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Effect of *Spirulina platensis* on the immunological and hematological factors in comparison with levamisole in Persian shepherd dogs

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ABSTRACT

Background & Aim: *Spirulina microalgae* is known to has beneficial effects in the treatment of many animals diseases. Effects of *Spirulina platensis* microalgae, singly or in comparison with levamisole drug are not still studied in dogs. Thus, the present study was conducted to investigate the effects of this microalgae on hematological and immunological parameters in dogs.

Experimental: In this research, 24 native breed dogs were used for 30 days. The dogs were divided into three groups of eight randomly, including a control group, treatment group (received 2 g *Spirulina* orally*)* and the third group received 5-mg/kg Levamisole. At the end of mentioned period, hematocrit percent, Hb, RBC, WBC, Neutrophil, Lymphocyte, Eosinophil, monocyte, basophil levels and immunological factors including IgM, total protein and phagocytic precent were measured.

Results: Hematocrit in levamisole receiving group increased significantly (P<0.05). Results showed that RBC and Hb levels in *Spirulina* receiving group were significantly higher than the control and levamisole groups. Alb and Neutrophil levels in *Spirulina* receiving group were lower than the other two groups. In addition, Eosinophil and WBC levels in control group were significantly lower than the other two groups, but monocyte and Lymphocyte levels in control group increased significantly (P<0.001). Also, on the 30th day, total protein and phagocytic percent in *Spirulina* receiving group were significantly higher than others groups. In addition, in *Spirulina* and levamisole groups a significant increase in IgM level was observed (P<0.001).

Recommended applications/industries: It can be concluded that the use of *Spirulina* in dogs for a short time could improve hematological and immunological factors and the effects of *Spirulina alga* in the most factors is comparable to levamisole drug.

1. Introduction

Spirulina platensis is a blue-green alga, that is economically important. Because of high amounts of protein, vitamins D, E, B_1, B_2, B_6, B_{12}, C and Pantothenic acid, minerals, fatty acids, essential amino acids, antioxidant, pigments as phycocyanin *Spirulina* are considered as a probiotic and immune system booster (Cohen, 1997; Shams *et al.*, 2017; Goksan *et al.*, 2007; Henrikson, 1989; Dasgupta, 2001). Recently, algae are as sources of bioactive components and they showed anticancer, antioxidant, antidiabetic, and anti-inflammatory features (Shams *et al.*, 2017). Various factors including aging, stress and diseases affect the immune and blood systems. Regard to hematological results, we can understand about health of other organs

and biological and metabolic changes in the body, as well as the effects and comparison of chemical and algal medicines. To strengthen the immune system and clear the blood of infectious agents and free radicals, studies have been performed on seagrasses and algae, due to their low side effects, its economic value and lack of resistance to pathogens. Herbal and algal medicines have a special value and place in treatment. The most important pigment in *Spirulina* is phycocyanin. This blue pigment has very important effects as stimulating the immune system (Kulshreshtha *et al*., 2008). Therefore, the aim of this study was to investigate and compare the effect of *Spirulina* on the hematological changes of Persian Shepherd dogs in comparison with levamisole, which is one of the immunoplas drugs. It is suitable for generalizing the positive results of research to humans (Mani *et al*., 2000). Salighehzadeh *et al*. (2014) concluded that *Spirulina* dietary supplement at the level of 10% improves blood factors, safety and biochemical parameters of mesopotamichthys sharpeyi (Salighezadeh *et al*., 2014). Soltani *et al.* (2012) found that consumption of *Spirulina platensis* extract (800-mg/kg orally for three days) had an immunogenic effect on infectious cells of mice infected with *Candida albicans* and breast cancer (Soltani *et al*., 2012). Studies by Sahan *et al*. (2015) and Shokri *et al*. (2014) concluded that the use of *Spirulina platensis* increased the phagocytic activity of macrophages as well as the activity of NK cells; lysozymes; production of antibodies; Interferon gamma and cytosines such as interleukins 1,4 and 17 (Sahan *et al*., 2015; Shokri *et al*., 2014). Ragap *et al*. (2012) reported that consumption of *Spirulina* in *tilapia* fish (*Oreochromis niloticus*), increased immune function by the bacterial response to *Aeromonas hydrophila* and increased their phagocytic and lysozyme activities. Research by Krishnaveni *et al*. (2013) on the effect of *Spirulina* supplement probiotic on the hemato-immunological function of *Catla crabs* showed that the addition of *Spirulina maximus* to the crab diet improved hematological parameters such as RBC, WBC, hemoglobin, MCV and MCH; and in addition to immunological factors such as lymphocytes; Lysosomes, monocytes, IgM and phagocytic index also grew well (Krishnaveni *et al*., 2013).

