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Effects of *Spirulina Platensis* Algae on Growth Performance, Antioxidative Status and Blood Metabolites in Fattening Lambs

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Abstract

The objective of this study was to investigate the effects of *Spirulina platensis* powder (SPP) supplementation on growth performance, antioxidative status and blood metabolites in fattening lambs. Ten healthy lambs (46.5 ± 1.06 kg BW) were randomly assigned to one of two treatments (5 lambs per treatment) and received either no supplementation or supplemented with SPP at a rate of 1 g/10 kg BW/day. The feeding experiment was conducted for 35 days with body weight recorded and blood samples collected on days 0, 17 and 35 of the experiment. The paired Student's *t*-test for means was used for statistical analysis. The results showed that SPP supplementation improved final live body weight, daily live weight gain, feed intake and feed conversion ratio, compared to the control group ($P<0.05$). Also, haemoglobin, total white blood cell count, serum globulin, vitamin A and reduced glutathione were higher ($P<0.05$), while the aspartate amino transferase, alanine amino transferase, cholesterol, glucose and serum malondialdehyde levels were lower ($P<0.05$) in SPP supplemented group compared with the control. In conclusion, the findings of the present study clearly demonstrate that the SPP could be incorporated in fattening lambs diets as an antioxidant, immune-stimulant and growth promoter feed additive.

Keywords: fattening lambs, spirulina platensis, performance

1. Introduction

Intensive livestock production systems may be associated with multiple stressful incidents that negatively impact immune response and animal performance. The high metabolic rate during intensive feeding is accompanied by an increased production of free radicals, and any imbalance between production of these molecules and their safe disposal may culminate in oxidative stress, which can damage cells and tissues (Miller et al., 1993; Lykkesfeldt & Svendsen, 2007). Therefore, under oxidative stress conditions, there is an increased demand for antioxidants to reduce the deleterious effects of free radicals on the immune system (Carroll & Forsberg, 2007). Interestingly, feeding natural, rather than synthetic, antioxidant could be advantageous to animal welfare and consumer safety (Call et al., 2008; Makkar et al., 2007). The blue-green algae, *Spirulina platensis*, have been considered as a suitable natural antioxidant and immune-stimulant to humans and animals with fewer side effects and more cost effectiveness than synthetic products (Abdel-Daim et al., 2013; Belay, 2002; Khan et al., 2005). Recently, the impact of dietary Spirulina supplementation on animal health and productivity have been reported (Holman & Malau-Aduli, 2012). However, studies on use of *Spirulina platensis* as a feed additive in ruminant feeding are still quite limited. To our knowledge, moreover, no studies have been undertaken on its usage with high concentrate diets. Therefore, the objective of this study was to test whether *Spirulina platensis* had beneficial effects when included in a high concentrate fed to fattening lambs.

2. Materials and Methods

2.1 Animals and Diets

Ten healthy lambs (46.5 ± 1.06 kg BW) were randomly allocated into two groups of 5 lambs each. Control lambs (CON) received a diet without *Spirulina*, whereas in treated group, *Spirulina platensis* powder (SPP) was incorporated daily in the concentrate of each lamb at a rate of 1 g/10 kg BW.day. The SPP was obtained from a commercial retailer in a powdered form (HERBAFORCE LTD, UNITED KINGDOM). The basal diet was formulated to meet the lamb's nutrient requirements in order to balance the body weight gain at a rate of 0.3 kg/day (NRC, 1985). The composition of the basal diet is presented in Table 1. Diet was offered twice a day in the morning and evening with free access to water. The trial period was five weeks, with a pre-trial period of one week for adaptation to diets and facilities. Animals were weighed on days 0, 17 and 35 of experiment, after fasting for twelve hours before the morning feedings.

Table 1. Ingredients and calculated chemical composition of experimental diet

Item	%
Ingredient	
Berseem hay	14.65
Wheat straw	4.88
Corn grains	57.74
Cotton seed meal	13.07
Soybean meal	7.35
Limestone	0.79
Salt	0.49
Sodium bicarbonate	0.49
Ammonium chloride	0.24
Vitamin-mineral premix	0.29
Calculated chemical composition	
DM	88.73
CP	14.17
TDN	76.17
NDF	22.76
ADF	14.92
EE	4.14
Ca	0.73
P	0.37

