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Supplementary effect of spirulina on hematological status of rats during pregnancy and lactation

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Abstract. The effect of Spirulina on iron status was assessed based on hemoglobin, packed cell volume, serum iron, total iron binding capacity and ferritin levels of rats during pregnancy and lactation. Rats were fed 5 different kinds of diets (casein, Spirulina, wheat gluten, Spirulina + wheat gluten, Spirulina without additional vitamins and minerals) each providing 22 percent protein. Diets containing Spirulina alone or in combination with wheat gluten resulted in significantly higher iron storage and hemoglobin contents than casein and wheat gluten diets during the first half of pregnancy and lactation. Wheat gluten diet result in the smallest increase in hemoglobin levels and iron stores compared to other diets. The values of serum iron and iron binding capacity remained unchanged with different diets. Spirulina appears to be effective in improving the iron status of rats during pregnancy and lactation.

Key words: Blue green alga, Hematological response, Protein, Spirulina, Supplementation, Wheat gluten

Introduction

Spirulina, a blue green algae, has been used as human food for centuries by the natives of Mexico, Indo China, Japan and Central Africa where it grows naturally in the lakes. However, its human consumption as a food in other countries has not yet become popular.

Spirulina contains a substantial amount of iron and has been reported to produce good hemoglobin regeneration efficiency in anemic rats [1]. It has a unique blend of all hematopoietic nutrients; protein content ranges from 46 to 70 percent and it is a concentrated source of folic acid, cyanocobalamin, copper, vitamin E and available B-carotene [2, 3].

Incidence of anemia in India is very high, especially during pregnancy and lactation. Around 56 percent of women of reproductive age group are reported to be anemic [4]. Spirulina supplementation during these periods may be of great potential value. The present study was undertaken to assess

Table 1. Nutritional composition of *Spirulina platenses*

Nutrient	Amount
Moisture (%)	4.28 ± 0.26
Crude protein (%)	45.12 ± 0.81
Crude fat (%)	3.02 ± 0.12
Crude fiber (%)	2.28 ± 0.10
Ash (%)	0.93 ± 0.13
Iron (mg/100 g)	57.55 ± 2.50

Table 2. Composition of diet fed to rats during premating period

Ingredient	Amount (g/kg)
Casein	120
Oil	100
Dextrose	100
Cellulose	50
Mineral mixture	40
Vitamin mixture	10
Starch	680

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Materials and methods

Spirulina platensis (food grade) in the form of a fine powder with a mesh size 60 was procured from Murugappa Chettiar Research Centre, Madras, India. It was analyzed for proximate constituents and iron content [5] (Table 1).

Animals and experimental design. Female albino Wistar rats weighing 60–80 g were procured from Disease and Germ Free Animal House of Haryana Agricultural University, Hisar. The rats were housed individually in polypropylene cages in a room maintained at 22–24 °C with 12 hour light and dark cycles. The rats were fed a synthetic diet providing 10% protein until they attained 150 g body weight. Composition of the diet is given in Table 2.

Two mature female rats (weighing 150 ± 10 g) were housed with one male (weighing 200 ± 10 g) in a polypropylene cage. Vaginal smears of rats were tested each morning. For the preparation of vaginal smear, about 0.2–0.4 ml of sterile normal saline solution was introduced into the vagina with the help of a plastic Pasteur pipette. The same contents were immediately flushed out and spread on a glass slide. The smear was air dried, fixed with ethanol for 5 minutes and stained with giemsa stain for 30 minutes. Day 0 of pregnancy was designated after obtaining a combination of epithelial and/or cornified cells with sperm. The apparently pregnant rats were alloted to 5 dietary regimens, each dietary group consisting of 10 rats. The vaginal smears of apparently pregnant females were tested daily for the next 3 days. The presence of 100 percent leukocytes in the vaginal smears during these 3 consecutive days was considered to be an indication of pregnancy. At confirmed pregnancy, females were separated from males and placed in independent polypropylene cages. Two days before the expected date of delivery, paper cuttings were spread in the cages to facilitate the parturition and to provide warmth and cushioning to the pups.