Qureshi *et al*. (1996), in the study of the effects of *Spirulina* as a safety factor in broilers, concluded that chickens fed with *Spirulina* had IgG levels; PHA-P lymphocyte response, macrophage phagocytic activity and activity of natural NK killer cells were higher than control group and *Spirulina*-treated macrophages showed higher percentage of phagocytic activity for red blood cells (SRBC) (Qureshi *et al*., 1996). Also, Krishan *et al*. (2015) showed that the use of *Spirulina* has protective effects against the infectious virus anemia in broilers (Krishan *et al*., 2015). Al-Batshan *et al*. (2001) showed that dietary supplement *Spirulina platensis* improved phagocytic function of chick macrophages as well as metabolic pathways leading to increased nitric oxide synthase activity (Qureshi *et al*., 1996). Mathew *et al*. (1995) found that the immunological properties of *Spirulina* play an important role in anti-cancer properties such as oral cancer (Mathew *et al*., 1995). Pankaj and Varma (2013) found that *Spirulina platensis* protects hematological parameters against diabetes-induced alloxan in mice (Pankaj and Varma, 2013). In addition, Seyidoglua *et al*. (2017) documented that consumption of *spirulina* increased the production of cytokines CD^{4+} and CD^{8+} T lymphocytes in rabbits (Seyidoglua *et al*., 2017). In addition, Kim *et al*. (2013) in a study on the protective effects of *Spirulina* in *Parrots* (*Oplegnathus fasciatus*) showed that the addition of *Spirulina* in the diet of fish increased the hematocrit (Kim *et al*., 2013). The effects of *Spirulina platensis* algae as an immune stimulant on immunological parameters in the various aquatic animals, especially in the crab *Cyprinus carpio*, were reported by Watanuki *et al.* (2006); and the immune system was strengthened by these algae. In humans; mammals; chickens and fish; *Spirulina* showed an immunostimulatory effect by increasing resistance to infections due tostimulation of antibodies and cytokinin production (Moorhead *et al*., 2005). Also, *Spirulina* has macrophage activity (B, T cells). *Spirulina*-derived sulfolipids have great effects on the HIV, herpes virus, cytomegalovirus and influenza virus.

With regard to the lack of relevant studies on the hypoglycemic-hypolipidemic effects of *Spirulina* extract and general changes in serum biochemical factors in dogs; the present study aims at investigating the effects of *Spirulina* on the decreasing of the parameters related to lipids profile and hepatica enzymes in dogs.

2. Materials and Methods

2.1. Preparation of algae

Spirulina *platensis* algae as oral tablets were prepared from Reyhan Naghshe Jahan Pharmaceutical Company, Iran.

2.2. Animals

In the present study, 24 adult dogs from the Persian Shepherd breed with a weight range of 25-30 kg were used. During the experiment, the dogs were kept in separate cages under standard conditions at 25-30°C and in the 12-hour light-darkness cycle, so that they could easily access to water and food. The dogs were fed with fixed diet (cooked chicken, as chicken heads and paws) for 1 month. Also, they were given anti-parasitic medications of Mebendazole and Praziquantel (based on recommended dosage) for two weeks before the start of the study to remove any digestive parasitic disease. They have also been cleansed of skin parasites through being injected twice with Ivermectin hypodermic and their skin washed with anti-parasitic shampoo. Then, their health was thoroughly and carefully examined before start of the study. All of their vital signs, such as heart rate, respiratory rate, body temperature, and blood pressure, were checked and recorded. All of the dogs were being examined at least twice a day and were kept in separate cages; the place they were kept, was rinsed every day; its floor and the cages were disinfected once every 4 days and all of the reports were recorded. This study was carried out in Veterinary Hospital of Islamic Azad university of Shahrekord located in Kian city, Shahrekord (Iran) and observed all of the ethical guidelines to manipulation of animals (Mosallanejad *et al*., 2016). Animals were fed every day at 9 A.M., then after one hour, they were given the specified dosage of the extract as follows:

The first group was a control group that did not receive anything other than water and food.

The second group received 2g of *Spirulina* algae, equivalent to 4 tablets of 500mg.

Levamisole was administrated orally to third group at a dose level of 5mg/kg.

