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The preventive role of different doses *Spirulina platensis* on lipid peroxidation and antioxidant status in healthy rats

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ABSTRACT

There are several antioxidant supplements using for reproductivity and life quality, especially herbal ones. Nowadays, herbal antioxidants especially *Spirulina platensis* has been still interested due to protective role on oxidant antioxidant balance and health. The present study, we aimed to evaluate the effects of different doses of *S. platensis* on important oxidant molecule MDA (TBA, oxidant malondialdehyde), and individual antioxidants as GPx (glutathione peroxidase), CAT (catalase) and SOD (superoxide dismutase) in healthy rats. For this purpose, we used thirty Wistar Albino male rats in three groups: Control, Low Dose Spirulina (500 mg/kg) and High Dose Spirulina (1000 mg/kg). *S. platensis* additives were given by oral gavage daily under a long forty five day of trial. At the end of the study, interestingly, all the antioxidants GPx, CAT, SOD and the oxidant MDA lipid peroxidation values were decreased in group high dose Spirulina compared to Control ($p < 0.05$). In spite of these decreases, testis weights and indexes were increased in group high dose Spirulina compared to Control significantly. The testis weights and indexes were evaluated for normal health of animals. It can be considered that due to the excessive protein and antioxidants features of *S. platensis*, oxidant and antioxidant mechanisms may be changed. However it can be said that Spirulina can compensate the homeostasis and health of animals. It is also suggested that the applications and different doses of *S. platensis* are needed to be assayed for further studies .

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Introduction

Free radicals are important reactive molecules which designate for oxidative stress imbalance between oxidative and antioxidative status. Although the oxidant molecules have a role on cellular damage with radical oxygen species, the antioxidant molecules suppress and scavenge free radicals. The most important oxidative molecule known as malondialdehyde (MDA) damages cells or tissues in stressful situations such as diseases, over nutrition or high protein, and thereby oxidative-antioxidative balance reduces. Also, all biological molecules in cells such as proteins, lipids, DNA or RNA can be damage during oxidative status. Especially, if protein gets oxidation, several functional changes can be existed in

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organism for example inactivation of DNA, decompose the protein peroxides and damage molecules (Halliwell and Whiteman, 2004). Nevertheless, antioxidants have a protective role on cellular mechanism against to oxidative damage (Misra and Niyogi, 2009; Firat et al., 2011). Glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) are the best-known antioxidant markers called as enzymatic antioxidants. These enzymatic molecules are capable of either removing or scavenging free radicals and their actions.

Spirulina platensis is the most popular antioxidant herbal due to its rich features and protection efficiency against to diseases. This natural antioxidant includes 60-70 % proteins, 4-7 % essential fatty acids (a-linoleic acid), 20% carbohydrates, 6-7% minerals, pigments (phycocyanin, b-carotene) and some special vitamins (Goksan and Kilic, 2009; Yang and Zhang, 2009; Yusuf et al., 2016). The antioxidant, anti-inflammatory, antimicrobial activities of *S. platensis* were reported by several researchers (Kitada et al., 2009; Mahdi et al., 2019; Seyidoglu et al., 2019). *S. platensis* contains special antioxidant molecules such as carotenoids, phycocyanin, xanthophylls, and phycobilins which have a key role on cardiotoxicity, hepatotoxicity, carcioneogenesis, tumor destruction and cancer (Mohan et al., 2006; Karkos et al., 2011; Ibañez et al., 2012; Abdel-Daim et al., 2013; Wu et al., 2016). All these researchers reported that especially phycocyanin, an extract of Spirulina, could protect the body against to oxidative stress. Martin et al. (2007) indicated that when the reactive oxygen species is occurred because of lipid peroxidation, antioxidant substances of Spirulina can prevent peroxidation and oxidative stress. Also, Mansour et al. (2006) explained that Spirulina may serve as an antioxidant by its oxygen quenching properties for free radicals due to its carotenoid content.

