



## SHORT COMMUNICATION

### Immunostimulatory Properties of *Spirulina platensis* against Rabbit Hemorrhagic Disease Virus

Elena Kistanova<sup>1\*</sup>, Mihail Chervenkov<sup>1</sup>, Kiril Shumkov<sup>1</sup>, Rayko Peshev<sup>2</sup>, Krasimira Todorova<sup>1</sup>, Soren Hayrabedyan<sup>1</sup>, Desislava Abadjieva<sup>1</sup>, Almantas Shimkus<sup>3</sup> and Aldone Shimkiene<sup>3</sup>

<sup>1</sup>Institute of biology and immunology of reproduction, BAS, 73 Tzarigradsko shose, 1113 Sofia, Bulgaria; <sup>2</sup>National diagnostic and research veterinary medicine institute, Bul. Pencho Slaveikov 15a, 1606 Sofia, Bulgaria; <sup>3</sup>University of health sciences, A. Mickevičiaus g. 9, 44307 Kaunas, Lithuania

\*Corresponding author: kistanova@gmail.com

#### ARTICLE HISTORY (14-131)

Received: March 10, 2014  
Revised: January 24, 2015  
Accepted: January 26, 2015

#### Key words:

Antibody titer  
Immunostimulation  
Rabbit hemorrhagic disease virus (RHDV)  
*Spirulina platensis*  
Vaccination

#### ABSTRACT

Given the high risk, hemorrhagic disease virus (RHDV) leading to 100% mortality of the infected rabbits, special attention has to be paid to form an adequate and timely immunity after vaccination. The present investigation aimed to analyze the ability of the *Spirulina (S.) platensis*, enriched of phycocyanin, carotenoids, iron and selenium through modified technology of growth, to boost the immunity of the vaccinated rabbits against RHDV. The experimental white rabbits were fed 1g dry biomass *S. platensis* per head daily one month before and three months after vaccination with PESTORIN (Bioveta, Czech). The antibody titers against RHDV were determined at days 15, 30, 60 and 90 post-vaccination. Faster and higher production of the specific antibodies against RHDV was observed in animals treated with whole biomass of algae. The *S. platensis*, enriched with bioactive components, could be beneficial for supporting the immunization against RHDV.

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**To Cite This Article:** Kistanova E, M Chervenkov, K Shumkov, R Peshev, K Todorova, S Hayrabedyan, D Abadjieva, A Shimkus and A Shimkiene, 2015. Immunostimulatory properties of *Spirulina platensis* against rabbit hemorrhagic disease virus. Pak Vet J, 35(3): 379-381.

#### INTRODUCTION

The use of the green-blue algae *Spirulina (S.) spp.* in human medicine and animal breeding has a long-term history. It is an excellent, non-toxic food and has positive effect on human and animal healthy state. The wide interest in *S. platensis* is concentrated mainly on its rich content of vital and biologically active compounds including: proteins (55-70%), carbohydrates (15-25%), essential fatty acids (18%) vitamins, minerals and pigments like carotenes, chlorophyll *a* and phycocyanin (Gershvin and Belay, 2012).

In the scientific literature there are not strong confirmations related to the antiviral properties of *S. platensis*, obtained from in vivo studies. The main data reported that the fraction of sulfated polysaccharides from *Spirulina spp.* inhibits the *in vitro* replication of several enveloped viruses (Hayashi *et al.*, 1996; Babu *et al.*, 2005). The researchers underlined that the effect of algal products against viruses should be realized by immunomodulation (Karkos *et al.*, 2011). The scientific evidences related to the immunostimulatory and immunomodulatory properties of *S. platensis* had

