Original Research

Effect of *Spirulina* (*Spirulina platensis*) Supplementation on Antioxidant Status and Immunity in Growing Barbari Goats

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Abstract

Effect of supplementation of spirulina on antioxidant status and immunity was studied in growing Barbari goats. Eighteen male Barbari goats were divided into three groups, control (Group 1) and treatment groups (Group 2 and Group 3), consisting of six animals in each group as per completely randomized design. All the animals were fed with complete pellet feed having Bengal gram straw and concentrate mixture in 60:40 ratio. In Group 1 no Spirulina was supplemented while in Group 2 spirulina was supplemented @ 0.25% of DMI and in Group 3, spirulina was supplemented @ 0.50% of DMI. The duration of experimental feeding was 90 days. There was significant (P<0.05) increase in catalase and superoxide dismutase (SOD) activity in the spirulina supplemented group (Group 2 and Group 3) as compared to control group (Group 1) after 90 days of spirulina supplementation. There was improvement in the total immunoglobulin concentration and cell mediated immune response against PHA-p in spirulina supplemented group of goats. From present study it can be concluded that supplementation of Spirulina @ 0.25 and 0.50 of DMI improved the antioxidant and immune status in growing Barbari goats.

Key words: Antioxidant Enzymes, Barbari Goats, Immunity, *Spirulina*

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Introduction

Demand for animal products is increasing with changes in consumer tastes and expanding markets; particularly in developing countries where affluence is spreading. So there is challenge for animal scientist to meet the demand of people with limited feed resources. Many chemical feed additives have been used to improve the productivity of the animals but they have drawbacks of residue and health hazards to consumers. So natural feed supplements have to search upon. Azolla, an aquatic fern supplementation in the ration been reported to improve the performance of chicks (Yadav and Chhipa, 2016); bucks (Kumar

et al., 2016) and buffalo calves (Indira *et al*., 2009). Consequently, *spirulina* is emerging as a potential candidate to fulfill these criteria. *Spirulina* (*Arthrospira platensis*) is a blue-green microalgae, a source of protein of high biological value, vitamins, minerals and essential fatty acids, along with a number of bioactive compounds. Its dietary supplementation has been found to improve the animal health and productivity (Holman and Malau-Aduli, 2012). *Spirulina* supplementation has shown to reduce oxidative stress and enhance humoral and cell-mediated immune functions in human being. *Spirulina* is widely available in the market as food supplement for both humans and animals. Keeping the above facts in view, the present study was conducted to explore the potential of *Spirulina platensis* to improve immunity and reducing oxidative stress in Barbari goats.

Materials and Methods

Eighteen male Barbari goats were divided into three groups, control (Group 1) and treatment groups (Group 2 and Group 3), consisting of six animals in each group in a completely randomized design (CRD). All the goats were kept under uniform management conditions by housing them in well ventilated sheds. All the goats were fed with complete pellet feed having Bengal gram straw and concentrate mixture in 60:40. In case of control group no supplementation of *Spirulina platensis* (SP) was done, while in treatment groups of goats *Spirulina* was supplemented @ 0.25% (Group 2) and 0.50% (Group 3) of DMI respectively. An experimental feeding on three groups of goats was carried out for 90 days. Blood was collected before feeding from each animal of all three groups at 90th days of experimental feeding by puncturing the jugular vein with the help of a clean sterilized needle. Blood was collected in acid citrate dextrose buffer (@ 1.5 ml/10 ml of blood) in 2 ml eppendorf tube on ice. Blood samples collected in acid citrate dextrose (ACD) buffer for assays of oxidative stress indices were centrifuged in refrigerated condition at 2000 rpm for 20 min to harvest the erythrocytes. Buffy coat was removed and erythrocytes were washed thrice with ice cold phosphate buffer saline (PBS) solution. Packed erythrocyte was used for estimation of reduced glutathione (GSH). Erythrocytic suspension was prepared by mixing equal volume of erythrocytes and normal saline solution (NSS). This suspension was used for haemoglobin estimation. Exactly, 0.5 ml of erythrocytic suspension was mixed with 4.5 ml of stabilizing solution [0.5025 g EDTA (2.7 mM, pH-7.0) and β-Mercaptoethanol (0.7 mM) 24.55 µL in 500 ml distilled water] to form haemolysate. Haemolysate and RBC suspension were kept at -70^{o}C and used for antioxidants assay. Catalase was estimated in the RBC haemolysate as per the method of Cohen *et al.* (1970). Superoxide dismutase (SOD) activity of RBC haemolysate samples was measured using Nitro blue tetrazolium (NBT) as a substrate after suitable dilution according to the method of Marklund and Marklund (1974), with certain modifications as suggested by Minami and Yoshikawa (1979).