Physiological changes in oxidative status have been correlated with differences of organ weights and functions due to production of higher reactive oxygen species, such as testis (Vernet et al., 2004; Aitken and Roman, 2008; Bashandy et al., 2016). It was reported that food utilization and protein catabolism increase in oxidative reaction, and thereby organ weights decrease (Aitken and Roman, 2008). Sarkar et al. (2003) observed the reduction in the testicular weight due to germ cell loss in rats. Testicular weight loss seems to be a feature of infertility belongs to oxidant-antioxidant imbalance. Also, it was reviewed that in spite of other organ weights, testis weights may provide a sensitive alert for studies. Especially in immature animals, testicular weights measurement is accepted to interpretation the closely linking with body weight, testicular size and testis index (Greaves, 2007). Also, increase of the testis weights and testicular index are accepted as the normal growth of the animals.

As the use of natural antioxidants increases, more studies are necessary to evaluate the protective effects for health. In this study, it's aimed to investigate the oxidant and antioxidant efficiency of *S. platensis* on health and to identify the effects on testicular weights in healthy rats which fed by low and high doses of *S. platensis* under a long period of forty five day trial.

Materials and Methods

Animal housing and diets: The experimental protocols were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of University. The study was carried out with the permission of University Animal Experimentation Local Ethics Committee (Approval no: 2017/04-4).

Thirty, adult Wistar albino, male rats aged 7-8 weeks old and average weight 180-200 g were used in this study. Rats were housed under standard laboratory conditions (Lights: 12-hour light/dark/day, Humidity: 55% and Temperature: 24 ± 25 °C). The animals were housed in stainless steel cages and divided into three groups forty five day of trial. The groups were as follows: 1.Control (with basal diet); 2. Low dose Spirulina-LSp (with 500 mg/kg *S. platensis*); 3.High dose Spirulina-HSp (with 1000 mg/kg *S.platensis*). Rats were given ad libitum access to commercial rodent diet (Table1). *S. platensis* (Egert, Izmir-Turkey) was applied to rat by oral gavage daily was provided and modified according to the literatures (Nagaoka et al., 2005; Moreira et al., 2011).

Measurements: Blood samples were collected by puncture of heart under short (2-3 minutes) isoflurane anesthesia at the end of the study. Laparotomy was done for conceiving the reproductive system. Both testes (without epididymis) and gonadal fats were

Table 1. Basal diet formulation*

Contents	
Metabolize energy (ME-kj)	2000-2500
Crude protein (%)	23
Crude fat (%)	3
Crude fiber (%)	7
Crude ash (%)	8

* The basal diet was formulated and projected to take on maintenance requirements according to the NRC, 1995.

removed and weighted with precision weighing. (Sartorius, BL210S) immediately.

Blood specimens were centrifuged in the same days within 30 minutes at 2000 × g for 10 min at 4 °C and the plasma was stored at -80 °C until analyses day.

Antioxidant enzymes in plasma SOD, GPx and CAT enzyme activities were determined with commercial kits by microplate reader. MDA levels were measured according to the colorimetric method by spectrophotometer (Epoch, Biotek, Vermont, USA) that reported by Yoshoiko et al. (1979).

Antioxidant Parameters Kit Methods for SOD, GPx, CAT: Antioxidant enzymes activities SOD (Cat. No:706002), GPx (Cat. No:703102), and CAT (Cat. No:707002) in plasma were determined with commercial kits (Cayman Chemical Company, Michigan, USA) by microplate spectrophotometer (Epoch, Biotek, Vermont, USA). SOD Assay Kit uses tetrazolium salt for detection of superoxide radicals produced by xanthine oxidase and hypoxanthine. GPx Assay Kit measures GPx activity indirectly by two reactions with glutathione reductase (GR). Oxidized glutathione (GSSG), produced on reduction of hydroperoxide by GPx and is turned to reduced state by GR and NADPH. Catalase (CAT) assay kit utilizes the peroxidation function of CAT for determination enzyme activity. The method is based on the reaction of enzyme with methanol in the presence of H2O2 optimal concentration.