2.3. Sampling and tests

The dosage assigned was based on the prescribed dose for lab rats (Mosallanejad *et al*., 2016). To blood let the dogs, blood vessels of hand (Cephalic veins) were used in this manner: the dogs were restrained; hairs covering the target place were shaved; then it was disinfected with alcohol. Collecting blood specimens were carried out for each dog individually three times on 0, 15th and 30th days during the study by syringes of 5ml blood capacity. Afterwards, the specimens were transferred to the separate and marked test tubes, then,the blood samples immediately transferred to the specialty laboratory of clinical pathology in Ayatollah Kashani hospital in Shahrekord, Iran. Finally, Hematological factors including hematocrit precent, Hb, RBC, WBC, Neutrophil, Lymphocyte, Eosinophil, monocyte, basophil and immunological factors including IgM, total protein and phagocytic precent were measured.

2.4. Statistical analysis

Data analysis was performed through the SPSS (SPSS Inc., Chicago, Ill., USA) version 22. The results were described as Mean±SD. For the statistical analyses the Anova and Tukey's post-hoc tests were used and the significance level adopted was $P<0.05$.

3. Results and discussion

In addition to good nutritional value, *Spirulina* has many therapeutic properties, including antioxidant and anti-inflammatory properties (Deng and Chow, 2010). Adding *Spirulina* to animal diets can improve some immunological and hematological factors. The results of the present study showed that the mean of all hematological factors in dogs before the intervention did not differ significantly between the two groups ($P>0.05$). In contrast, on 15th days after intervention, the hematocrit level in the control group (39.25) was significantly lower than the *Spirulina* and levamisole receiving groups (42.75). On 30th day, the hematocrit level in the control and *Spirulina* receiving groups were not significantly different ($P>0.05$); but hematocrit level in levamisole receiver group with a mean of 43.25 was more than the two other groups ($P<0.05$). In contrast, in the study of intragroup changes, it was found that over time within 30 days, none of the three groups showed significant changes in the hematocrit level ($P> 0.05$) (Table 1).

Kim *et al.* (2013) studied the protective effects of *Spirulina* in Parrots (*Oplegnathus fasciatus*) and they showed that adding *Spirulina* to the diet of these fish increased hematocrit level (Kim *et al*., 2013). In

addition, Watanuki *et al*. (2006) reported the positive effects of *Spirulina platensis* as an immune stimulant on immunological parameters in various aquatic animals, especially the crab *Cyprinus carpio* (Watanuki *et al*., 2006).

Table 1. Comparison of the average level of hematocrit in three studied groups.

Variables	Time	Control	*Spirulina algae*	Levamisole	SL1	SL2	SL3
Hematocrit (%)	**Baseline**	40.37±2.26	41.25±3.06	42.50±2.72	0.524	0.131	0.365
	15 days	39.25±1.67	42.75±1.67	42.75±2.37	0.002	0.002	1.00
	30 days	42.00±0.76	41.88±1.55	43.25±1.04	0.832	0.043	0.028
	L4	0.058	0.387	0.668			

Significance level 1 (SL1): Comparison of the means between the control group and Spirulina receiving group
Significance level 2 (SL2): Comparison of the means between control and Levamisole receiving group
Significance level 3 (SL3): Comparison of the means between Levamisole and Spirulina receiving groups
Significance level 4 (SL4): Comparison of the means over time in each of the three groups

On the other hand, on the 15th day, the level of RBC in *Spirulina* receiving group (7.69) was significantly higher than two other groups (P<0.001). On the 30th day, the control group with a mean of 7.50 was significantly higher than the group that received *Spirulina* alga (6.61, P =0.001). In addition, over time within 30 days, the algae *Spirulina* had significant effects in RBC, but these changes were not significant in the control and levamisole receiver groups (P> 0.05) (Table 2). At 15th and 30th days, the Hb level in the control group was significantly lower than the two other groups (P<0.05). In addition, In the study of the trend of changes within the groups, it was found that over time within 30 days, significant changes in Hb level was observed in the *Spirulina* receiving group; however, these changes were not significant in the control and levamisole receiving groups (P>0.05). Qishen *et al*. (1989) found that *Spirulina* had a reducing effect on the abundance of micronuclei in the bone marrow polychromatic erythrocytes of gamma-exposed mice. Similarly, *Spirulina* polysaccharides activated cell nucleus enzymes and enhanced the DNA repair process. Significant changes were observed in Alb during 30 days in *Spirulina* receiving group (P=0.006); however, these changes were not significant in the control and levamisole receiving groups (P>0.05). On the other hand, at 15th and 30th days, the level of WBC in the control group was significantly lower than the two other groups (P<0.01). In contrast, the two intervention groups showed a significant difference with each other (P<0.05). Also, over time within 30 days, in the *Spirulina* and levamisole receiving groups significant changes in the WBC were observed (P=0.001); but these changes were not significant in the control group (P>0.05) (Table 2). In a study of the effect of *Spirulina* supplementation on the hemato-immunological function of *Catla* crabs Krishnaveni *et al*. (2013) showed that the addition of *Spirulina maximus* to the crab diet improved hematological parameters such as RBC, WBC, hemoglobin, MCV, MCH.and improvement in immunological factors such as lymphocytes; Lysosomes, monocytes, IgM and phagocytic index was observed (Krishnaveni *et al*., 2013). Nasirian *et al*. (2017) showed that 30 mg/kg oral solution of *Spirulina* improved the levels of MCHC, MCV and white blood cells in rats.

