

## RESEARCH ARTICLE

# Body Weight and Liver Profile Status for Female Rabbits Administration DXN-spirulina and Combination of Folic acid, B6 and B12

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### ABSTRACT

**Introduction:** Spirulina is widely used as a nutritional supplement and provides new hope for millions or more nutritional deficiencies for people suffering from nutritional deficiencies and promises many health benefits to those people. The present study aimed to evaluate the effects of oral administration of DXN-spirulina (a food additive) on weight parameters and liver function in female rabbits.

**Materials and Methods:** Thirty mature female rabbits (16–18 weeks of age) were divided into three groups (10 rabbits/group). These groups include the first group served as a control group and; the second group was given DXN-spirulina in doses (0.5 g/kg of body weight) and the third group was given a mixture of folic acid, B6 and B12 (2.5 + 50 + 1 mg/kg of body weight). All groups were administered for a period of four weeks as a treatment. At the beginning and end of the treatment, the animals' initial and final body weights (BW) were recorded and liver enzymes were analyzed at the end of treatment.

**Results:** The results showed that the DXN-spirulina dose in final body weight was heavier ( $p < 0.05$ ) in GII than in GI and GIII and changed positively in BW (absolute or relative to initial BW). Aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase decreased ( $p < 0.05$ ) in GI and GII compared with GIII.

**In conclusion:** DXN-spirulina taken orally (0.5 mg/kg of body weight) as a natural antioxidant has been shown to improve body weight and liver enzyme status without adverse effects on these enzymes.

**Keywords:** FA, B6, B12, Body weight, Liver enzymes, Rabbits, Spirulina.

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### INTRODUCTION

Spirulina known as arthrospira is multicellular blue-green micro-algae, spiral-shaped, filamentous, photosynthetic, and naturally grows in alkaline water. Spirulina, a "little spiral" medium, has high protein content (about 65–70%) and value-added biochemical components. It is a superfood because it has an unfamiliar nutritional profile and has emerged as dieting has great potential. Spirulina is used for more nutritional security, energy, cleansing, and weight control. It is a natural green energy and good for every one.<sup>1</sup> The spirulina's green color is derived from chlorophyll, while the blue color is derived from the exotic pigment called phycocyanin (7–8%), a potent health-building pigment that helps produce stem cells.<sup>2</sup> In the recent, spirulina is now a healthy food all over the world. As one of the oldest living plants on the planet, spirulina is the (best of tomorrow) declared by the United Nations World Food

Conference. Recently, it is gaining popularity as a nutritional supplement.<sup>3</sup>

Maternal folic acid has been shown to be important in preventing neural tube defects.<sup>4</sup> In India, there is a policy to provide all pregnant women with iron and folic acid (60 mg and 500 mg/day) for the prevention of neural tube defects.<sup>5</sup> Vitamins are organic substances that are indispensable for the normal metabolic processes of animals, and vitamins are required in very small quantities and are classified as micronutrients. They are essential for maintaining health and performance and must be provided as part of the general diet. Vitamins can also be taken as pro-vitamins, which are converted into the corresponding vitamins by the animal organism. In most cases, the animal organism cannot synthesize vitamins.<sup>6</sup>

This research aims to study the effect of DXN-spirulina oral intake and also the effect of the combination of folic acid

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+ B6 + B12 on growth performance and liver enzymes in female rabbits.

## MATERIALS AND METHODS

The experimental animals for this study included 30 adult females, which were randomly divided into three groups as follows:

**Gr. 1 (control):** 10 adult female rabbits were orally administered distilled water (5 mL/animal) by gavage daily for 30 days.

**Gr. 2:** 10 adult female rabbits were orally administered DXN-spirulina (0.5 gm/ kg BW) dissolved in 5 mL distilled water by gavage daily for 30 days.

**Gr. 3:** 10 adult female rabbits were orally administered of combination of folic acid + B6 + B12 (2.5 + 50 + 1 mg/Kg BW) dissolved in 5 mL distilled water by gavage daily for 30 days.

### Body Weight and Body Weight Change Measurements

The experiment lasted for 30 days, and the animals were weighed at zero days (treatment) and at the end of the experiment. Body weight change after 30 days of treatment was estimated according to the following equation:

$$\text{BW change (kg)} = \text{Final BW (kg)} - \text{Initial BW (kg)}$$

### Relative Organs Weight Measurement

The following organs (liver, kidneys, ovaries, and uterus) were removed immediately from each animal sacrificed and weighed with an electronic scale (Tokyo/Japan/A and D Co., Ltd.), the weight was then determined by body weight such as relative weights.