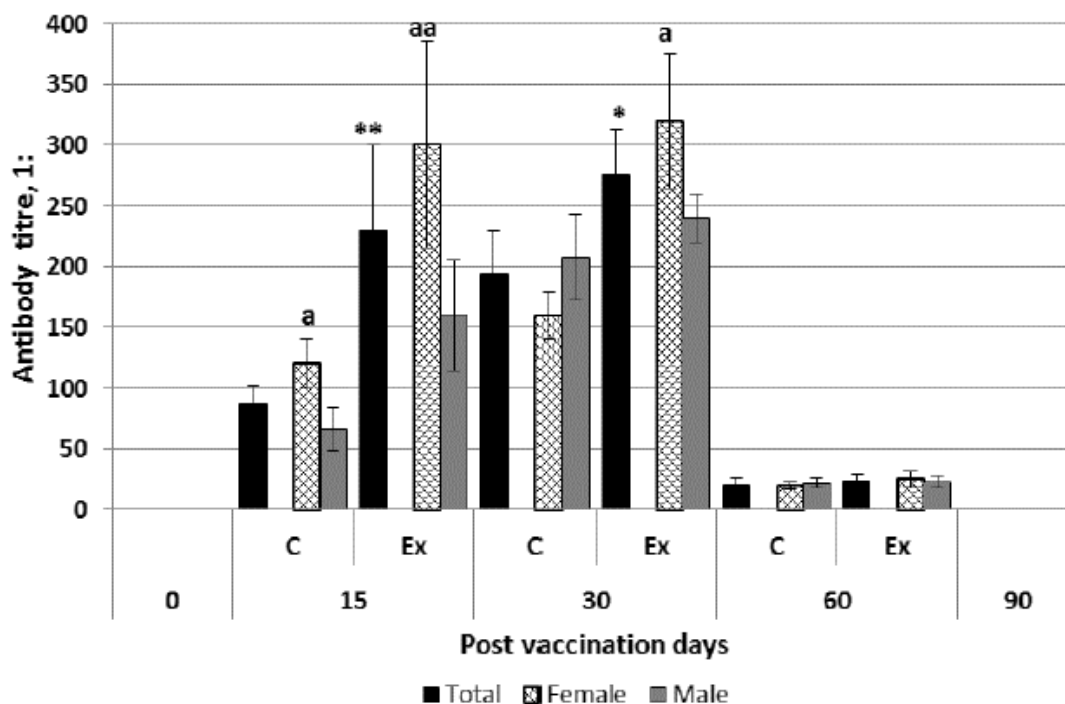
appeared during last decades. The *Spirulina spp.* promotes an activation of T and B cells followed by enhancement of cytokines and antibody production (Nemoto-Kawamura *et al.*, 2004; Ravi *et al.*, 2010; Karkos *et al.*, 2011).

Rabbit hemorrhagic disease virus (RHDV) causes high mortality of adult animals. The rabbits succumb during the 48-72 h post-infection (Abrantes *et al.*, 2012). Immunoprophylactic measures such a vaccination is of utmost importance for the prevention and control of the disease.

The scientific data allowed supposing that *S. platensis* could ensure the better immune response to the antigens during the immunization against dangerous diseases like RHDV. The present investigation aimed to study the effect of *S. platensis* on the production of the specific antibodies against RHDV in post-vaccination period.

#### MATERIALS AND METHODS

The producer of the investigated *Spirulina platensis* is company UAB MAZURO AD, Lithuania. As a maternity cell line the strain IBSS-31 was used for *Spirulina* production through the modified technology.



**Fig. 1:** Antibody response to vaccination against RHDV in control and treated with *S. platensis* rabbits in dependence on sex. C-control, Ex-experimental rabbits. The black bar presents the average titer for whole group (total). \*,a=P<0.05; \*\*,aa=P<0.01: compared to the control or females compared to males.

The chemical analysis of dry biomass was done in the Laboratory of Agrochemical Investigations of Lithuania. The evaluation of mineral content in dry biomass of *Spirulina platensis* was done by inductively coupled plasma mass spectrometry (ICP-MS), according to certified methodology (European standard LST EN 15763:2010. Microelement evaluation). In the experiment the whole dry biomass of algae was applied.

Immunostimulatory effects of *Spirulina platensis* were studied in 16 one-month old white rabbits (8 males and 8 females) those were divided into two groups - control and experimental. Both groups were fed on standard diets and water *ad libitum*. The experimental animals additionally received 1g dry biomass of *S. platensis* per head daily throughout the experimental period. After one month of feeding with *S. platensis*, animals were vaccinated against RHDV (PESTORIN, Bioveta, Czech), once subcutaneously with 1 ml dose. The animals were reared and handled in accordance with Bulgarian Veterinary Law (25/01/2011) related to the life conditions and welfare of experimental animals adapted to the European Union regulation 86/609. The experiment was conducted within "The permission for use of the experimental animals" validated by National Ethics commission for animals.

