JRVM
Journal of Research in Veterinary Medicine

RESEARCH ARTICLES

J Res Vet Med. 2019: 38 (1) 71-76
DOI:10.30782/uluvfd.422576

The Effect of Saccharomyces Cerevisiae and Spirulina Platensis on Glutathione and Leucocytes Count in Rabbits

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Received 06.02.2018 Accepted 22.02.2018

Abstract

Glutathione is the important antioxidant agent that is used for body detoxification system. Because of the fact that ıt is crucial for protecting health. A feeding trial was conducted to evaluate the effect of natural additives such as live yeast culture Saccharomyces cerevisiae (SC) and microalgae Spirulina platensis (SP) on the glutathione and leukocytes counts of rabbits. Forty male New Zealand white rabbits, aged 5-6 weeks, were studied in 4 groups. Treatments were control group, SC (added 3 g/kg diet), SP (added 5% of the diet) and, SC and SP (added 3 g/kg diet and added 5% of the diet) respectively. The experiment lasted for 90 days and the blood samples were obtained by ear venipuncture on the 90th day.

In conclusion, according to the results of this study, although not statistically significant, supplementing rabbit with S. cerevisiae or S. platensis had increased on glutathione values. Glutathione tend to be positively correlated with the addition of SC or SP. No significant difference in white blood cell counts was evidenced, even if lymphocyte counts tended to increase and neutrophil counts to decrease in rabbits fed SC or SC+SP. The determination of biological consequences (antioxidant potential, resistance to diseases, and improvement of nutritional status) requires further investigations.

Keywords: Saccharomyces cerevisiae, Spirulina platensis, glutathione, leukocyte.

Introduction

Antioxidants such as glutathione provide a defence against the damage of cells by reactive oxygen species (ROS) in living systems.[1-3] Glutathione (GSH) has several biological roles: detoxifies electrophiles, maintains the essential thiol by preventing oxidation, modulates the cellular prosess (DNA synthesis), and modulates immunity.[4,5] Blood glutathione protects cellular proteins against oxidation through glutathione redox cycle.[6]

It is suggested that ROS has a role in the development of various diseases such as cardiovascular, arteriosclerosis, cancers, and many others, and the process of aging. Therefore, in recent years, researchers' increasing attempts to prevent diseases caused by ROS have been drawing attention.[7,8] Therefore, there have been many attempts to prevent against chronic diseases, by reducing cellular oxidative stress.[9,10] There are positive correlation between dietary intake of natural antioxidants and health. Especially, presence of antioxidants in the plant foods have a beneficial role in resistance of the some cardiovascular diseases, cancer or degenerative diseases of aging.[11]

Spirulina platensis (SP, S. platensis) is rich in fatty acids, amino acids, vitamin and selenium.[12] Recently, attention has been placed on the antioxidant potential of spirulina. Indeed, many of the chemical components of spirulina, such as phenolic compounds, tocopherols, A-carotenes, and phycocyanins exhibit antioxidants properties.[13] S.

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platensis recommended for health due to their effects on growth,[14] immunity[15,16] and antioxidant mechanism.[14,17-20] In our previous studies serum CD4+/CD8+ increased in the animals fed SP, and it was concluded that S. platensis may be used as an immune enhancer.[16]

The yeast culture, Saccharomyces cerevisiae (SC, S. cerevisiae) is a well-known probiotic having positive effects in the treatment and prevention of diseases.[21] S. cerevisiae, inparticular, has proven to benefit health in several ways including stimulation of the growth of intestinal microflore in mammals; pH modulation in ruminants as well as reduction in the number of pathogenic microorganisms in monogastric animals.[22-24] In addition, in our previous experimental studies on S. cerevisiae, addition of SC at a level of 3 g/kg of feed in rabbits increased the total mucosa, villus height, and gland depth of duodenal mucosa,[25] improvement of ruminal cellulolytic activity[26] and haematopoiesis.[27]

The combined effect of S. platensis and S.cerevisiae on antioxidant mechanism and defense cells in the blood have not been addressed yet. Therefore, the aim of this study is to investigate the effect of natural additives such as live yeast culture S. cerevisiae and microalgae S. platensis on the glutathione and leukocytes counts of rabbits.

