, the normality of the data was verified by Kolmogorov-Smirnov and the uniformity of variance by Bartlett test. When necessary Duncan multiple range test was used to determine differences between means, also, the viability was analyzed through the comparison of proportions. All data were analyzed by the statistical software SPSS version 22.1.

RESULTS AND DISCUSSION

Table 2 shows the effect of red algae powder on growth performance of broilers. Mortality and body weight (BW) at 1, 21 and 32 days did not show significant differences (p>0.05) among the treatments. However, T1 reduced (p<0.05) FI and FCR at 21 days of age with relation to T0 and T2. Although, from the 22nd to the 32nd day of age and during the whole experimental period these productive indicators increased by algae effect.

Mortality (Table 2) demonstrated the safety of red algae powder supplemented up to 0.40% on broiler diets. It seems that this marine organism does not present highly toxic secondary metabolites that cause morbidity and mortality to birds, especially in the first days of life, due to the high susceptibility of these animals (Taha-Abdelaziz *et al*., 2018). Other results using 2% red algae in laying hens diets showed similar results (Kulshreshtha *et al*., 2014). Moreover, few studies have been developed to know the nutraceutical effects of red algae in the diets of non-ruminant animals, especially birds. Studies in humans have shown that algae are advisable to control or decrease body weight (Ibañez & Cifuentes, 2013), mainly by their prebiotic characteristics, because they are sources rich in polysaccharides and dietary fiber (Kulshreshtha *et al*., 2014; Liu *et al*., 2015).

The use of these marine bioactive appears to have the same effect on the BW of growing (Table 2) and adult birds (Kulshreshtha *et al*., 2014). It is known that prebiotics stimulate the growth of bacterial species and improve host health, especially intestinal health (Iser *et al*., 2016), however, some studies in birds with these non-digestible products found no benefits in

Table 2 – Effect of dietary supplementation with red alga powder (*Chondrus crispus*) on growth performance in broiler.

Age, days	Dietary supplementation with red alga (%)			SEM±	*p* value
	Basal diet	0.30	0.40		
Mortality (%)					
0-21	2.00	2.00	4.00	0.897	0.943
22-32	3.00	1.00	1.00	0.936	0.571
1-32	5.00	3.00	5.00	1.023	0.093
Body weight (g)					
0	41.25	40.75	40.50	0.589	0.669
21	595.56	615.93	624.68	24.222	0.694
32	1166.52	1087.73	1091.87	33.427	0.226
Feedintake (g/bird)					
0-21	915.12^{a}	772.42^{b}	899.75^{a}	13.542	0.001
22-32	1532.5^{b}	1756.7^{a}	1752.5^{a}	24.344	0.001
1-32	2448^{b}	2528.7^{a}	2652.25^{a}	28.257	0.002
Feed conversion ratio (kg/kg)					
0-21	1.66^{a}	1.34^{b}	1.54^{a}	0.555	0.008
22-32	2.69^{b}	3.76^{a}	3.19^{a}	0.233	0.013
1-32	2.18^{b}	2.42^{ab}	2.53^{a}	0.078	0.029

^{a,b} Means within the same row with different superscript differ significantly ($p<0.05$).

body weight (Torres-Rodriguez *et al*., 2005; Zhang *et al*., 2005), it seems that growth promotion must be combined with other beneficial chemical compounds (Iser *et al*., 2016).

Also, table 2 shows T1 increases productive efficiency, with a decrease in FI and FCR, but only in the first three weeks of life, which is the most critical period of birds, characterized by an immature digestive and immunological system (Anand *et al*., 2016; Aroche *et al*., 2018). A decrease in FI in T1 may be due to the fact that red algae have a high concentration of mucilages (80%) (Kulshreshtha *et al*., 2014), these polysaccharides are soluble in water and indigestible, which reduces gastrointestinal transit and increases the feed satiety (Solominski *et al.*, 2011).