The peripheral blood was investigated at day 0 (before vaccination) and at days 15, 30, 60 and 90 post-vaccination for the rising of antibodies against RHDV. A hemagglutination inhibition (HA/HI) test was used to determine the antibody titers against RHDV (Veleva *et al.*, 1995). The results were developed by statistical package STATISTIKA (Stat Soft version 6.0). Differences were considered significant with  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

The obtained through modified technology *Spirulina* was with higher content of total phycobillins (23%), particularly, phycocyanin (17.1 versus 15.6% usually) as well as of carotenoids (6.0 versus 4.5 mg/g usually), iron (1.1 versus 0.53 mg/g usually) and selenium (120  $\mu\text{g/g}$  - no data) than mainly used *Spirulina platensis* (Gershwin and Belay, 2012).

Animals fed with this *S. platensis* elicited faster ( $P < 0.01$ ) immune response against the virus of haemorrhagic disease (Figure 1). At 15<sup>th</sup> day post-vaccination, they had shown an antibody titer higher than 1:160, which is considered sufficient for animal infection resistance (Veleva *et al.*, 1995). The animals of control group reached this level between 15 and 30 days post-vaccination. Average antibody titer in the experimental group's animals was higher between 0 and 60 days. However, in both groups at 60<sup>th</sup> day post-vaccination, the antibody titer started to decline and reached zero at day 90. If the animals are in danger of the epidemic of hemorrhagic disease, the revaccination should be done close to day 90.

The analysis of results by sex of animals indicated stronger effect of *Spirulina* on females (Fig. 1). The antibody titers of experimental group's females at 15<sup>th</sup> and at 30<sup>th</sup> days post-vaccination were almost twice higher in comparison with males of experimental group and with females of control group on same day. It was interesting to notice that the average weight gain and some blood parameters (alkaline phosphatase activity, protein content) were also higher in females treated with *S. platensis* than in males (data not shown).

It seems that *S. platensis*, enriched with bioactive components, acted as a powerful tool for the rabbit immune system. Our data confirmed the results from studies in laboratory animals, which showed that *Spirulina* spp. improves functions of immune system through enhanced interleukin-1 (IL-1) production from peritoneal macrophages (Belay, 2002) and increased numbers of splenic antibody producing cells as a primary immune response to sheep red blood cells (Hayashi *et al.* 1998). Our experiments *in vivo* for the first time had clearly shown that the whole biomass enriched with bioactive component *S. platensis* enhanced the immune response after vaccination and ensured faster and higher production of the specific antibodies against RHDV. The use of whole biomass for agricultural animals has a double benefit: it contents' sulfated polysaccharides possess an antiviral activity *in vitro* (Babu *et al.*, 2005) and it can cover possible nutritional deficiencies. The lack of important nutrients results in changes of T-cells production and IgA antibody secretion (Karkos *et al.*, 2011). The results of epidemiologic studies of HIV/AIDS in humans are in support of this hypothesis. The populations with high algae consumption have correspondingly low rates of HIV infection (Teas *et al.*, 2004 cited by Gershwin and Belay, 2012).

**Conclusion:** Feeding *Spirulina platensis* along with the vaccination against RHDV ensured faster preventive effect especially in female rabbits. It is anticipated that the use of the *S. platensis*, enriched with biological compounds as phycocyanin, carotenoids, iron and selenium, could be beneficial for supporting immunization and the prevention of RHDV.

Further investigations are required to define the metabolic pathways affected by *S. platensis* and lead to improving the immune response to vaccination against RHDV, especially in females. Also there is interesting to check, if immunostimulatory effect of *Spirulina* spp.

works similar for other specific rabbit viral pathogens, for example *Myxoma* virus (MYXV).

**Acknowledgement:** The gift of *Spirulina platensis* dry biomass from UAB MAZURO AD, (Lithuania) for this study is gratefully acknowledged. The research was supported by grants № DKOF7RP02/17 and BG051PO001-3.3.06-0059 under Ministry of Education and Science of Bulgaria.

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