Materials and Methods

Animals, Groups and Feeding

Forty male New Zealand white rabbits aged 5-6 weeks were randomly allocated on a weight basis to four groups: I. Control, II. Saccharomyces cerevisiae (SC, added 3 g/kg diet), III. Spirulina platensis (SP, added 5% of the diet), IV. Combination of SC and SP (added 3 g/kg diet and 5% SP of the diet), respectively. The rabbits were housed individually in metal cages and, feed and water were offered ad libitum to the rabbits throughout the 90 day trial. Basal diet (pelleted) was formulated to contain 2.500 kcal ME/kg metabolizable energy, 16% crude protein and was designed to meet maintenance requirements according to the NRC.28 Chemical composition and ingredients of the diet are provided in Table 1 and Table 2. Chemical analyses of diets were carried out according to AOAC.29 Basal diet was supplemented with Saccharomyces cerevisiae live yeast culture (Yea Sacc1026 Altech. Nicholasville: 1x109 CFU g-1) and/or Spirulina platensis.

The experimental protocols were approved by the Animal Care and Use Committee of Uludag University and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The study was carried out with the permission of Uludag University Animal Experimentation Local Ethics Committee (Approval No: 2010-09/01).

Measurements

The blood samples were collected by ear venipuncture on the 90th day. Counts of leukocytes were estimated according to the methods reported by Jain[30]. Reduced glutathione in erythrocytes was determined spectrophotometrically (Shimadzu UV 1201 V, Japan). The absorbances were read in a spectrophotometer at 412 nm.[31] The values were determined from standard curve. The results were expressed as micromole per gram of protein (μmol/g protein).

Statistical Analysis

Statistical analyses were performed with SPSS (Version 17.0; Chicago.IL).[32] 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 the parameters to examine the difference between groups.[33]

Results

Glutathione concentration in erythrocytes and leukocytes counts of blood of the control and experimental groups (SC, SP and SC+SP) are presented in Table 3. There was no significant difference in the glutathione and leukocyte counts in all groups ($P<0.05$) but glutathione (control, SC and SP; 5.75, 5.96 and 5.91 (μmol/g protein) , respectively) and leukocytes counts (control, SC and SP; 6860, 7011 and 7010, respectively) tended to increase with the addition of SC or SP . In addition, lymphocyte counts tended to increase, and neutrophil counts to decrease in rabbits fed SC or SC+SP ($P>0.05$; Table 3). As a result, S. cerevisiaea and S. platensis had changed glutathione and leukocytes counts.

Table 1. Chemical composition of basal diet (%DM)

	Diet
Dry matter%	88.89
Crude protein%*	16.00
Ether extracts%*	3.52
Crude fiber%*	10.95
Ash	7.68

* Based on %Dry Matter

Table 2. Ratio of feed ingredients (%)

Ingredients	Usage rate, %
Barley	30.00
Alfalfa meal	25.00
Corn14	17.61
Soybean meal 46	10.83
Rice bran	10.00
Corn bran	3.60
Limestone	1.40
Salt	0.80
Dicalcium phosphate 18	0.28
Vitamin premix*	0.25
Methionine	0.09
Anticoccidial	0.03
Antioccidial	0.03
Total	100.00

* Premix: Vit A 4.800.000 IU, Vit D 800.000 IU, Vit E 14.000 mg, Biotin 18 mg, CH-CL 50.000 mg, Folic acid 400 mg, Niacin 8.000 mg, Pant.Acid 4.000 mg, Riboflavin 2.800 mg, Thiamin 1.200 mg, Pyridoxine 2.000 mg, Vit K 1.600 mg, Zinc 24.000 mg, Iron 2.000 mg, Iodine 400 mg, Manganese 32.000 mg, Selenium 60 mg, Copper 24.000 mg.

Table 3. Glutathione values (µmol/g protein) and leukocytes counts (mm3 of blood) in Rabbits fed yeast and spirulina supplemented diets (mean±standard error).

Treatment groups				
Parameters	**C[1]**	**SC[2]**	**SP[3]**	**SC + SP[4]**
GSH[5]	5.75±0.23	5.96±0.30	5.91±0.33	5.75±0.24
Leukocytes	6860 ± 192	7011 ± 228	7010 ± 212	6780 ± 223
Lymphocytes	4328 ± 385	5126 ± 234	4430 ± 332	4712 ± 229
Neutrophils	2127 ± 299	1605 ± 279	2243 ± 350	1844 ± 226
Eosinophils	213 ± 87	91 ± 62	147 ± 75	95± 50
Basophiles	55 ± 33	39 ± 24	49 ± 26	47 ± 21
Monocytes	137 ± 00	148 ± 39	140 ± 36	81 ± 33

1Control group, 2Saccharomyces cerevisiae , 3Spirulina platensis , 4Saccharomyces cerevisiae and Spirulina platensis, 5Reduced glutathione values in erythrocytes.

