

### EFFECT OF DIETARY SUPPLEMENTATION WITH SEAWEED AND POLYPHENOLS MIXTURE ON ANTIOXIDANT STATUS, CONCENTRATION AND MOTILITY OF RABBIT SPERMATOZOA

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#### ABSTRACT

In recent years, many studies have been focused on natural substances that could have effect on health of animals. We investigated effect of extract consisting mainly of polyphenols, brown algae and plant polysaccharides on the reproduction potential of male rabbits (New Zealand white breed) during 60 days long dietary experiment. The rabbits were divided into three groups. Control was fed a basal diet, whereas the second and third group were supplemented with seaweed and polyphenols mixture: T1 = 0.3% and T2 = 0.6% respectively. We observed that sperm concentration in both experimental groups increased in comparison to the control group. Results of the CASA analysis showed enhanced motility (C = 85.09±7.53%, T1 = 87.21±8.25%, T2 = 89.38±8.02%) and progressive motility (C = 74.28±12.6%, T1 = 79.07±13.89%, T2 = 81.28±11.37%) in experimental groups supplemented with combination of algae and polyphenols in comparison with the control group. While monitoring ferric reducing ability of plasma (FRAP), we found the highest value in T1 group, changes were insignificant. An increase in GPx activity was measured in experimental groups in comparison with the control group with major difference in T1 group. In experimental groups, we determined an increase in activity of superoxide dismutase (SOD) in comparison with control group, the difference was significant in T2 group (C = 0.26±0.11 U/mg TP; T2 = 0.31±0.09 U/mg TP). In conclusion, our studies suggest that dietary supplementation with brown seaweed and plant polyphenols mixture may be potentially useful for enhancement of sperm motility and protection against oxidative stress.

**Keywords:** antioxidant, motility, polyphenols, rabbit, spermatozoa concentration, seaweed

#### INTRODUCTION

Oxygen free radicals are molecules forming in aerobic cellular metabolism, containing one or more unpaired electron. Reactive free radicals could bind to different molecules and lead to damage to lipid membranes, nucleic acids and proteins (Aladag et al., 2009). Over the years, plants have served as sources of bioactive compounds for both humans and animals (Pellegrini et al., 2007). Nutritional factors play an important role in the protection against consequences of free radicals (Sikora et al., 2008). Dietary supplementation of antioxidant can enhance the physiological and productive statuses of animals by modifying metabolic processes (Elwan et al., 2019). Consumption of seaweed as a food additive in order to treat diseases has centuries of history in East Asian territories including Japan, China, Vietnam and Korea. In recent decades, due to the extremely rich nutritional values as well as pharmacological properties, the usage of edible seaweed has risen in Western and developing countries (Hong et al., 2007; Hamed et al., 2015; Ruocco et al., 2016).

Although, the production of reactive oxygen species (ROS) is a physiological event in various organs including the testis, various antioxidants have proven helpful in treating infertility of males (Sinclair, 2000). Baker et al. (1996) reported, that antioxidants can be beneficial against the damaging effect of leukocyte-derived ROS on sperm motility due to association of overproduction of ROS with male infertility (Akiyama, 1999).

Despite its importance, the effect of this supplement on reproduction has not been reviewed in the past, therefore we investigated its effect on the reproduction potential of male rabbits. The purpose of the present study was to determine the effects of brown seaweed and polyphenols additives on antioxidant status (SOD, GPx, FRAP), concentration and motility of rabbit spermatozoa.