Lipid Peroxidation Manual Method for MDA: MDA levels were measured for lipid peroxidation according to the colorimetric method that reported by Yoshoiko et al. (1979) with microplate spectrophometer. Thiobarbituric acid (TBA) method were evaluated using the spectrophotometer. The reaction of thiobarbituric acid (TBA) with MDA, one of the aldehyde products of lipid peroxidation (Hodges et al., 1999). 0.5 plasma was mixed with 2.5 ml of 20% trichloroacetic acid (TCA) in a 14 ml centrifuge tube. 1ml of 0.6 % TBA was added to the mixture and warmed for 30 min in a boiling water bath then done cooling procedure. Then it was mixed into a 4 ml of nbutyl-alcohol layer in a separation tube and MDA content in the plasma was determined from the absorbance at 532 nm by spectrophotometer. Thiobarbituric reactive substances (TBARS) in the plasma was determined from the absorbance at 532 nm by spectrophotometer.

Statistical Assessment: Statistical analyses were performed with SPSS (Version 20.0). Data were tested for normality distribution and variance homogeneity assumptions. All the values were grouped, and the means and standard errors were calculated. One-way ANOVA was applied to all parameters to examine the differences between groups. Differences were considered significant at $p < 0.05$. If the differences between groups was provided to be significant ($p < 0.05$), differences evaluated by Tukey's test. On the other hand, in non-homogenous groups, differences between means were analyzed by Kruskal Wallis and following by Mann Whitney U test between groups one by one.

Results

The MDA, SOD, CAT and GPx values of all groups were provided in Table 2. Although there was no statistical difference between groups LSp and Control, there was a significant decrease in MDA in group HSp compared to Control (p: 0.001; 8.05 ± 0.06 and 4.39 ± 0.04 µmol L-1 group Control and HSp, respectively). Besides that, the antioxidants parameters (SOD, CAT and GPx) were tended to increase in group LSp than Control ($p > 0.05$, Table 2). However, interestingly the statistical decreases were determined in group HSp compared to Control (p: 0.001; p: 0.001 and p: 0.001 SOD, CAT and GPx, respectively) as shown in Table 2.

The testis and gonadal fats weights and testis indexes were shown in Figure 1. There were significant changes in testis weight in group HSp compared to Control (p: 0.017; 4.16 ± 0.16 and 4.91 ± 0.19 g, Control and HSp respectively) as figured in Figure 1a. Also, there was significant increase in testis index in group HSp than Control (Figure 1b; p: 0.008; 1.49 % and 1.83 % , Control and HSp respectively). However, no differences were observed about gonadal fat in all groups (Figure 1c; 1.27±0.08 , 1.30±0.10 and 1.40±0.13 Control, LSp and HSp respectively).

Discussion

This study, we assessed the oxidant and antioxidant efficiency of *S. platensis* in healthy rats. We also provide evidence that forty five day of trial feeding with low and high doses of Spirulina observed a protective effect on testis weight and gonadal fat.

Lipid peroxidation changes are linked to MDA and oxidative damage in cell, tissue and organs. Insight of the literatures, MDA levels increase due to free radicals activation resulting from fatty acids in tissue damage. Thereby, the antioxidant defense system cells are activated and the oxidant activities are reduced (Urso and Clarkson, 2003). In the present study, the MDA value in high dose Spirulina group was found lower compared to both control and low dose Spirulina groups. It's thought that *S. platensis* may

Table 2. Serum oxidant and antioxidant parameters in control and experimental groups (mean±standard error, n=30).

Parameters	Groups		
	Control	**Low dose Spirulina** (LSp-500 mg kg^{-1})	**High dose Spirulina** (HSp-1000 mg kg^{-1})
MDA (µmol L^{-1})	8.05 ± 0.06	7.70 ± 0.10	4.39 ± 0.04[ab]
SOD (U L^{-1})	1.20 ± 0.04	1.22 ± 0.02	0.79 ± 0.03[ab]
CAT (nmol min^{-1})	120.54 ± 1.73	120.57 ± 1.21	75.26 ± 2.80[ab]
GPx (nmol min^{-1})	34.68 ± 0.92	34.95 ± 1.45	18.42 ± 1.23[ab]

MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase. *Different superscripts a, and b show differences: (a); p<0.05, High dose Spirulina group versus Control group. (b); p<0.05, High dose Spirulina group versus Low dose Spirulina group