2.2 Sampling and Analysis

Feed and refusals were recorded daily. Body weight was recorded, and blood samples were collected from the jugular vein for each lamb on days 0, 17 and 35 of the experiment. A portion of each blood sample was used for white blood cell (WBC) counts and to measure hemoglobin (Hb) (Linne & Ringsrud, 1992) and reduced glutathione (GSH) (Beutler et al., 1963) concentrations using commercial kits (Biodiagnostic, Egypt). Remained blood samples were then centrifuged at 3000 x g for 20 min. The obtained sera were separated and stored at -20°C until assayed spectrophotometrically (Spekol 11, Carl Zeiss Jena, Germany) for total protein (TP) and albumin concentrations as well as biochemical parameters, including serum enzymes activities as aspartate amino transferase (AST) and alanine amino transferase (ALT), blood urea nitrogen (BUN), triglyceride (TG) and cholesterol (CHO) levels (Young, 2001) according to the instructions of manufacturer (Endpoint kits from Diamond Diagnostics, Egypt). Globulin was calculated by subtracting albumin values from total serum protein. The albumin/globulin (A/G) ratio was also determined. Serum malondialdehyde (MDA) concentration was measured according to (Ohkawa et al., 1979) and the instructions of manufacturer (Biodiagnostic, Egypt). Retinol concentration in plasma was determined by modifying the method described by Suzuki and Katoh (1990). In brief, 50 ml of ethanol and 150 ml of hexane were added to 50 ml of plasma, and the hexane phase was recovered after

40-min mixing and 10-min centrifugation at 6500 x *g*. Retinol concentrations were calculated based on the absorbance of hexane extracts at 325 nm and 453 nm using the equations described (Suzuki & Katoh, 1990).

3. Statistical Analysis

All data are presented as mean ± SEM. Mean comparisons were performed using Wilcoxon-Mann-Whitney test and considering $P<0.05$ as level of significance.

4. Results and Discussion

4.1 Growth Performance

The growth performance of lambs is presented in Table 2. SPP supplementation to the diets of fatting lambs significantly increased ($P<0.05$) the final live body weights, daily live weight gain and feed intake compared with the control group. Moreover, the feed conversion ratio decreased ($P<0.05$) for SPP fed lambs compared with the control group. These results are consistent with previous reports that *Spirulina* supplementation induced greater live weights in cattle (Kulpys et al., 2009) and sheep (Holman et al., 2012). The better growth performance in lambs fed SPP supplemented diet may be a subsequence of high nutrient density of *Spirulina* as well as stimulation of the secretion of extracellular enzymes by the gut microflora (Tovar-Ramírez et al., 2002). *Spirulina* contains several nutrients, especially vitamins, minerals, essential fatty acids, amino acids and other nutrients that may promote faster growth (Gershwin & Belay, 2008). Furthermore, *Spirulina* has previously been shown to decrease rumen protein degradation and produce changes in bacterial community composition with a subsequent increase the efficiency of rumen microbial crude protein production in steers (Panjaitan et al., 2010).

Table 2. Effect of Spirulina platensis powder on growth performance of fattening lambs

Item	Treatments	
	CON	SPP
Initial weight (kg)	46.0 ± 0.50	47.0 ± 2.25
Final weight (kg)	50.4 ± 0.92^a	55.3 ± 2.10^b
Daily weight gain (kg/day)	0.127 ± 0.012^a	0.236 ± 0.015^b
Daily feed intake (kg/day)	1.64 ± 0.002^a	1.72 ± 0.010^b
Feed conversion ratio	13.2 ± 1.38^a	7.35 ± 0.41^b

CON = Control. SPP = Spirulina platensis powder.

Mean values in the same row with different superscripts differ ($P<0.05$).

4.2 Haematology

The Hb concentration and total WBC count of fattening lambs during the experiment are shown in Table 3. The Hb concentration and total WBC count were higher ($P<0.05$) in SPP fed group compared to the control. Leucocytes play an important role in non-specific or innate immunity and their count can be considered as an indicator of relatively lower disease susceptibility (Matanović et al., 2007). The increased WBC production may be due to the presence of phycocyanin and polysaccharides components in *Spirulina* (Zhang, 1994). The WBC counts and Hb concentration were increased with supplementation of polysaccharide of *Spirulina* in mice (at a dose of 30-60 mg/kg) and dogs (at a dose of 12 mg/kg) (Zhang et al., 2001). Similarly, *Spirulina* was found to enhance immunity in chickens fed 10 g/kg of *Spirulina platensis* (Qureshi et al., 1996) and fish (Watanuki et al., 2006).