#### MATERIALS AND METHODS

##### Experimental animals and natural extract

The experiment included 14 males of New Zealand white breed rabbits that have been bred at NPPC-National Agricultural and Food Centre, Research Institute for Animal Production Nitra (Lužianky, Slovak Republic). Management of animals and experimental procedures were managed in accordance with European Community guidelines no. 86/609/EEC. A cycle of 16 h of light and 8 h of darkness was maintained throughout the experiment. Temperature was set between 20 – 24°C and humidity level at 65%. In the experiment, which lasted for 60 days, adult males rabbits were used as an excellent model for assessing the effects of toxic agents on semen quality and fertility. The dietary supplement was analyzed and the composition of the polyphenols profile is reported in Table 1. During our experiments, animals were divided into 3 homogeneous groups (18.5±1.5 months old; 4.90±0.87 kg). Control group (C, n = 8) rabbits were fed by commercial feed. Rabbits from first experimental group (T1, n = 8) were fed by feed supplemented with 0.3% of natural extract mixture and animals from second experimental group (T2, n=8) were fed by feed supplemented with 0.6% of natural extract mixture. Natural extract mixture was provided by Lombarda Trading (Italy) and polyphenol content was measured using HPLC.

##### Semen sampling and analysis

The semen samples were collected on 60th day using artificial vagina. The obtained semen samples were diluted with physiological solution (1:5). Each of prepared samples were evaluated using a Computer Assisted Semen Analyzer (CASA) system – Sperm Vision (Minitub, Tiefenbach, Germany) equipped with

a microscope (Olympus BX 51, Japan) to assess the spermatozoa motility. Each sample was placed into Makler Counting Chamber. We analysed spermatozoa concentration, total motile spermatozoa and progressive motile spermatozoa using the rabbit specific set up. SOD activity as well as activity of GPx were analysed by RANSOD assay (Randox Laboratories, Crumblin, Great Britain). FRAP assessment was guided by the original method described by Benzie and Strain (1996).

**Statistical analysis**

All the data was expressed as mean ± standard deviation (SD). The means of various parameters of control and experimental animals were compared using ANOVA & Tukey test for statistical significance in GraphPad Prism. P<0.05 was considered to be statistically significant.

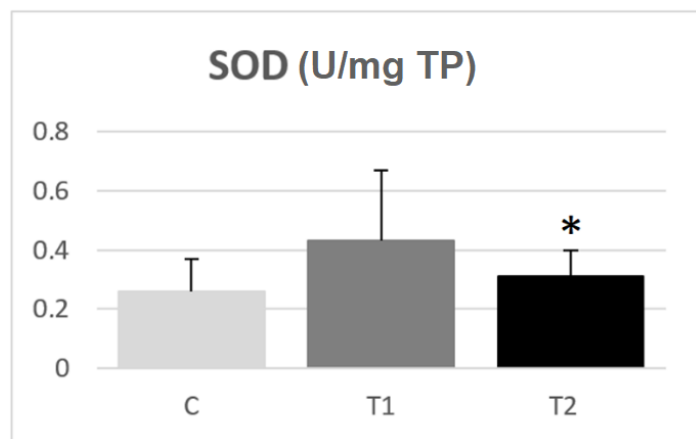
**Table 1** Polyphenols content in the natural extract supplement.

Neochlorogenic acid (mg/kg)	7979.23
Elaigic acid (mg/kg)	2440.88
Syringic acid (mg/kg)	1059.79
Cynaroside (mg/kg)	566.72
Rutin (mg/kg)	272.37
Rosmarinic acid (mg/kg)	126.54
Trans-sinapic acid (mg/kg)	105.54
Myricetin (mg/kg)	53.88
Chlorogenic acid (mg/kg)	21.45
Protocatechuic acid (mg/kg)	<LOD
Vitexin (mg/kg)	<LOD
Trans p-coumaric acid (mg/kg)	<LOD
Trans-ferulic acid (mg/kg)	<LOD
Resveratrol (mg/kg)	<LOD
Daidzein (mg/kg)	<LOD
Quercetin (mg/kg)	<LOD
Apigenin (mg/kg)	<LOD
Kaempferol (mg/kg)	<LOD