On the other hand, in the first days of life, birds are exposed to various stressful conditions such as climate, pathogenic bacteria and management that cause intestinal inflammation, mainly postprandial that depress the animal response (Fang *et al*., 2017). The algae have anti-inflammatory properties by inhibiting phospholipase A2 and the formation and/or liberation of prostaglandins and leukotrienes (Lee *et al*., 2013). Also, in laboratory it is known that macroalgae regulate the production of cytokines and the activation of macrophages (Robertson *et al*., 2015), this beneficial action could enhance the feed efficiencyat 21 days of age (T1)

Likewise, dietary supplementation with red algae could decrease the proliferation of intestinal pathogenic bacteria such as *Salmonella* ssp. and *E. coli.* In this sense, Kulshreshtha *et al*. (2014) found a higher colonization of intestinal lactic acid bacteria when supplemented with these algae in the diets of laying hens due to its prebiotic effect. Likewise, Cox *et al.* (2014), Dhas *et al*. (2014) and Shanmugam *et al*. (2014), reported antimicrobial properties in macroalgae extracts. Also, other studies with algae observed that supplementation of *Spirulina platensis* and *Ascophyllum nodosum* extract in laying and pig diets improved cellular and humoral immunity and slightly increased growth performance, respectively (Qureshi *et al.* 1996; Turner *et al*., 2002).

However, a higher supplementation with red algae (0.40%) and therefore polysaccharides (as mucilages) and other secondary metabolitescould provoke some antinutritional effects, which reduced this productive indicator. In this sense, Aguilar *et al*. (2013) inform that usually the medicinal effects of natural products as algae are for their content in secondary metabolites. Studies of Savón *et al*. (2007) have confirmed that a dietary excess of secondary metabolites inhibits the absorptionof sulfur amino acids, minerals and vitamins in poultry. In this sense, other studies with natural products rich in secondary metabolites such as tannins, glucosinolates, carvacrol, and polysaccharides have found similar results when they were used in the diets of broilers (Kubena *et al*., 2001; Botsoglou *et al*.; 2002; Solominski *et al.*, 2011; Woyengo *et al*., 2011). Perhaps an increase in algae intake produces metabolic alterations that lead to antinutritional influence, mainly in young birds.

Also, the increase of FI and FCR with the use of the red algae from the 21st to the 32nd day of age is contradictory. It seems that daily consumption of red algae rich in secondary metabolites caused a decrease in weight growth, because at this stage the chickens increased in a proportion of 1.74 to 1.95, with respect to the first phase (1-21 days). According to Solominski *et al.* (2011) the continued use of mucilages (rich in algae) in poultry diets, increase intestinal viscosity, which reduces energy utilization, fat digestibility and growth performance.However, more studies are needed to corroborate this hypothesis.

Table 3 shows that the dietary supplementation of 0.30% with red algae improved the carcass relative weight with relation to T0 and T2 and the breast relative weight compared with T2 ($p<0.05$). However, the relative weight of the other edible portions did not show significant differences ($p>0.05$) among treatments.

The chemical benefits of this natural product increased the carcass and breast yields at 32 days (T1) (at slaughter), perhaps due to better intestinal health and absorption of biomolecules in the gastrointestinal tract, as amino acids. An improvement in the absorption of lysine increases the yield of the breast (Zhai *et al*., 2016) and therefore the carcass yield, as was observed in our experiment.

The T1 caused a decrease in abdominal fat pad in broilers; a decrease of this indicator is associated with the concentration of very low density lipoprotein (VLDL) and its influence on intestinal inflammation (Dong *et al*., 2015; Van den Borne *et al*., 2015). Thus, a high concentration of mucilages in these algae and its hypolipidemic effect could decrease this harmful serum lipoprotein and its incorporation in the tissues (Solominski *et al.*, 2011; de Jesus Raposo *et al*., 2015). In addition, the practical point of view is very beneficial for poultry slaughterhouses, where abdominal fat is an undesirable component (Fouad & El-Senousey, 2014).

However, T2 depressed the carcass relative weight (Table 3). It found a relationship betweenthe high concentration of secondary metabolites and a decrease of BW and carcass yield, because these phytochemicals apparently develop anti-nutritional factors, which decrease digestion and absorption of nutrients and inflame the small intestine (Woyengo *et al*., 2011; Martínez *et al*., 2015).

Table 3 – Effect of dietary supplementation with red alga powder (*Chondrus crispus*) on carcass traits in broiler.