### Hormones Assay (Enzyme-Linked Immunosorbent Assay (ELISA))

The basic principle of this method according to Ma *et al.*<sup>7</sup>

### Serum Aspartate Aminotransferase (AST) and Serum Alanine Aminotransferase (ALT) (U/I) estimations

Aspartate aminotransferase or (alanine aminotransferase) is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazine.<sup>8</sup>

### Serum Alkaline Phosphatase (ALP) Estimation (U/I)

This estimation was done by using the colorimetric determination of alkaline phosphatase activity (Biomerieux, France). Principally an estimation of ALP activity according to Tietz.<sup>9,10</sup>

### Statistical Analysis

Results were expressed as mean  $\pm$  standard deviation (M  $\pm$  SD), and experiments were analyzed with one-way ANOVA by SPSS (Special Program for Statistical System) version 22.0. The lowest significant test difference (LSD) was used to determine the differences between groups in the ANOVA test and the large group level on ( $p < 0.05$ ).<sup>11</sup>

## RESULTS

Study the effect of DXN-spirulina and combination of (FA + B6 + B12) on BW and BW changes of mature female rabbits shown in Table 1 appeared that the initial BW were

no significant differences in all groups while the final BW increased significantly ( $p < 0.05$ ) in group 2 compared to control and combination groups. Whereas in the G3 group there were unremarkable differences compared to the control group. BW changes in group management DXN-spr. It showed a significant ( $p < 0.05$ ) increase more than the control and combination group, also in the combination group, the BW changes significantly ( $p < 0.05$ ) increased more than that in the control group.

Effect of DXN-spirulina. and combination of (FA. + B6 + B12) on the weight of the relative organs in mature female rabbits: The relative organs weights alliterated in Table 2 that appeared significant ( $p < 0.05$ ) changes increment in group administration DXN-spr. in liver, kidney, ovary and uterus weights as compared with the GI (control) and group of combination tri-B vitamins (F + B6 + B12) while there were no significant changes between GI and GIII groups. On the other hand, relative organ weights were also documented in Table 2, which shows a significant increase ( $p < 0.05$ ) in liver weight in the DXN-spr group. More than the treated group and the other control group (GI). In the same line the weight of kidneys revealed a significant ( $p < 0.05$ ) increase in group DXN-spr. administration compared with combination (F + B6 + B12) and control. Also, ovaries and uterus weight showed an increase in GII as compared to other groups in this experiment.

Effect of DXN-spr. and combination of (FA. + B6 + B12) on liver enzymes in mature female rabbits: These enzymes aspartate aminotransferase (AST) alanine transaminase (ALT) Alkaline phosphatase (ALP) levels which indicated the health of liver and body against any treatment or material administration. The results in Table (3) revealed that AST level increment significantly ( $p < 0.05$ ) in the group III treated with combination as compared with control and DXN- spr. groups. Also the levels of ALT decreased significantly ( $p < 0.05$ ) in GII (DXN-spr.) group more than GIII and similarly to that reported in control group. ALP levels showed in the same table revealed significantly ( $P < 0.05$ ) decreased in group treated with DXN-spr. and control compared to GIII group.

## DISCUSSION

The data in Table 1 showed a significant increase in the BW and BW changes in the DXN-spr group and also in relative organs 30 days after taking DXN-Spr. The improvement of BW may be due to the enhancement of appetite and nourishing intake of this increased muscle mass. These results agreement with Vasudhevan and Tames<sup>12</sup> Those who documented that consuming spirulina (30 g/kg in the diet) alone or with different levels of vitamin C (50, 100, 300 mg/kg diet) for 30 days resulted in increased body weight and increased reproductive weight of the female system in goldfish. Spirulina has been identified as sources of protein in animal feed.<sup>13</sup> Also Ragab *et al.*<sup>14</sup> who reported that *S. plainsis* (0.6 g/kg diet) significantly enhanced the reproductive affinity for adult and pediatric rabbits. Seyidoglu *et al.*,<sup>15</sup> Spirulina has been documented as a feed additive used to improve the digestive system and potential genetic arrest of growth performance Spirulina, so

**Table 1:** Effect of DXN-spr. and combination of (FA + B6 +B12) on BW and BW changes in mature female rabbits

Groups Parameters	GI (Control)	GII (DXN-Spr.)	GIII (F+B6+B12)	LSD
Initial weight (Kg)	1.68 Aa ± 0.10	1.58 Aa ± 0.075	1.62 Aa ±0.08	N.S
Final weight (Kg)	1.67 Ba ± 0.08	1.92 Ab ± 0.079	1.79 Ba ± 0.075	0.23
BW. changes (Kg)	0.10 B ± 0.042	0.33 A ± 0.09	0.16 B ±0.05	0.22

Values expressed in capital letters mean significant differences at (p <0.05) levels (M ± SD).