Table 3. Effect of Spirulina platensis powder on Hb concentration (g/dl) and total WBC counts (per cmm) of fattening lambs

Item	1st Sampling (0 day)		2nd Sampling (17^{th} day)		3rd sampling (35^{th} day)	
	CON	SPP	CON	SPP	CON	SPP
Hb	12.0 ± 0.55	13.5 ± 0.60	10.7 ± 0.50^a	12.9 ± 0.27^b	12.2 ± 0.20^a	13.0 ± 0.09^b
WBC	11383 ± 1490	10883 ± 1482	8217 ± 784	9783 ± 683	7600 ± 891^a	9900 ± 908^b

CON = Control. SPP = Spirulina platensis powder.

Hb = Haemoglobin. WBC = White blood cells.

Mean values in the same row with different superscripts differ ($P<0.05$).

4.3 Biochemical Parameters

Plasma TP, albumin, globulin, A/G ratio, AST, ALT, BUN, TG, CHO and glucose concentrations are presented in Table 4. Significant differences (P<0.05) in the serum globulin, AST, ALT, CHO, TG, BUN and glucose were found between the treatment groups (Table 4). There was no significant difference in the concentrations of TP, albumen and A/G ratio between treatment groups. Supplementation with SPP induced significant elevation (P<0.05) in plasma globulin while significantly reduced (P<0.05) the AST, ALT, CHO and blood glucose concentrations. Furthermore, there was a significant increase in BUN with an unexpected increase in TG concentrations in lambs fed SPP supplemented diets.

Table 4. Effect of Spirulina platensis powder on biochemical parameters of fattening lambs

Item	1st Sampling (0 day)		2nd Sampling (17^{th} day)		3rd sampling (35^{th} day)	
	CON	SPP	CON	SPP	CON	SPP
TP (g/dl)	4.34 ± 0.21	4.52 ± 0.05	4.71 ± 0.09	4.96 ± 0.16	5.37 ± 0.11	5.80 ± 0.28
Albumin (g/dl)	2.08 ± 0.05	2.16 ± 0.12	2.50 ± 0.15	2.33 ± 0.21	2.99 ± 0.17	2.86 ± 0.17
Globulin (g/dl)	2.36 ± 0.10	2.29 ± 0.08	2.26 ± 0.06^a	2.62 ± 0.09^b	2.37 ± 0.09^a	2.93 ± 0.21^b
A/G ratio	1.02 ± 0.05	0.91 ± 0.08	1.09 ± 0.10	0.89 ± 0.11	1.27 ± 0.12	0.98 ± 0.09
AST (U/ml)	51.2 ± 2.00	45.3 ± 4.91	50.0 ± 2.55^a	40.5 ± 1.50^b	85.7 ± 14.5^a	39.3 ± 1.86^b
ALT (U/ml)	21.3 ± 0.67	22.3 ± 1.45	30.7 ± 2.73^a	20.3 ± 0.95^b	65.3 ± 12.8^a	25.7 ± 4.84^b
Urea (mg/dl)	19.3 ± 0.39	23.8 ± 3.37	25.3 ± 3.75^a	40.0 ± 1.42^b	19.4 ± 2.05	25.7 ± 1.92
Triglycerides (mg/dl)	78.4 ± 3.94	76.5 ± 2.42	68.3 ± 4.41^a	80.1± 1.34^b	79.4 ± 1.0	73.6 ± 5.54
Cholesterol (mg/dl)	45.9 ± 2.46	50.1 ± 6.70	62.8 ± 1.73^a	49.6 ± 2.48^b	81.2 ± 10.4	77.2 ± 12.5
Glucose (mg/dl)	40.2 ± 1.13	35.4 ± 5.78	68.65 ± 2.85 ^{a}	52.72 ± 6.15 ^{b}	54.9 ± 10.6	58.33 ± 2.90

CON = Control. SPP = Spirulina platensis powder.

TP = Total protein. A/G ratio = Albumin/globulin ratio. AST = Aspartate amino transferase. ALT = Alanine amino transferase.

Mean values in the same row with different superscripts differ (P< 0.05).

In this study, higher globulin concentration was found in the SPP supplemented group. The increased concentrations of plasma globulin may be related to the high protein contents in *Spirulina* (Gershwin & Belay, 2008). Increased plasma globulin levels are thought to be associated with a stronger innate response in lambs and indicate higher resistance (Matanović et al., 2007). This result is supported by increased total leukocytic count in SPP fed group.