Yields (%)	Dietary supplementation with red alga (%)			SEM±	*p* value
	Basal diet	0.30	0.40		
Carcass	51.57[b]	53.59[a]	51.77[b]	0.554	0.039
Breast	15.76[ab]	16.14[a]	14.63[b]	0.463	0.048
Neck	3.16	2.57	2.63	0.233	0.176
Thigh+leg	19.63	19.41	19.27	0.269	0.157
Heart	0.65	0.66	0.65	0.043	0.962
Liver	2.73	2.39	2.98	0.341	0.480
Gizzard	2.35	2.42	2.79	0.166	0.172
Abdominal fat	1.42[a]	1.07[b]	1.53[a]	0.121	0.015

[a,b] Means within the same row with different superscript differ significantly ($p<0.05$).

Table 4 shows that the thymus relative weight increased in T2, this is determined by the activation of the immune system, which could increase the production of T cells by jointly destroying the macrophages (Smith & Hunt, 2004; Chen *et al*., 2013). In this sense, the bursa of Fabricius also increased (Table 4) with the new natural product (red algae), this organ stimulates humoral immunity and produces memory antibodies with great specificity. Usually, a high size of this organ means more immunological activity, taking into account that both lymphoid organs involute at early ages (Liang *et al*., 2015; Yasmin *et al*., 2015).

The supplementation with red algae had no effect on the relative weight of the spleen (Table 4). In this sense, Huang *et al*. (2005) and Al-Khalifa *et al*. (2012) found no relationship between beneficial nutrients and spleen weight in broiler chickens. This is explained,

Table 4 – Effect of dietary supplementation with red alga powder (*Chondrus crispus*) on lymphoid organs in broilers.

Lymphoid organs (%)	Dietary supplementation with red alga (%)			SEM±	*p* value
	Basal diet	0.30	0.40		
Bursa of Fabricius	0.20[b]	0.24[ab]	0.33[a]	0.064	0.041
Spleen	0.14	0.14	0.20	0.037	0.436
Thymus	0.55[b]	0.52[b]	0.62[a]	0.085	0.016

[a,b] Means within the same row with different superscript differ significantly ($p<0.05$).

because the primary lymphoid organs (Bursa of Fabricius and thymus) have the highest immunological activity and production of antibodies (B and T cells) (Smith & Hunt, 2004).

Currently, it is known that immuno stimulation leads to a higher energy costs, mainly for the production of antibodies, activation of macrophages and for anti-inflammatory activity (Van den Borne *et al*., 2015), this could have increased the FI in T2, especially to supply the energy requirements for maintenance and production. Studies with phytochemicals in birds have found that lymphoid organ growth is not sometimes related to the best experimental treatment (Aguilar *et al*., 2013). However, further experiments are needed to justify this hypothesis in birds.

The decrease in intestinal pH is determined by the increased colonization of lactic acid bacteria (LAB) such as *Lactobacillus* and *Bifidobacterium* in TGI, which increases the production of volatile fatty acids (VFA), which emit protons and acidify the intestine (Latorre *et al*., 2015). The use of up to 0.40% of red algae in broiler diets did not increase the proliferation of LAB in such a way that it modified the intestinal pH (p<0.05), as was observed in Table 5. In young birds, there are some contradictions about the effect of phytochemicals on intestinal pH, mainly by the late proliferation of LAB and because an excess of these chemical compounds causes metabolic disturbances; therefore, their effects will depend on the concentration of these secondary metabolites in the biological material and their supplementation in the diet.

Table 5 – Effect of dietary supplementation with red alga powder (*Chondrus crispus*) on intestinal pH in broiler.

pH	Dietary supplementation with red alga (%)			SEM±	*p* value
	Basal diet	0.30	0.40		
Small intestine	6.84	6.82	6.84	0.084	0.897
Left cecum	7.17	7.03	6.89	0.197	0.609

CONCLUSIONS

These results showed that dietary supplementation of 0.30% of red algae improved the growth performance (at 21 days of age) and some edible parts; also, dietary supplementation of 0.40% increased the relative weight of the lymphoid organs, without modifying the intestinal pH of broiler.

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