**Table 2:** Effect of DXN-spr. and comb. of (FA.+B6+B12) on the relative organs weight in mature female rabbits

Groups Parameters	GI (Control)	GII (DXN-Spr.)	GIII (FA.+B6+B12)	LSD
Liver weight (g)	65.66 B ± 5.20	70.31 A ± 4.28	66.35 B ± 5.01	3.52
Kidney weight (g)	8.65 B ± 1.45	9.72 A ± 2.05	8.90 B ± 1.63	1.10
Ovary weight (g)	3.42 B ± 1.21	5.83 A ± 2.03	3.92 B ± 1.56	1.76
Uterus weight (g)	10.55 B ± 3.57	14.23 A ± 5.62	11.78 B ± 4.70	2.91

Values expressed in capital letters mean significant differences at (p <0.05) levels (M ± SD).

**Table 3:** Effect of DXN-spr. and combination of (FA.+B6+B12) on liver enzymes in mature female rabbits

Groups Parameter	GI (Control)	GII (DXN-Spr.)	GIII (FA+B12 +B6)	LSD
AST (U/I)	65.00 B ± 5.58	60.166 B ± 6.52	69.16 A ± 1.47	9.00
ALT (U/I)	69.66 B ± 3.07	64.33 B ± 5.75	70.00 A ± 6.87	7.33
ALP (U/I)	31.37 B ± 4.27	33.62 B ± 3.56	40.67 A ± 5.27	5.61

Values expressed in capital letters mean significant differences at (p <0.05) levels (M ± SD).

in recent years it is great as a natural additive, and it has also been shown that rabbits fed spirulina free in the diet cause significant increases in body weight, BG, total and food intake and also daily food intake as similar to results in this study. The results of Bonos *et al.*<sup>16</sup> Which did not record any significant increase in the growth performance or body weight in rabbits or broiler chicken spr. management in the diet. Also Khanradeh *et al.*<sup>17</sup> reported that the growth factors (including: final B.W, BW. Change and other growth factors of fish) didn't significant differences between group intake spirulina and control group.

The increase in weight organs may be due to increased muscle mass and increased metabolic activities of organs. These results are consistent with El-Ratel<sup>18</sup> that reported the weight of organs (liver and kidney) of the doe rabbit when Spirulina alga was administered in two doses (300 and 600 mg/kg of body weight) which resulted in a significant weight gain and showed a normal tissue structure. Liver in rabbits at all doses, and these observations indicate normal liver function in the spirulina-treated group and also in kidney and tissue weight, and these improvements due to spirulina contain natural antioxidant agents that have strong scavenging activity to give spirulina as hepato-protective potential. Also, the kidney weight of the group treated with spirulina increased with a normal range similar to what was shown by El-Ratel,<sup>18</sup> the normal tissue section of the kidneys in both doses of spirulina, which indicates the normal function of the kidneys and histology. Spirulina may act as an antioxidant agent against any substance toxicity by reducing MDA in the kidneys, liver, and blood. Spirulina also causes a decrease in lipid peroxidation (increased antioxidant enzymes such as: GSH, Septo-optic dysplasia and GPX levels).<sup>19,20</sup> Abu Aita<sup>21</sup> who showed the effect of spirulina on body weight and liver in rats, showed any statistically significant differences between groups compared to the control group, and also indicated the

recovery of spirulina body weight and liver weight because spirulina contains many important elements of the composition such as carbohydrates proteins, vitamins, essential fatty acids, carotenoids, alpha-chlorophyll, minerals and phococyanin, this combination enables spirulina to act as the main sources as food additives. It is an ideal food in the fourth century and as a diet of any supplement for this century from the Food and Agriculture Organization (FAO).<sup>22</sup>