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The cell mediated immune (CMI) response was assessed in the goats after 90 days of experimental feeding by delayed type hypersensitivity (DTH) reaction by measuring the increase in skin thickness (Kim *et al*., 2000) in response to intra-dermal inoculation of phytohaemaglutinin-p (PHA-p). The skin on both the sides of the neck was shaved with the help of a razor and injected with 25 µg of PHA-p in 100 µL of PBS (pH 7.4) at two different sites 4 cm apart from each other on one side of neck and 100 µL of PBS at two different sites 4 cm apart on other side of the neck as a control. The skin thickness/ induration were measured at 0, 24, 48, 72 and 96 h post sensitization with the help of a vernier calliper. All the injections and skin measurements were made by the same person to minimize the variations. Immunoglobulins in plasma sample were estimated by Zinc turbidity method (Mc Ewan *et al*., 1970).

The data collected during study was statistically analyzed using generalized linear model (GLM) procedures and difference between treatments and periods was analyzed by using analysis of variance (ANOVA) as per Snedecor and Cochran (1989) using SPSS 1995.The difference between mean was obtained using Duncan's test at 95% level of significance.

Results and Discussion

The effect of *Spirulina platensis* supplementation on erythrocytic antioxidants enzymes catalase and superoxide dismutase (SOD) activity studied during feeding trial (0, 45 and 90th days) is presented in Table 1.

Table 1: Effect of *Spirulina platensis* supplementation on erythrocytic antioxidant profile of different groups of goats

Attributes	Group 1	Group 2	Group 3	Mean	SEM	P value
Catalase (U/mg Hb)						
0 day	47.01^{b}	45.66^{b}	44.39^{b}	45.68^{Y}	1.58	0.049
90 days	45.49^{b}	63.86^{a}	61.74^{a}	57.03^{X}		
Mean	46.25^{B}	54.76^{A}	53.06^{A}	51.35		
SEM	1.58					
P value	0.007			**G*P** = 0.028		
Superoxide dismutase (U/mg Hb)						
0 day	49.43^{b}	55.36^{b}	58.67^{b}	54.48^{Y}	3.22	0
90 days	49.91^{b}	65.34^{a}	67.72^{a}	60.99^{X}		
Mean	49.67^{B}	60.35^{A}	63.19^{A}	57.79		
SEM	3.22					
P value	0.004			**G*P** = 0.000		

Means bearing different superscripts (a,b) differ significantly; Means bearing different superscripts (A and B) in a row differ significantly Means bearing different superscripts (X and Y) in a column differ significantlyy at (P<0.05)

The catalase activity (U/mg Hb) was 47.01, 45.66 and 44.39 in Group 1, 2 and 3 respectively at 0 day which changed to 45.49, 63.86 and 61.74 at 90 days of experimental feeding. There was significant (P<0.05) increase in catalase activity in the *Spirulina* supplemented group (Group 2 and Group 3) as compared to control group (Group1). The catalase activity (U/mg Hb) was significantly (P< 0.05) higher in Group 2

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(63.86) that was statistically similar to Group 3 (61.74) and lowest for Group 1 (45.49) after 90 days of *Spirulina* supplementation. Similar trend was reported for superoxide dismutase activity. The superoxide dismutase activity (U/mg Hb) was 49.43, 55.36 and 58.67 in Group 1, 2 and 3 respectively at 0 day which changed to 49.91, 65.34 and 67.72 at 90 days of experimental feeding. There was significant (P<0.05) increase in superoxide dismutase activity in the *Spirulina* supplemented group (Group 2 and Group 3) as compared to control group (Group 1). The mean superoxide dismutase activity (U/mg Hb) was highest for Group 3 (63.19) that was statistically similar to Group 2 (60.35) and was lowest for Group 1 (49.67). The findings are in agreement with Abdel-Daim. (2014) who reported that buck fed concentrates with *Spirulina platensis* (SP) had significantly higher levels SOD concentration compared to the control group. Treatment with SP offered protection through attenuation of lipid peroxidation and decreased production of free-radical derivatives, as evident from the decreased levels of serum MDA, and normalization of GSH and SOD levels. Reddy *et al*. (2004) also suggested that *Spirulina* supplementation resulted in significantly higher activities of superoxide dismutase and catalase in the erythrocytes with concomitant increase in reduced tripeptide glutathione content in broiler chickens. The antioxidative effect *Spirulina* is related to several active ingredients, notably phycocyanin, polysaccharides, α-tocopherol and β-carotene that have potent antioxidant activities working, individually or synergy, directly on free-radicals (Riss *et al*., 2007) The effect of *Spirulina platensis* supplementation on total immunoglobulin concentration in blood serum studied during feeding trial (0, 45th days and 90th days) is presented in Table 2.