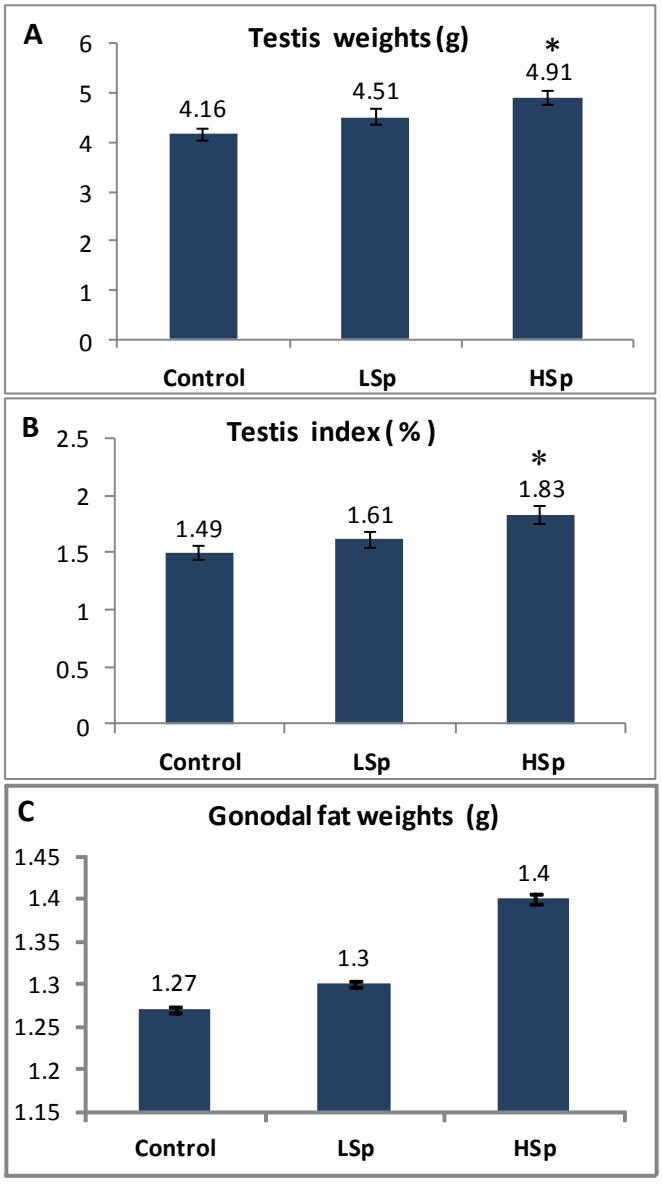

Figure 1: The effect *S. platensis* on testis and gonadal fat weights parameters at the end of experiment.

A) Testis weights; B) Testis index; C) Gonadal fat weights. Groups: Control, LSp: Low dose Spirulina , HSp: High dose Spirulina, respectively. * p < 0.05, group HSp versus group Control.

reduce the accumulation of lipid peroxidation in organism. Nevertheless, it was suggested that CAT, SOD and GPx are the crucial endogenous antioxidants molecules which closed to free radicals and oxidative damages. All these enzymes are accepted for the antioxidant balance and homeostasis. In the present study, the individual antioxidants CAT, SOD and GPx values were determined than expected. All these enzymes were decreased in high dose Spirulina group compared to groups control and low dose Spirulina. Similarly our results, Yu et al. (2018) reported that increasing dose of Spirulina may decrease the hepatic CAT, SOD and GPx values in coral trout due to its excessive activation on enzymatic antioxidant system. Also it was indicated that *S. platensis* play role as an antioxidant system due to possess the single oxygen quenching properties.

Testicular weight and index are important valuable parameters for measuring reproductive toxicity and health of animals. It was reported that testis index increases in healthy condition and shows the normal growth of animals (Greaves, 2007). In the present study, testis weights and indexes of rats were increased in high dose Spirulina group compared to groups control and low dose Spirulina. It's thought to that high dose of *S. platensis* may increase testis weight due to its high protein content. Similarly our results, Afkhami-Arkadani et al. (2018) found that testis weights were increased feeding Spirulina in testicular injury rats. They suggested that Spirulina effects positively on oxidative stress and protects the rats against to testicular damages due to its protein contents.