The mean of neutrophils on 15th and 30th days in the control group was significantly higher than *Spirulina* and levamisole receiving groups (P<0.01) but the two intervention groups did not differ significantly from each other (P>0.05). Also, there were significant changes in neutrophils level between *Spirulina* and levamisole groups over time, (P<0.001); but these changes were not significant in the control group (P>0.05). Lymphocyte level on 15th day in levamisole group with a mean of 54.63 was significantly higher than the two control and *Spirulina* groups 33.13 and 36.25 respectively (P<0.001). On 30th day, the lymphocyte level in the control group with an average of34.00 was still significantly lower than the other two groups (P<0.001). In addition, in the study of intragroup changes, it was found that in *Spirulina* and levamisole receiving groups significant changes in lymphocytes were observed over time (P<0.001); but these changes were not significant in the control group (P>0.05). Seyidoglua *et al*. (2017) documented that consumption of *Spirulina* increased the production of cytokines $CD4^+$ and $CD8^+$ T lymphocytes in rabbits (Seyidoglua *et al*., 2017). In contrast, the level of monocytes and Eosinophils on day 15 was not significant in comparison with two other groups

(*P*>0.05). However, on 30th day after the intervention, the monocytes level was significantly higher in the control group and the level of Eosinophils was significantly lower than from *Spirulina* and levamisole receiving groups (*P*<0.05), but the two intervention groups did not differ significantly from each other (*P*>0.05). In addition, in the study of intragroup changes, it was found that over time within 30 days of the intervention, none of the three groups showed significant changes in monocytes and Eosinophils levels (*P*>0.05). On the other hand, basophils and band cells were not significant in the comparison of the two groups on days 15 and 30 (*P*>0.05). None of the three groups showed significant changes in these two factors over period of 30 days (P>0.05).

Table 2. Comparison of the hematological factors in three studied groups

Variables	Time	Control	*Spirulina*	Levamisole	SL1	SL2	SL3
RBC (u/µl)	**Baseline**	7.07±0.32	6.93±0.22	7.22±0.41	0.408	0.368	0.092
	15 days	6.69±0.30	7.69±0.39	6.84±0.30	0.001<	0.379	0.001<
	30 days	7.26±0.44	6.61±0.44	7.06±0.46	0.001<	0.064	0.057
	SL4	0.065	0.004	0.215			
Hb (g/dl)	**Baseline**	14.19±0.63	14.00±0.71	14.76±0.62	0.571	0.092	0.420
	15 days	13.63±0.58	14.51±1.03	14.89±0.61	0.031	0.004	0.341
	30 days	14.29±0.47	16.05±0.47	15.34±0.54	0.001<	0.001<	0.009<
	SL4	0.059	0.001	0.355			
Alb (g/dl)	**Baseline**	4.15±0.54	4.75±0.87	4.29±0.56	0.09	0.688	0.185
	15 days	3.60±3.40	3.19±0.01	4.23±0.97	0.190	0.053	0.003
	30 days	4.09±0.64	4.11±0.97	3.85±0.50	0.946	0.523	0.480
	SL4	0.105	0.006	0.354			
WBC (u/µl)	**Baseline**	13425.0±319.59	13637.50±420.67	1383.50±719.00	0.419	0.124	0.446
	15 days	13012.50±1007.74	14143.75±684.23	17500.00±417.48	0.006	0.001<	0.001<
	30 days	13600.00±1128.84	17075.00±604.15	16250.00±444.01	0.001<	0.001<	0.047
	SL4	0.419	0.001<	0.001<			

Significance level 1 (SL1): Comparison of the means between the control group and Spirulina receiving group
Significance level 2 (SL2): Comparison of the means between control and Levamisole receiving group
Significance level 3 (SL3): Comparison of the means between Levamisole and Spirulina receiving groups
Significance level 4 (SL4): Comparison of the means over time in each of the three groups