Legend: <LOD - below LOD, mg/kg - milligram/kilogram

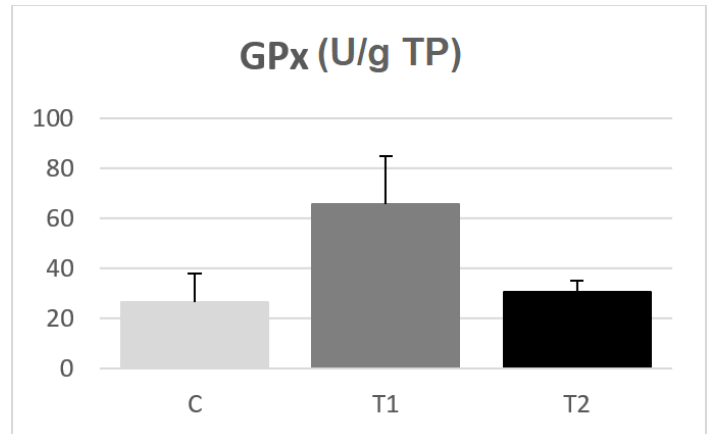
**RESULTS**

In this study we analysed antioxidant status, concentration and motility of rabbit spermatozoa following oral administration of polyphenols and seaweed mixture at different doses (groups T1, T2) and compared them against the control group without any addition (C). The results of the analysis are shown in Figures 1 - 6. As seen in Figure 1, administration of seaweed and polyphenols mixture in individual doses (T1 - received 0.3 %, T2 - received 0.6 %) resulted in increase of enzyme activity in both experimental groups, increase was significant in T2 group (P<0.05) when compared to the control group.



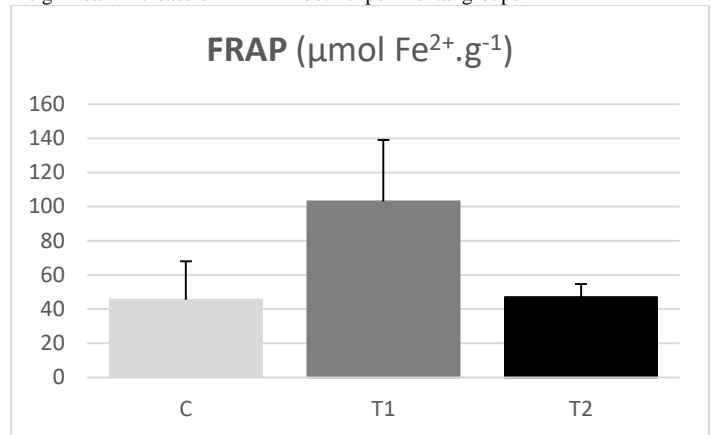
**Figure 1** The values of the activity of superoxide dismutase (C = 0.26±0.11 U/mg of total protein; T1 = 0.43±0.24 U/mg of total protein, T2 = 0.31±0.09 U/mg of total protein)  
**Legend:** Groups of rabbit with doses of seaweed and polyphenols mix: C – 0%; T1 – 0.3%; T2 – 0.6%; \*statistical significant at P<0.05 in comparison to control group

Figure 2 shows the values of the activity of glutathione peroxidase. Administration of brown seaweed and polyphenols mix in individual doses (T1 – 0.3 %, T2 – 0.6 %) resulted into increasing trend of enzyme activity in both experimental groups.



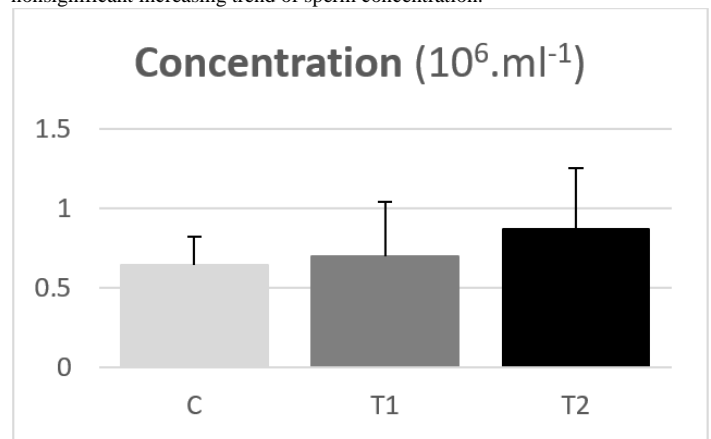
**Figure 2** The values of the activity of glutathione peroxidase (C = 26.6±11.25 U/g of total protein; T1 = 65.58±19.31 U/g of total protein; T2 = 30.53±4.53 U/g of total protein)  
**Legend:** Groups of rabbit with doses of seaweed and polyphenols mix: C – 0%; T1 – 0.3%; T2 – 0.6%

As Figure 3 shows, the administration of seaweed and polyphenols mix in individual doses (T1 - received 0.3 %, T2 - received 0.6 %) resulted in insignificant increase of FRAP in both experimental groups.