Khanzadeh *et al.*<sup>17</sup> who reported no significant changes in ovarian weight for treatment groups with *Spirulina platensis* actually for female fish. The combination of (F + B6 + B12) did not significantly increase weight performance but did enhance and prevent anemia, and it may be due to this combination that it could not enhance appetite and therefore food intake was stable and did not significantly increase the mechanism of this formula on body weight it isn't clear yet. The weight of the ovaries and the uterus of the female rabbits in the spirulina group increased significantly compared to the other treated group, and these results may be due to the spirulina that enhanced the nutrition and hormonal environment of the reproductive system and the increase in the maturation of the ovaries and the uterus by increasing the weight and also attributed spirulina to contain natural antioxidants. There are restrictions on the date with respect to DXN-spr. on reproductive weight organs and some reproductive characteristics, these results agree with Khanzadeh *et al.*<sup>17</sup> who used spirulina as fish food in different doses, which showed that all meals contained spr. in the meal that caused the stages of reproductive maturity as well as increased ovarian weight with extension to its circumference and statistical differences with the longer diameter. Spirulina administration is associated with improved serum or blood levels of thyroid hormones because they contain antioxidant compounds in large quantities (these compounds include: beta-carotene, the active

form of phycocyanin, the di-protein that plays a role as radical scavenger proteins and the body's antioxidant capacity.<sup>23</sup> DXN-Spr and the combination of (F + B6 + B12) effect on liver enzymes in female rabbits, the results of liver enzymes (AST, ALT, and ALP) were significantly decreased in the DXN-Spr group more than other groups after 30 days, meaning that spr. taking it improves liver function, and has been shown by significantly lowering levels of these enzymes. These results similar to that documented by Abu Eita (21) He indicated that the activation of serum AST and ALT was decreased in the AlCl<sub>3</sub> group treated with spr. and compare it to the AlCl<sub>3</sub> administration group without treatment. Spirulina has a protective effect on the liver, which can be noted that it contains active ingredients such as: Phycocyanin, β-carotene, Vit. C and E, linolenic acid and sulfur polysaccharide, as well as selenium, which stimulates free radical activity in cleaning enzymes, a system responsible for protecting the liver.<sup>21,24</sup>

In conclusion for these results showed SPR. Taking it orally reduces and enhances liver function and protects the liver through the role of spr. They act as antioxidant agents, reduce lipids, increase HDL-C (the good cholesterol) and reduce liver enzyme activity (AST, ALT and ALP). These results are also documented by Abu Eita<sup>21</sup> which indicates a study for the role. From DXN-spr. It increases lipid peroxidation and the activities of antioxidant enzymes such as SOD and CAT. Ismail *et al.*<sup>25</sup> and Rajab *et al.*<sup>26</sup> who also spoke about the role of spr. in decreasing AST and ALT concentrations of DXN-spr treated group to the normal level compared to the untreated groups in rabbit's dose.