Conclusion

According to our results, rats seemed to tolerate the oxidant antioxidant differences under long trial period

and normal rearing conditions. This could be the ability of rats to compensate the S. platensis and endogenous antioxidants together on health and organ metabolism. In conclusion, insight of the literatures, within our results, total antioxidant status should be determined to evaluate the individual antioxidant better. However, it's thought that S. platensis may be one of the most important herbal food and represents an antioxidant supplement for homeostasis. It's also thought that the doses must be determined due to antioxidant properties of Spirulina. However, more studies are necessary to clarify the efficiency of testicular health and antioxidant correlation, and for future works.

References

Abdel-Daim, M. M., Abuzead, S. M. & Halawa S. M. (2013). Protective role of Spirulina platensis against acute deltamethrin-induced toxicity in rats. *Plos One, 8*(9), e72991.

Afkhami-Arkadani, M., Hasanzadeh, S., Shahrooz, R., Delirezh, N. & Malekinejad, H. (2018). Antioxidant effects of Spirulina platensis (Arthrospira platensis) on cyclophosphamide-induced testicular injury in rats. *Veterinary Research Forum,* 9, 35-41.

Aitken, R. J. & Roman, S. D. (2008). Antioxidant systems and oxidative stress in the testes. *Oxidative Medicine and Cellular Longevity,* 1, 15-24.

Bashandy, S. A. E., El Awdan, S. A., Ebaid, H. & Alhazza, I. M. (2016). Antioxidant potential of *Spirulina platensis* mitigates oxidative stress and reprotoxicity induced by sodium arsenite in male rats. *Oxidative Medicine and Cellular Longevity*, 27174351.

Devasagayam, T., Tilak, J., Boloor, K., Sane, K. S., Ghaskadbi, S. S. & Lele, R. (2004). Free radicals and antioxidants in human health: current status and future prospects. *Journal of the Association of Physicians of India*, 52, 4-10.

Firat, O., Cogun, H., Yuzereroglu, T., Gok, G., Kargin, F. & Kotemen, Y. (2011). A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, Oreochromis niloticus. *Fish Physiology and Biochemistry,* 37, 657-666.

Goksan, T. & Kilic, C. (2009). Growth and biochemical composition of Spirulina platensis Geitler in summer period under the conditions of Çanakkale Turkey. *Asian Journal of Chemistry*, 21, 4947-4950.

Greaves, P. (2007). Male genital tract. In: *histopathology of preclinical toxicity studies. 3rd ed.* (661-716). US: Elsevier Science Press.

Halliwell, B. & Whiteman, M. (2004). Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? *British Journal of Pharmacology, 142* (2), 231–255.

Hodges, D. M., DeLong, J. M., Forney, C. F. & Prange, R. K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta,* 207, 604-611.

Ibañez, E., Herrero, M., Mendiola, J. A. & Castro-Puyana, M. (2012). Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates. In *Marine Bioactive Compounds*. (pp. 55-98). US: Springer.

Kalafati, M., Jamurtas, A. Z., Nikolaidis, M. G., Paschalis, V., Theodorou, A. A., Sakellariou, G. K., Koutedakis, Y. & Kouretas, D. (2010). Ergogenic and antioxidant effects of spirulina supplementation in humans. *Medicine & Sciences in Sports & Exercise*, 42, 142-151.

Karkos, P. D., Leong, S. C., Karkos, C. D., Sivaji, N. & Assimakopoulos, D. A. (2011). Spirulina in clinical practice: evidence-based human applications. *Evidence-Based Complementary and Alternative Medicine*, 2011, 1-4.

Kitada, K., Macmudah, S., Sasaki, M., Goto, M., Nakashima, Y., Kumamoto, S. & Hasegawa, T. (2009). Antioxidant and antibacterial activity of nutraceutical compounds from Chlorella vulgaris extracted in hydrothermal condition. *Separation Science and Technology,* 44, 1228-1239.

Mahdi, T., Akineh, Y., Ghodrat, R. M., Mojtaba, N. & Soleiman, M. (2019). The effect of Spirulina platensis meal on antioxidant gene expression, total antioxidant capacity, and lipid peroxidation of rainbow trout (Oncorhynchus mykiss). *Fish Physiology and Biochemistry,* 45, 977-986.