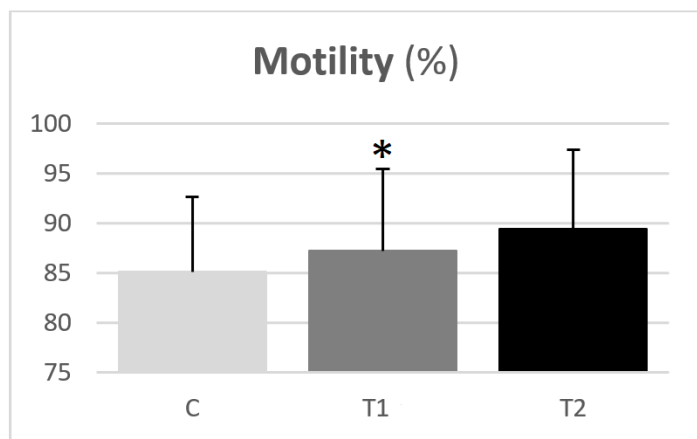
**Figure 3** The values of the ferric reducing activity of plasma (C = 45.46±22.51; T1 = 103.00±36.10; T2 = 47.11±7.53)  
**Legend:** Groups of rabbit with doses of seaweed and polyphenols mix: C – 0%; T1 – 0.3%; T2 – 0.6%

As seen in Figure 4, administration of seaweed and polyphenols mixture in individual doses (T1 - received 0.3 %, T2 - received 0.6 %) resulted in nonsignificant increasing trend of sperm concentration.



**Figure 4** The values of the spermatozoa concentration (C = 0.647±0.16; T1 = 0.699±0.35; T2 = 0.871±0.39)  
**Legend:** Groups of rabbit with doses of seaweed and polyphenols mix: C – 0%; T1 – 0.3%; T2 – 0.6%

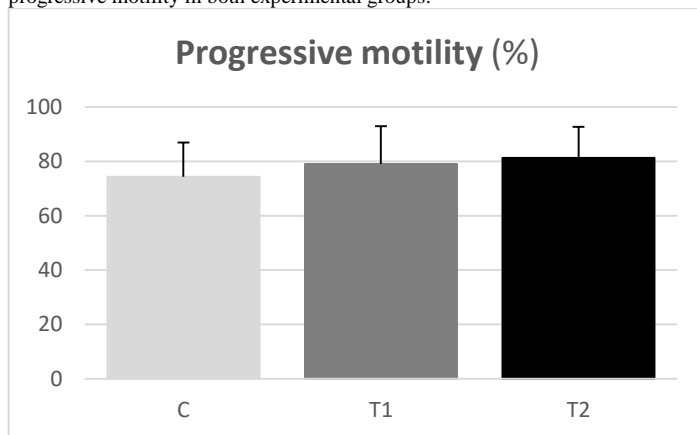
Data obtained from CASA analysis are shown in Figure 5, which shows the values of sperm motility. Administration of brown seaweed and polyphenols mix in individual doses (T1 – 0.3 %, T2 – 0.6 %) resulted in significant increase of motility in T1 group and nonsignificant increase in T2 group in comparison with the control group.



**Figure 5** The values of the sperm motility (C = 85.09±7.53%, T1 = 87.21±8.25%, T2 = 89.38±8.02%)

**Legend:** Groups of rabbit with doses of seaweed and polyphenols mix: C - 0%; T1 - 0.3%; T2 - 0.6%; \*statistical significant at  $P < 0.05$  in comparison to control group

Data obtained from CASA analysis are shown in Figure 6. Administration of seaweed and polyphenols mixture in individual doses (T1 - received 0.3 %, T2 - received 0.6 %) resulted in nonsignificant ( $p > 0.05$ ) increasing trend of progressive motility in both experimental groups.