Litter size was adjusted to 8 within 48 hours of parturition. Food offered and leftover was weighed daily from day 3 of pregnancy to day 14 of lactation. About 1 to 2 ml of blood were collected at days 0, 10 and 20 of pregnancy and day 14 of lactation from the retrorbital plexus.

Diet and feeding. The composition of the 5 diets provided during pregnancy and lactation is given in Table 3. The protein content of Spirulina, casein and wheat gluten were analyzed [5] and found to be 45, 74 and 60 percent, respectively. Three diets were prepared to contain 22 percent protein from casein, wheat gluten and Spirulina, respectively. A fourth diet contained equal amounts of both Spirulina and wheat gluten protein with total protein being equal to the rest of the groups. The fifth diet was similar to the Spirulina diet but did not contain mineral and vitamin mixtures. This was included to study the qualitative and quantitative effectiveness of minerals and vitamins contained in Spirulina itself to support pregnancy and lactation performance in rats. Food and deionized water were provided *ad libitum*.

Hematological analysis. Hemoglobin and packed cell volume were determined in fresh blood using standard procedures [8]. Serum iron was analyzed by atomic absorption spectrophotometry [9]. Unsaturated iron binding capacity (UIBC) and total iron binding capacity (TIBC) were determined employing the method of Persijn et al. [10]. Estimation of ferritin levels from serum samples was performed by the sandwich enzyme linked immunosorbent assay [11]. Rabbit antiserum to rat ferritin and its horseradish peroxidase enzyme

Table 3. Composition of diets fed to rats during pregnancy and lactation

Ingredient (g/kg diet)	Casein	Spirulina	Wheat gluten	Spirulina + wheat gluten	Spirulina (-VM[c], MM[d])
Casein	300	–	–	–	–
Spirulina	–	480	–	240	480
Wheat gluten	–	–	374	187	–
Dextrose	100	100	100	100	100
Oil	100	85	100	93	85
Cellulose	50	40	50	45	40
Mineral mixture[a]	80	80	80	80	–
Vitamin mixture[b]	10	10	10	10	–
Starch	360	205	286	245	295

[a] The mineral mixture was as described by Sampson et al. [6] and contained g/kg; calcium hydrogen phosphate 500, potassium citrate 220, sodium chloride 74, potassium sulfate 52, magnesium oxide 24, manganese sulfate 3.5, ferric citrate 6.0, zinc carbonate 1.6, copper carbonate 0.3, potassium iodate 0.01, sodium selinate $5H_2O$ 0.01, chromium potassium sulfate 0.55, sucrose 118.

[b] The vitamin mixture was as described by Grigor et al. [7] and contained g/kg; Vitamin A concentrate (200,000 IU retinyl acetate/g) 4.5, Vitamin D concentrate (400,000 IU calciferol/g) 0.25, -tocopheryl acetate 20.0, menaquinone 0.05, choline chloride 200, ascorbic acid 100, nicotinic acid 2.5, calcium pantothenate 2.0, thiamin hydrochloride 1.2, riboflavin 0.75, pyridoxine hydrochloride 0.5, cyanocobalamine 0.25, folic acid 0.1, D-biotin 0.02, sucrose to make 1000.

[c] Without vitamin mix added.

[d] Without mineral mix added.

conjugate were obtained from the National Institute of Immunology, New Delhi. Rat ferritin was procured from Sigma.

Statistical analysis. The data were analyzed statistically using analysis of variance. The significance of heterogeneity of variance was assessed by application of the Fmax test at the 1% level. The critical difference procedure was used in comparison of group means [12].