Discussion

Neutrophils, monocytes and macrophages derived monocytes work under inflammatory sites of high oxidative stress because of the production of ROS. Yang et al.[34] found that glutathione protects monocytes and macrophages against to hypochlorite formed by neutrophils and macrophages within inflammatory environments. In the same study, it was postulated that glutathione protects these cells from oxidative stress. Also, Kim et al.[35] indicate that the GSH status of cells play a crucial role in the differentiation and phagocytosis of macrophages. Leukocytes release chemical oxidants that have potent antibacterial, antiviral, and antifungal, but over production of these oxidants may cause the cellular damage that can lead to disease such as arthritis, cardiovascular disease, cancers and many others[36,37]. Antioxidants have a beneficial role in resistance of many diseases and aging process.[11]

S. platensis is a rich source of protein and has been found to be a rich source of vitamins, minerals, essential fatty acids, and antioxidant pigments such as carotenoids.[12,13,38] In particular, this alga is a rich source of phycocyanin, an antioxidant biliprotein pigment.[17,39,40] Recently, it was reported that the protein phycocyanin played a crucial role in the antioxidative action of Spirulina.[41] In studies, it was focused on its therapeutic properties, especially antioxidant effects.[42,43] Riss et al.[41] observed that SP (7.14 mL/kg/ day) significantly increased plasma antioxidant capacity compared with controls. Also, Kim et al.[18] found that 5% Spirulina significantly increased glutathione in erythrocytes compared with controls in rabbits fed a high cholesterol diet, and they suggested that dietary supplementation with Spirulina may be useful to protect the cells from lipid peroxidation and oxidative DNA damage. In addition, Kalafati et al.[44] reported significant increases of glutathione level in red blood cell in human supplemented with spirulina (6 g/day, for 4 wk) at rest and 24 h after exercise, and they suggested that these increases would be related to enhance glutathione synthesis by SP.

Al-Masri et al.[24] indicated that blood glutathione level significantly increased in groups administered S.cerevisiae in rats, and these researchers proposed that yeast beta glucan enhances the glutathione synthesis. On the other hand, in a study related to nicotine reported that nicotine caused significant reductions in the glutathione levels in rat when compared to control group, while beta glucan treatment significantly reversed the glutathione back to control level, and they suggested that beta glucan, S. cerevisiae cell wall component, protects against chronic nicotine- ınduced oxidative damage in tissue.[45]

The present study is an attempt to identify natural effects of SP, SC and SP+SC combinations for rabbits. According to the results of this study, although not statistically significant, supplementing rabbit with S. cerevisiaea and S. platensis had increased on glutathione values (Table 3).

A study related to aflatoxin, glukomannan, derived from cell wall of Saccharomyces cerevisiae, at a dose of 2g/kg did not statistically significant effect on reduced glutathione value in the blood compared with controls in quails , but it reverse the aflatoxin decreased glutathione.[46] Relating the present experimental results with our previous

studies in which rations of high forage or high concentrate and sheep were used, we observed that the addition with a daily dose of 4g SC for every animal had no significant effect on reduced glutathione in erythrocytes.[27]

According to some researchers feeding Spirulina enhanced nonspecific cellular immune responses such as chemotaxis and phagocytosis.[47,48] In a study involving the effect of Spirulina in fish, Duncan and Klesius[15] found that fish fed Spirulina had a higher percentage of lymphocytes than fish fed a control diet. Şahan et al.[49] found that the addition of 5.0 g/kg, 7.5 g/kg and 10 g/kg spirulina elevated leukocytes levels. In the same study, percentages of lymphocytes were significantly higher in the groups fed with 5.0 and 10 g/kg of spirulina whereas percentages of monocytes level were significantly lower in the same groups.

Onifade et al.[50] studied in rabbit with a diet containing 0 g/kg, 1.5 g/kg, and 3.0 g/kg S.cerevisiae yeast, and they found that leucocyte counts were similar on the treatments.

Also in the present study, addition of S.cerevisiae and S.platensis alone or in combination had no significant effect on leucocyte count (Table 3). Although not statistically significant, we found the higher counts of leucocytes in rabbits fed SC and SP, the higher counts of lymphocytes and the lower counts of neutrophils in rabbits fed SC or SC+SP.

In conclusion, according to the results of this study, although not statistically significant, supplementing rabbit with S. cerevisiaea and S. platensis had increased on glutathione values. Glutathione values are likely to rise as a result of feeding rabbit with SC and SP. No significant difference in white blood cell counts was evidenced, even if lymphocyte counts tended to increase and neutrophile counts to decrease in rabbits fed SC or SC+SP. The determination of biological consequences (antioxidant potential, resistance to diseases, and improvement of nutritional status) requires further investigations.

Acknowledgements

This study was supported by a grant from the Research Foundations of Uludag University (Project number: 2011/19)

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