**Figure 6** The values of progressive motility (C = 74.28±12.6%, T1 = 79.07±13.89%, T2 = 81.28±11.37%)

**Legend:** Groups of rabbit with doses of seaweed and polyphenols mix: C - 0%; T1 - 0.3%; T2 - 0.6%

## DISCUSSION

To our knowledge, the present study is the first to report of the antioxidant effect of brown seaweed and plant polyphenols mixture on male reproductive system. Our experiment indicates, that application this mixture caused an increase of selected reproductive parameters (sperm concentration, motility, progressive motility), as well as antioxidant parameters (SOD, GPx, FRAP). Our findings demonstrated, that mixture of brown seaweed and plant polyphenols might be capable of protecting spermatozoa against oxidative stress, since rabbit spermatozoa in treated groups exhibited increase in concentration, motility and progressive motility in comparison with the control group. Natural antioxidants-rich diets are capable of reducing the risk of some diseases induced by free radical generation. A significant amount of antioxidants in a diet is able to encourage immunological processes and elevate the defensive abilities of the cell (Sikora et al., 2008). The presence of phenolic compounds and flavonoids in the diet provides health benefits, however these benefits of phenolic compounds depend on their consumption and bioavailability. Phenolic compounds display a wide range of biological and chemical activities, including excellent scavenging activities (Rodríguez et al., 2009). Our research might support this finding as all of parameters monitored in our experiment slightly increased.

Sharma and Agarwal (1996) reported that seminal plasma confers some protection against ROS damage because it contains enzymes that scavenge ROS, such as SOD and GPx. Sperm membranes must be protected by a highly effective antioxidant system to prevent the peroxidative damage. The antioxidant system in semen includes enzymes such as glutathione GPx, SOD and catalase (Partyka et al., 2012). The first line of defence against the superoxide radicals are the SODs. Highly toxic hydrogen peroxide formed during superoxide dismutation is removed by GPx and catalase (Delamirande et Gagnon, 1995; Griveau et al., 1995). The present results showed an increase ( $P > 0.05$ ) in SOD and GPx levels as well as FRAP in seminal plasma of animals treated with natural mixture

(Figures 1-3). Pavin et al. (2018) reported, that supplementation with extract of *Tribulus terrestris*, which is high in antioxidants caused an increase in SOD and GPx levels.

Figures 4-6 showed a slight increase in sperm concentration, motility and progressive motility. Similar results were found by Brzezinska-Slebodzinska et al. (1995), Geva et al. (1996), Hsu et al. (1998), Surai et al. (1998), and Akiyama (1999), who reported antioxidant supplementation (vitamin E, vitamin C, selenium) reduced ROS generation and improved semen quality. The beneficial effect in improving fertilization rate was possibly due to a reduction in lipid peroxidation potential. Yousef et al. (2003) investigated effects of ascorbic acid and vitamin E supplementation on semen quality of male rabbits. According to their results, antioxidant supplementation led to improvement in ejaculate volume, total motile sperm, sperm motility index and sperm concentration. These results are in agreement with our results, however Weng et al. (2018) reported that supplementation with *Astragalus* polysaccharides did not affect sperm motility.

## CONCLUSION

The present study is the first to investigate effect of dietary supplementation with seaweed and polyphenols mixture on antioxidant status, concentration and motility of rabbit spermatozoa. In conclusion, this experiment indicates, that application of brown seaweed and plant polyphenols mixture caused an increasing trend of selected male reproductive parameters (sperm concentration, motility, progressive motility) as well as antioxidant parameters (SOD, GPx, FRAP), which points to the positive effect of this mixture added to feed. Considering that this is a pilot study, we may suggest further studies in order to understand effects of this mixture on male reproduction, other organ systems, and different animal species, as well as the mechanism of this extract in relation to its potential beneficial effects. This study indicates the practical potential of using seaweed and polyphenols mixture as an dietary supplement.

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