Results

Maternal weight gain and litter size. The effect of different diets on weight gain and litter size is given in Table 4. Spirulina + wheat gluten diet produced the maximum maternal weight gain response during pregnancy followed by

Table 4. Maternal weight gain during pregnancy and litter size of rats fed different diets

Diet	weight gain (g)	No. of pups/litter
Casein	95.2 ± 9.1 [c]	9.6 ± 1.0 [a]
Spirulina	96.2 ± 10.41[c]	12.6 ± 0.8 [b]
Wheat gluten	51.6 ± 4.67[a]	9.8 ± 1.46[a]
Spirulina + wheat gluten	105.2 ± 10.4 [d]	13.0 ± 1.09[b]
Spirulina (VM, -MM)	81.9 ± 5.47[b]	11.5 ± 1.02[b]

Values are mean ± SD of observations of 10 rats.
Means within column (a, b, c, d) bearing different letters are significantly different at $p < 0.05$.

Spirulina, casein, Spirulina (-VM-MM). The least was noted in animals fed the wheat gluten diet. The number of pups born per litter was higher in Spirulina fed groups compared to casein and wheat gluten fed groups.

Hemoglobin and packed cell volume. The effects of different diets on maternal hemoglobin and packed cell volume are shown in Table 5. Hemoglobin levels of rats fed casein and Spirulina containing diets were maintained until day 10 but decreased at day 20, whereas, rats fed wheat gluten showed a decrease in hemoglobin at both 10 and 20 days of pregnancy. At day 14 of lactation, the hemoglobin levels increased from those at day 20 of pregnancy in all the dietary groups.

Comparing the hemoglobin (Hgb) levels among the different dietary groups at day 10 of pregnancy, the wheat gluten diet group had lower Hgb than all other diet groups. At day 20, no significant differences were observed among different groups. At day 14 of lactation, hemoglobin levels in Spirulina and Spirulina + wheat gluten fed groups were higher compared to other groups. Of the remaining 3 groups, casein and Spirulina (-VM-MM) fed animals showed higher values than those consuming wheat gluten.

Like hemoglobin, packed cell volume was maintained at similar levels from day 0 to day 10 in all the groups except the rats fed wheat gluten in which the value decreased significantly ($p < 0.01$). From day 10 to 20 of pregnancy, the packed cell volume showed significant reduction in all the dietary groups. At day 14 of lactation, the values were comparable to initial values in all the dietary groups, except wheat gluten which had a significantly ($p < 0.01$) lower value. Comparison of packed cell volume among different dietary groups at day 10 of pregnancy and day 14 of lactation revealed lower

Table 5. Hemoglobin and packed cell volume of rats fed different diets during pregnancy and lactation

Diet	Hemoglobin (g/100 ml)			
	Pregnancy			Lactation
	0d	10d	20d	14d
Casein	$14.9 \pm 1.0^{a}_{z}$	$13.8 \pm 0.97^{b}_{yz}$	$9.6 \pm 0.84^{a}_{x}$	$13.1 \pm 0.12^{a}_{y}$
Spirulina	$15.5 \pm 0.98^{a}_{yz}$	$14.3 \pm 0.67^{b}_{y}$	$10.3 \pm 0.97^{a}_{x}$	$16.22 \pm 0.41^{c}_{z}$
Wheat gluten	$14.3 \pm 1.32^{a}_{z}$	$12.1 \pm 1.05^{a}_{y}$	$9.6 \pm 1.0^{a}_{x}$	$11.5 \pm 0.52^{a}_{y}$
Spirulina + wheat gluten	$15.5 \pm 0.98^{a}_{yz}$	$14.1 \pm 0.36^{b}_{y}$	$11.0 \pm 0.34^{a}_{x}$	$16.3 \pm 0.36^{c}_{z}$
Spirulina (-VM, -MM)	$14.8 \pm 0.66^{a}_{y}$	$13.7 \pm 0.77^{b}_{y}$	$10.5 \pm 0.47^{a}_{x}$	$14.4 \pm 0.45^{b}_{y}$
Diet	**Packed cell volume (%)**			
	Pregnancy			Lactation
	0d	10d	20d	14d
Casein	$41.8 \pm 1.16^{a}_{y}$	$39.0 \pm 1.09^{b}_{y}$	$32.0 \pm 1.78^{a}_{x}$	$41.2 \pm 0.91^{b}_{y}$
Spirulina	$41.4 \pm 1.33^{c}_{z}$	$39.8 \pm 1.93^{b}_{y}$	$32.6 \pm 3.2^{a}_{x}$	$41.8 \pm 1.03^{b}_{y}$
Wheat gluten	$40.2 \pm 1.32^{a}_{z}$	$36.0 \pm 1.16^{a}_{y}$	$31.4 \pm 2.9^{a}_{x}$	$34.2 \pm 0.8^{a}_{xy}$
Spirulina + wheat gluten	$42.0 \pm 1.09^{a}_{y}$	$39.4 \pm 2.2^{b}_{y}$	$34.0 \pm 2.6^{a}_{x}$	$40.0 \pm 0.83^{b}_{y}$
Spirulina (-VM, -MM)	$40.9 \pm 1.02^{a}_{y}$	$39.0 \pm 1.09^{b}_{y}$	$33.4 \pm 2.57^{a}_{x}$	$39.2 \pm 2.57^{b}_{y}$