Mansour, N., Mcniven, M. & Richardson, G. (2006). The effect of dietary supplementation with blueberry, a-tocopherol or astaxanthin on oxidative stability of Arctic char (*Salvelinus alpinus*) semen. *Theriogenology,* 66, 373-382.

Martin, D. A., Afonso, L. O., Hosoya, S., Lewis-Mccrea, L. M., Valente, L. M. & Lall, S. P. (2007). Effects of moderately oxidized dietary lipid and the role of vitamin E on the stress response in Atlantic halibut (Hippoglossus hippoglossus L.). Aquaculture, 272, 573-580.

Misra, S. & Niyogi, S. (2009). Selenite causes cytotoxicity in rainbow trout (Oncorhynchusmykiss) hepatocytes by inducing oxidative stress. Toxicology In Vitro, 23, 1249-1258.

Mohan, I. K., Khan, M., Shobha, J. C., Naidu, M. U., Prayag, A., Kuppusamy, P. & Kutula, V. K. (2006). Protection against cisplatin-induced nephrotoxicity by Spirulina in rats. Cancer Chemotherapy Pharmacology, 58, 802-808.

Moreira, L. M., Rocha, A. S. R., Ribeiro, C. L. G., Rodrigues, R. S. & Soares, L. S. (2011). Nutritional evaluation of single-cell protein produced by Spirulina platensis. African Journal of Food Science, 5, 799-805.

Nagaoka, S., Shimizu, K., Kaneko, H., Shibayama, F., Morikaw, K., Kanamaru, Y., Otsuka, A., Hirahashi, T. & Toshimitsu, K. (2005). A novel protein C-phycocyanin plays a crucial role in the hypocholesterolemic action of Spirulina platensis concentrate in rats. *Journal of Nutrition,* 135, 2425-2430.

Sarkar, M., Chaudhuri, G. R., Chattopadhyay, A. & Biswas, N. M. (2003). Effect of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats. *Asian Journal of Andrology,* 5, 27-31.

Seyidoglu, N., Gurbanli, R., Koseli, E., Cengiz, F. & Aydin, C. (2019). The effects of Spirulina (Arthrospira) platensis on morphological and hematological parameters evoked by social stress in male rats. *Journal of Istanbul Veterinary Sciences,* 3, 21-27.

Urso, M. L. & Clarkson, P. M. (2003). Oxidative stress, exercise and antioxidant supplementation. *Toxicology*, 189, 41-54.

Vernet, P., Aitken, R. J. & Drevet, J. R. (2004). Antioxidant strtategies in the epidydimis. *Molecular Cellular Endocrinology*, 216, 31-39.

Yang, L. & Zhang L. M. (2009). Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources. *Carbohydrate polymers,* 76, 349-361.

Yoshoiko, T., Kawada, K. & Shimada, T. (1979). Lipid peroxidation in maternal and cord blood and protective mechanism against active-oxygen toxicity in the blood. *American Journal of Obstetrics & Gynecology,* 135, 372-376.

Yu, W., Wen, G., Lin, H., Yang, Y., Huang, X., Zhou, C., Zhang, Z., Duan, Y., Huang, Z. & Li, T. (2018). Effects of dietary Spirulina platensis on growth performance, hematological and serum biochemical parameters, hepatic antioxidant status, immune responses and disease resistance of Coral trout Plectropomus leopardus (Lacepede, 1802). *Fish and Shellfish Immunology*, 74, 649-655.

Yusuf, M. S., Hassan, M. A., Abdel-Daim, M. M., El Nabtiti, A. S., Ahmed, A. M., Moawed, S. A., El-Sayed, A. K. & Cui, H. (2016). Value added by Spirulina platensis in two different diets on growth performance, gut microbiota, and meat quality of Japanese quails. *Veterinary World,* 9,1287-1293.

Wu, Q., Liu, L., Miron, A., Klimova, B., Wan, D. & Kuca, K. (2016). The antioxidant, immuno-modulatory, and anti-inflammatory activities of Spirulina: An overview. *Archive Toxicology*, 90, 1-24.