Supplementation with SPP reduced the total plasma CHO. This is in consistent with previous findings in rats (Kato et al., 1984) hamsters (Riss et al., 2007) rabbits (Cheong et al., 2010) and human (Ruitang & Chow, 2010). Although the mechanism by which the SPP reduces CHO has not been fully examined, the hypocholesterolemic actions of SPP involve reducing plasma and liver CHO levels due to the increase in lipoprotein lipase and hepatic triglyceride lipase activity (Karkos et al., 2008), inhibition of both jejunal CHO absorption and ileal bile acid resorption (Nagaoka et al., 2005), in addition to modifying lipoproteins metabolism (decrease of low density lipoprotein and increase of high density lipoprotein; Torres-duran et al., 2007). Alternatively, the hypocholesterolemic activity of *Spirulina* is related to the large amount of cystine found in the C-phycocyanin protein of *Spirulina* (Nagaoka et al., 2005). A negative correlation was reported between the blood CHO concentrations and the level of cystine in dietary protein in rats fed a high CHO diet (Sugiyama et al., 1986).

Spirulina has been reported to have a hypolipidemic effect due to the C-phycocyanin protein which inhibits the pancreatic lipase activity in a dose-dependent manner (Torres-Duran et al., 2007). Controversy, the unexpected increase in the TG in SPP fed lambs in this study may imply that the *Spirulina* dose might be not enough to affect plasma TG or the supplementation period was not long enough for *Spirulina* to exert its lipid-modulating properties. Ishimi et al. (2006) reported that *Spirulina* may affect plasma lipids only in hyperlipidemic conditions. Further trails are required to characterize the efficacy of *Spirulina* in lowering blood lipid in ruminants.

The activity of AST and ALT are indicators of hepatotoxicity (Azab et al., 2013). In the present study, treatment with *Spirulina* showed a significant decrease in AST and ALT indicating that *Spirulina* may play a protective role against liver dysfunctions (Bhattacharyya & Mehta, 2012).

4.4 Antioxidative Status

Vitamin A, blood GSH and serum MDA concentrations are presented in Table 5. There was a significant increase ($P<0.05$) in vitamin A and GSH and a significant decrease ($P<0.05$) in MDA levels in SPP supplemented diets compared with control. Increased vitamin A and GSH and decreased MDA concentrations are indicators of improved oxidative defense of animal tissues (Celli, 2010). These results are in an agreement with previous reports stated that treatment with *Spirulina* could reduce oxidative stress with a consequent decrease in lipid peroxidation (Reddy et al., 2004; Riss et al., 2007). Reddy et al. (2004) suggested that *Spirulina* supplementation resulted in significantly higher activities of superoxide dismutase and catalase in the erythrocytes with a concomitant increase in reduced tripeptide glutathione content in broiler chickens. The antioxidative effect of *Spirulina* is related to several active ingredients, notably phycocyanin, polysaccharides, α-tocopherol and ß-carotene that have potent antioxidant activities working, individually or in synergy, directly on free radicals (Riss et al., 2007). Gershwin and Belay (2008) reported that the antioxidant activity of phycocyanin is about 20 times more efficient than vitamin C. In addition, *Spirulina* contains superoxide dismutase that acts indirectly by slowing down the rate of oxygen radical generating reactions (Belay, 2002).

In conclusion from the above results it can be concluded that SPP increased body weight gain, total WBC count, plasma globulin, vitamin A and reduced glutathione concentration while decreased liver enzymes activities, cholesterol, glucose and plasma malondialdehyde concentration. The potential application of *Spirulina* in fattening lambs diet as antioxidants to protect against free radicals cellular damage from stress, to enhance growth, and as an immunomodulator is worth exploring. Further experiments with implementation of different levels of *Spirulina* with higher replications and varying feeding practices are worthwhile to evaluate the nutritional value of *Spirulina* more accurately and precisely.

Table 5. Effect of Spirulina platensis powder on Vitamin A, GSH and MDA concentrations of fattening lambs

Item	1st Sampling (0 day)		2nd Sampling (17th day)		3rd sampling (35th day)	
	CON	SPP	CON	SPP	CON	SPP
Vitamin A (μg/dL)	60.9± 2.5	61.5± 1.8	69.0± 5.1^{a}	106.3± 3.8^{b}	69.1± 0.35^{a}	71.4± 0.84^{b}
Blood GSH (μM/L)	2.8± 0.64	3.0± 0.74	5.2± 0.75^{a}	9.6± 0.73^{b}	8.9± 0.69^{b}	14.2± 1.7^{b}
Serum MDA (nM/ml)	4.8± 0.87	4.1± 0.93	12.4± 5.9	8.0± 2.6	99.1± 27.4^{a}	15.7± 3.6

CON = Control. SPP = Spirulina platensis powder.

GSH = Reduced glutathione. MDA = Reduced malondialdhyde.

Mean values in the same row with different superscripts differ ($P<0.05$).

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