Values are mean ± SD of observation of 10 rats.
Means within columns (a, b, c) and rows (x, y, z) bearing different letters are significantly different at $p < 0.01$.

values for animals fed wheat gluten than the rest of the groups. Other groups did not show variation.

Serum ferritin and iron. Changes in serum ferritin and iron levels with different diets are given in Table 6. Serum ferritin levels increased significantly at day 10 of pregnancy compared to the levels observed at day 0 in all the dietary groups except the wheat gluten group. By day 20, however, the levels had decreased dramatically in all the dietary groups during lactation, the levels increased from those observed at day 20 of pregnancy in all dietary groups except the one fed wheat gluten which showed only a slight increase that was insignificant. Among the different groups, at day 10 of pregnancy, the ferritin

Table 6. Serum ferritin and iron levels of rats fed different experimental diets during pregnancy and lactation

Diet	Serum ferritin (ng/ml)			
	Pregnancy			Lactation
	0d	10d	20d	14d
Casein	$210.0 \pm 43.59^{a}_{y}$	$355.0 \pm 55.22^{b}_{z}$	$90.0 \pm 27.4^{a}_{x}$	$180.0 \pm 66.33^{b}_{y}$
Spirulina	$217.0 \pm 54.03^{a}_{y}$	$487.0 \pm 73.9^{c}_{z}$	$27.5 \pm 14.79^{a}_{x}$	$200.0 \pm 57.22^{b}_{y}$
Wheat gluten	$247.0 \pm 47.63^{a}_{y}$	$215.0 \pm 99.12^{a}_{y}$	$75.0 \pm 16.58^{a}_{x}$	$100.0 \pm 25.98^{a}_{x}$
Spirulina + wheat gluten	$215.0 \pm 33.54^{a}_{y}$	$463.0 \pm 87.85^{c}_{z}$	$24.0 \pm 15.56^{a}_{x}$	$203.0 \pm 66.47^{b}_{y}$
Spirulina (-VM, -MM)	$231.0 \pm 69.02^{a}_{y}$	$434.0 \pm 60.77^{bc}_{z}$	$25.0 \pm 11.18^{a}_{x}$	$159.0 \pm 40.01^{ab}_{y}$
Diet	**Serum iron (μg/100 ml)**			
	Pregnancy			Lactation
	0d	10d	20d	14d
Casein	$249.0 \pm 30.67^{a}_{y}$	$253.0 \pm 29.44^{a}_{y}$	$232.0 \pm 19.2^{a}_{xy}$	$192.0 \pm 21.37^{b}_{x}$
Spirulina	$240.0 \pm 37.65^{a}_{xy}$	$267.0 \pm 22.77^{a}_{y}$	$214.0 \pm 32.06^{a}_{x}$	$211.0 \pm 25.63^{b}_{x}$
Wheat gluten	$233.0 \pm 42.76^{a}_{y}$	$220.0 \pm 29.52^{a}_{y}$	$185.0 \pm 15.26^{a}_{y}$	$115.0 \pm 23.74^{a}_{x}$
Spirulina + wheat gluten	$227.0 \pm 32.5^{a}_{xy}$	$248.0 \pm 25.14^{a}_{y}$	$196.0 \pm 15.3^{a}_{x}$	$190.0 \pm 15.3^{b}_{x}$
Spirulina (-VM, -MM)	$224.0 \pm 36.65^{a}_{x}$	$236.0 \pm 35.86^{a}_{x}$	$202.0 \pm 37.27^{a}_{x}$	$191.0 \pm 28.62^{b}_{x}$