On the 15th and 30th days, the percentage of phagocytosis was not significant between the two intervention groups (*P*>0.05). In addition, in the study of intragroup changes, it was found that after 30 days, the control and levamisole receiving groups did not have significant changes in the percentage of phagocytosis (*P*>0.05), but in the *Spirulina* group, a significant increase in the percentage of phagocytosis was observed. In the evaluation of total phagocytosis, it was found that on 15th day, total phagocytosis in the control group (24.88) was significantly lower than the two other groups (*P*<0.01). In addition, over 30 days *Spirulina* and levamisole receiving groups showed significant changes in total phagocytosis (*P*<0.05), but no significant change was observed in the control group (*P*>0.05). Also, on the 15th and 30th days, the total protein level in the control group was significantly higher than the two other groups (*P*<0.05). In addition, over period of 30 days, the control and levamisole receiving groups did not show any significant changes in total protein level (P>0.05), but in the *Spirulina* receiving group, a significant decrease was observed in total protein (P<0.001) (Table 3). Results showed that IgM level in the control group was significantly higher than the others groups on day 15 (*P*<0.001). However, on 30th day, the level of this factor in the control group was significantly lower than the two other groups (*P*<0.001). Also, IgM level in levamisole group was significantly higher than *Spirulina* group (*P*<0.001). In the study of intragroup changes, it was found that over period of 30 days, *Spirulina* and levamisole receiving groups had a significant increase in IgM level (*P*<0.001), but no significant changes in IgM level were observed in the control group.

Table 3. Comparison of the average level of Immunological factors of the three studied groups.

Variables	Time	Control	*Spirulina*	Levamisole	SL1	SL2	SL3
Phagocytosis percent (%)	**Baseline**	15.50±1.77	16.12±1.46	16.00±1.85	0.471	0.563	0.885
	15 days	16.00±3.34	15.63±1.69	17.25±3.99	0.815	0.437	0.315
	30 days	15.50±2.27	19.25±2.49	16.50±5.58	0.059	0.601	0.159
	SL4	0.874	0.011	0.661			
Total Phagocytosis (%)	**Baseline**	25.00±4.37	24.50±3.66	25±3.07	0.792	1.00	0.792
	15 days	24.88±4/61	32.13±3.48	33.25±4/43	0.002	0.001	0.598
	30 days	23.75±5/18	26.50±1.93	25.50±3.12	0.148	0.350	0.591
	SL4	0.433	0.001<	0.005			
Total Protein (%)	**Baseline**	8.62±1/98	8.12±0/56	7.57±1/37	0.492	0.157	0.450
	15 days	8.89±2.43	6.34±0.36	6.74±1.11	0.004	0.012	0.613
	30 days	9.20±1.55	6.58±0.40	7.03±1.21	0.001<	0.001<	0.446
	SL4	0.577	0.001<	0.249			
IgM (mg/dl)	**Baseline**	40.00±2.72	39.12±2.85	40.50±5.10	0.643	0.818	0.537
	15 days	37.75±2.38	44.00±3.38	141.25±22.1	0.347	0.001<	0.001<
	30 days	38.25±2.25	108.38±15.41	156.00±11.24	0.001<	0.001<	0.001<
	SL4	0.103	0.001<	0.001<			

Significance level 1 (SL1): Comparison of the means between the control group and Spirulina receiving group
Significance level 2 (SL2): Comparison of the means between control and Levamisole receiving group
Significance level 3 (SL3): Comparison of the means between Levamisole and Spirulina receiving groups
Significance level 4 (SL4): Comparison of the means over time in each of the three groups

Krishan *et al*. (2015), found that *Spirulina* possessed protective effects against the infectious virus anemia in broilers (Krishan *et al*., 2015). Mathew *et al*. (1995) observed that the immunological properties of *Spirulina* played an important role in anti-cancer properties such as oral cancer (Mathew *et al*., 1995). Pankaj and Varma (2013) reported that *Spirulina platensis* protected hematological parameters against diabetes-induced alloxan in mice and the hematological profile of *Spirulina* -fed rats differed significantly in WBC cells while neutrophil and lymphocyte levels decreased in mice treated with *Spirulina*. Hematology profile of *Spirulina* -treated rats for 45 days showed no toxic signs and it could be completely harmless in human and animal food preparation.

4. Conclusion

In general, in the present study, due to the significant positive changes and improvement in some immunological and hematological factors, it is recommended to administer Spirulina alga in the dog's diet. It was also found that Spirulina alga like levamisole can increase the activity of the immune system and phagocytosis, which can be due to the presence of antioxidant compounds in this alga. It also increased the level of total protein, which is a turning point in the ability of this algae as a dietary supplement in animal and poultry feed.

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