## REFERENCES

- Mahipal S. and Mahipal S. (2016). Spirulina as dietary supplement for health: A pilot study. *The Pharma Innovation Journal* 2016; 5(4): 07-09.
- Kulshreshtha A, Zacharia J, Jarouliya U, Bhadauriya P, Prasad GBKS, Bisen PS. Spirulina in Health Care Management. *Current. Pharm. Biotechnol.* 2008; 9(5):400–405.
- Earnest CP, Jordan AN, Safir M, Weaver E and Church TS. Cholesterol – lowering effects of bovine serum immunoglobulin in participants with mild hypercholesterolemia. *Ameri. J of Clinical Nutrition.* 2005; 81(4):792-798.
- Pitkin R.M. Folate and neural tube defects. *Am J Clin Nutr.* 2007; 85:285S–8S.
- Metz J, Sikaris KA, Maxwell EL and Levin MD. Changes in serum folate concentrations following voluntary food fortification in Australia. *Med J.* 2002; 176:90–91.
- Blum R, Brown G, Buyens A, Dersjant-Li Y, Miceli E, Nuyts C, Peisker M, Saibi L, Sainsbury T, Tredway E and Van Der Ven G. Vitamins in animal nutrition. Chairperson of FEFANA Working Group Vitamins. 2015; Pp:11.
- Ma H, Shieh KJ, and Lee S L. Study of ELISA technique. *Nat. and Sci.* 2006; 4(2):36-37.
- Schumann G and Klauke R New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: Preliminary upper reference limits obtained in hospitalized subjects. *Clin. Chim. Acta.* 2003; 327(1-2): 69-79.
- Tietz NW. *Textbook of clinical chemistry.* 3rd ed. C.A. Bruits E.R. Ashwood W.B. Saunders. 1999; Pp.:676-684.
- Odeh WD, Parlow AF, Cargille CM and Ross GT. Radioimmunoassay for human follicle-stimulating hormone. *Physiological studies. J. Clin. Invest.* 1981; 47(12): 2551.
- Abo-Allam R M. Data statistical analysis using SPSS Program. 1st ed. Publ. for the U. Cairo. 2003
- Vasudhevan I and James R. Effect of optimum spirulina along with different levels of vitamin C incorporated diets on growth, reproduction and coloration in goldfish *carassius auratus* (Linnaeus, 1758). *Indian J. Fish.* 2011; 58(2): 101-106.
- James R, Sampath K, Thangarathinam R and Vasudhevan I. Effects of dietary spirulina on growth, fertility, coloration and leucocytes count in red swordtail, *xiphophorus helleri*. *Israeli J. Aquacult.* 2006; 58(2): 97-104.
- Ragab MA, Bushara MM, Alazab AM, Fahim HN and El-Desoky AEI MI. Effect of spirulina platensis supplementation to Rabbits' Doe Diets on Reproductive and Economical Performance. *J. Animal and poultry prod.; Mansoura Univ.* 2019; 10(8): 237-242.
- Seyidoglu N, Gurbanli R, Koseli E, Cengiz F and Aydin C. The effect of spirulina (*Arthrospira*) platensis on morphological and hematological parameters evoked by social stress in male rats. *J Ist Vet Sci.* 2019; 3(1):21-27.
- Bonos E, Kasapidou E, Kargopoulos A, Karampampas A, Christaki E, Florou-Paneri P and Nikolakakis I. Spirulina as a functional ingredient in broiler chicken diets. *S. Afr. J. Anim. Sci.* 2016; 46(1): 94-102.
- Khanzadeh M, Fereidouni A E and Berenjestanaki SS. Effect of partial replacement of fish meal with spirulina platensis meal in practical diets on growth, survival, body composition and reproductive performance of three-spot gourami (*Trichopodus trichopterus*) (pallas 1770). *Aquacult. Int.* 2015. DOI 10. 1007/s 10499-015-9909-4.
- El- Ratel IT, Abdel-Khalek AE, El-Harairy MA, Fouda SF and Lamiaa Y and El-Bnawy S. Impact of green tea extract on reproductive performance, hematology, lipid metabolism and histogenesis of liver and kidney of rabbit does. *Asian J. Anim. Vet. Adv.* 2017;12:51-60.
- Karadeniz A, Yildirim A, Simsek N, Kalkan Y and Celebi F. Spirulina platensis protects against gentamicin-induced nephrotoxicity in rats. *Phytother. Res.* 2008; 22: 1506-1510.
- Elshazly MO, Abd El-Rahman SS, Morgan AM and Ali ME. The remedial efficacy of spirulin platensis versus chromium-induced nephrotoxicity in male Sprague-dawley rate. *PLoS ONE*, 2015; 10:10. 1371/ journal. Pone. 01267780.
- Abu-Aita NA. Hepatoprotective Effect of Spirulina platensis Against Aluminum Chloride Induced Liver Damage in Rats. *Global Veterinaria.* 2014;13(4):552-559.
- Pelizer LH, Pelizer EDG, Danesi CD, Rangel CEN, Sassano JCN, Carvalho SS and Moraes IO. Influence of inoculum age and concentration in Spirulina platensis. *Food Engin.* 2003; 56(4): 371-375.
- Ibrahim RM and Abdel-Daim MM. Modulating Effects of Spirulina platensis against tilmicosin-induced cardio-toxicity in mice cell. *J.* 2015; 17(1): 137-144.
- Abdel-Daim MM, Abuzead SMM and Halawa SM. Protective role of Spirulina platensis against acute deltamethrin-induced toxicity in rats. *PLoS One.* 2013; 8(9): e72991.
- Ismail MF, Ali DA, Fernando A, Abdraboh ME, Gaur RL, Ibrahim WM Raj MH and Ouhitit A. Chemoprevention of rat liver toxicity and carcinogenesis by spirulin. *Int. J. Biol. Sci.* 2009; 5(4):377-387.
- Ragab MA, Bushara MM, Alazab AM, Fahim HN and El-Desoky A. Effect of spirulina platensis supplementation to Rabbits' Doe Diets on Reproductive and Economical Performance. *J. Animal and poultry prod.; Mansoura Univ.* 2019; 10 (8): 237-242.