Table 2: Effect of *Spirulina platensis* supplementation on total immunoglobulin concentrations (mg/ml)

Attributes	Group 1	Group 2	Group 3	Mean	SEM	P value
0 day	36.62^{cd}	36.44^{bcd}	32.84 ^{d}	34.30 ^{Z}	0.98	0
45 day	35.60^{bcd}	39.76 ^{b}	38.86^{cb}	38.07 ^{Y}		
90 day	36.64^{bcd}	46.29 ^{a}	50.34 ^{a}	44.42^{X}		
Mean	38.28	40.83	40.68	39.26		
SEM	0.98					
P value	0.602			**G*P** = 0.000		

Means bearing different superscripts (a to d) differ significantly; Means bearing different superscripts (X to Z) in a column differ significantlyy at (P<0.05)

Mean total immunoglobulin concentration (mg/ml) changed from 36.62 to 36.64 in Group 1; 36.44 to 46.29 in Group 2 and 32.84 to 50.34 in Group 3 in 0 to 90 days of experimental feeding. There was significant increase in total immunoglobulin concentration in the *Spirulina* supplemented group (Group 2 and Group 3) as compared to control group (Group1). Qureshi *et al*. (1996) reported that no differences were observed in anti-sheep red blood cells antibodies during primary response. However, during secondary response K-strain chicks in all *Spirulina* dietary groups had higher total anti SRBC titers with 10,000 ppm SP group being highest versus the o ppm group. In broiler chicks IgG concentration was increased in 10,000 ppm SP

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group over the control. Qureshi *et al*. (1996) fed increasing levels of SP to chicken poults and evaluated immunological performance after injection of sheep red blood cells. No differences in total Ig were observed 7 days after the first injection. However, 7 days after a second injection, higher total Ig concentrations were observed in all poults fed SP. This suggests that the immune enhancement response is not immediate it means that to reach the final conclusion need longer duration of feeding trial. Hayashi *et al*. (1994) reported that in the primary immune response to sheep red blood cells (SRBC), significant increase of splenic PFCs per 10^6 cells was observed in mice fed *Spirulina* diet (SP-10 and SP-20), as compared to control group. However, HA titers observed in SP-10 and SP-20 was almost the same as the control group. While, immunoglobulin G (IgG)-antibody production in the secondary immune response hardly effected. The skin thickness (mm) measured at 0, 12, 24, 48, 72 and 96 h post sensitization with the help of a vernier caliper is presented in Fig. 1.

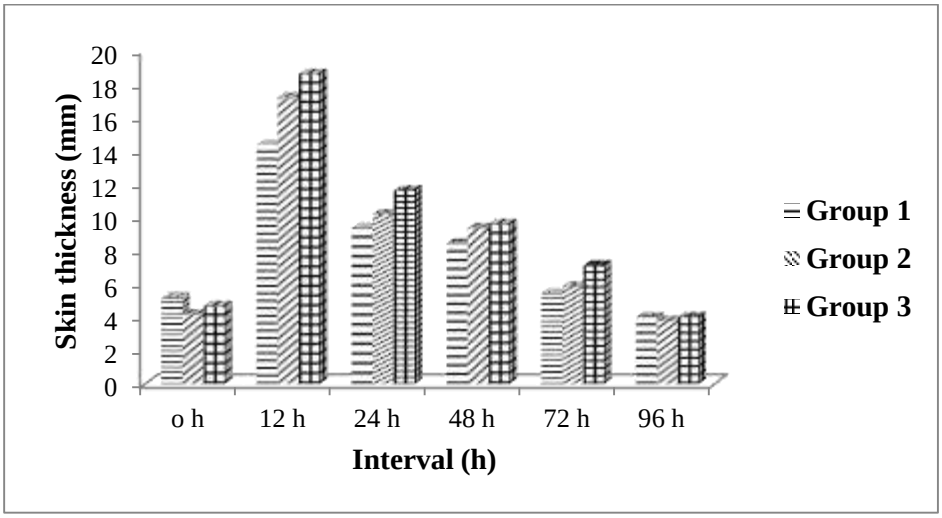

Fig.1: Effect of *Spirulina* (*Spirulina platensis*) supplementation on cell mediated immune response against PHA-p in different groups of goats

The skin thickness (mm) was 5.16, 4.16 and 4.66 at pre injection (0 hr) which increased to 14.33, 17.16 and 18.58 at 12 hr in Group 1, Group 2 and Group 3 respectively. After 12 hr to 96 hr post inoculation a statistically significant ($P<0.05$) decreasing trend in skin thickness was reported with period. The skin thickness (mm) was 9.33 (24h), 8.41 (48h), 5.41 (72h) and 5.32 (96h) in control group (Group 1) while 10.16 (24h), 9.33 (48h), 5.83 (72h) and 4.7 (96h) in Group 2 and 11.58 (24h), 9.58 (48h), 7.08 (72h) and 4.90 (96h) in Group 3. Overall mean skin thickness (mm) was 9.39 in 0.5% *Spirulina* supplemented group, 8.56 in 0.25% *Spirulina* supplemented group and 7.97 in un supplemented group of goats, showing improvement in cell mediated immunity on *Spirulina* supplementation. Qureshi *et al*. (1996) reported that 10,000 ppm *Spirulina* group chick had higher PHP-p mediated lympho proliferation response over the 0 ppm control group.

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Conclusion

From present study it can be concluded that supplementation of *Spirulina* @ 0.25 and 0.50 of DMI had significantly improved the antioxidant status and immunity in growing Barbari goats.

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