Values are mean ± SD of observation of 10 rats.
Means within columns (a, b, c) and rows (x, y, z) bearing different letters are significantly different at $p < 0.01$.

levels for animals consuming Spirulina containing diets were higher than with casein and wheat gluten; the casein group, in turn, was higher than the wheat gluten. At day 20, Spirulina containing diet groups had somewhat less ferritin than the casein and wheat gluten groups, although the differences were insignificant. At day 14 of lactation, rats fed casein, Spirulina and Spirulina + wheat gluten showed higher values than those consuming Spirulina (-VM-MM) and wheat gluten but differed significantly ($p < 0.05$) only with wheat gluten.

Serum iron levels remained unchanged through day 10 of pregnancy in all the groups. At day 20, the levels were significantly ($p < 0.05$) lower in Spirulina and Spirulina + wheat gluten groups compared with values at

Table 7. Unsaturated iron binding capacity (UIBC) and total iron binding capacity (TIBC) in rats fed different experimental diets during pregnancy and lactation

Diet	UIBC (μg/100 ml)			
	Pregnancy			Lactation
	0d	10d	20d	14d
Casein	$376.0 \pm 37.28^{a}_{y}$	$380.0 \pm 35.4^{a}_{y}$	$313.0 \pm 67.5^{a}_{x}$	$472.5 \pm 37.9^{a}_{z}$
Spirulina	$375.3 \pm 37.00^{a}_{y}$	$356.0 \pm 48.45^{a}_{y}$	$299.0 \pm 50.66^{a}_{x}$	$445.0 \pm 46.55^{a}_{z}$
Wheat gluten	$401.0 \pm 42.81^{a}_{y}$	$388.0 \pm 29.57^{a}_{y}$	$314.0 \pm 46.28^{a}_{x}$	$663.0 \pm 64.4^{b}_{z}$
Spirulina + wheat gluten	$409.0 \pm 30.92^{a}_{y}$	$366.0 \pm 33.2^{a}_{y}$	$307.3 \pm 25.89^{a}_{x}$	$480.0 \pm 31.96^{a}_{z}$
Spirulina (-VM, -MM)	$380.7 \pm 33.91^{a}_{y}$	$387.0 \pm 39.28^{a}_{y}$	$329.0 \pm 31.09^{a}_{x}$	$468.0 \pm 41.76^{a}_{z}$
Diet	TIBC (μg/100 ml)			
	Pregnancy			Lactation
	0d	10d	20d	14d
Casein	$625.0 \pm 59.1^{a}_{xy}$	$633.0 \pm 58.19^{a}_{y}$	$545.0 \pm 85.4^{a}_{x}$	$664.5 \pm 45.96^{a}_{y}$
Spirulina	$615.3 \pm 38.43^{a}_{y}$	$623.0 \pm 44.33^{a}_{y}$	$513.0 \pm 50.35^{a}_{x}$	$656.0 \pm 56.62^{a}_{y}$
Wheat gluten	$634.0 \pm 78.91^{a}_{y}$	$608.0 \pm 55.75^{a}_{y}$	$499.0 \pm 52.0^{a}_{x}$	$778.0 \pm 37.62^{b}_{z}$
Spirulina + wheat gluten	$636.0 \pm 51.51^{a}_{y}$	$614.0 \pm 51.42^{a}_{y}$	$503.3 \pm 54.87^{a}_{x}$	$670.0 \pm 39.24^{a}_{y}$
Spirulina (-VM, -MM)	$604.7 \pm 46.5^{a}_{xy}$	$623.7 \pm 57.6^{a}_{y}$	$531.0 \pm 37.31^{a}_{x}$	$659.0 \pm 49.7^{a}_{y}$

Values are mean ± SD of observation of 10 rats.
Means within columns (a, b, c) and rows (x, y, z) bearing different letters are significantly different at $p < 0.01$.

day 10. Casein, wheat gluten and Spirulina (-VM-MM) groups did not show any significant change. Serum iron observed at day 20 of pregnancy were maintained at day 14 of lactation in all the groups except the one fed wheat gluten, which showed a significant decline. No intergroup differences were observed at either day 10 or day 20 of pregnancy. At day 14 of lactation, only the wheat gluten group showed a lower value than the other dietary groups.

TIBC and UIBC. No significant changes were observed in TIBC and UIBC at day 10, compared to the initial values in any of the groups studied (Table 7). In comparison to day 10 values, UIBC and TIBC decreased significantly ($p < 0.05$) at day 20 in all the groups. The values again increased in all the groups during lactation in comparison to that observed at day 20. Intergroup differences were observed only during lactation with significantly higher values of UIBC and TIBC for the wheat gluten fed group compared to the other groups.

Discussion

Comparison of different parameters used as indicators of iron status revealed that hemoglobin, packed cell volume and serum ferritin levels responded to duration and dietary treatments, whereas, serum iron and TIBC were not found to be very sensitive to these variations. Serum iron, even in combination with TIBC, is not a reliable indicator of iron stores [13]. In considering the effect of duration, the iron content of rats was found to increase during the first 10 days of pregnancy as is evident from the increase in hemoglobin and serum ferritin levels during that period. This is consistent with observations of other workers who reported that maternal blood volume and red cell volume increase considerably during the end of pregnancy (day 13 to parturition) to meet the high iron demand of developing fetus [13–15].

The effect of different diets on iron status revealed that feeding of Spirulina resulted in much higher iron storage and hemoglobin contents than feeding of casein and wheat gluten diets during the first half of pregnancy and during lactation. The Spirulina (-VM, MM) group without added iron produced good hemoglobin and iron stores during pregnancy, although the serum ferritin levels were slightly less during lactation. The response of the casein fed rats was better than animals eating wheat gluten. Wheat gluten was the least effective diet in contributing to the iron status of the rats.

Iron status is intimately related with the dietary protein quality. Lysine supplementation of a wheat gluten diet fed to rats has been shown to result in an increase in spleen iron, hemoglobin, hematocrit and serum iron binding capacity [16]. Protein influences the hematological response by supplying amino acids for synthesis of porphyrin and globin or by regulating the synthesis of hemoglobin and release of red cells into the circulation by altering endocrine function [17].

Another interesting phenomenon observed in the present study was that the losses of iron storage and serum iron at day 20 were relatively higher in the Spirulina fed groups compared to other groups. This may be due to the increased iron demand of the larger litter size observed in rats fed Spirulina diets irrespective of vitamin or mineral addition. The results thus reveal that Spirulina diets produced a greater hematological response in comparison to wheat gluten and casein diets. The levels of hematopoietic nutrients supplied by Spirulina were adequate to meet the higher demands of pregnancy and lactation as the group fed the Spirulina diet devoid of vitamin and mineral supplements produced a hematological response comparable to those fed diets containing supplemental vitamins or minerals.

The present study shows a good hematological potential of Spirulina in rats during pregnancy and lactation. Thus it can serve as a good supplementary food during these crucial periods to combat anemia. However, the use of Spirulina as human food in India is very rare at present on account of its poor commercial availability and high cost. Though Spirulina can be cultivated at home using simple technology, extensive efforts are needed to popularize it.

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