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RESEARCH ARTICLE

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Effects of different selenium sources and levels on serum biochemical parameters and tissue selenium retention in rats

Abstract A total of 54 female Wistar rats were allotted to nine treatments by weight and fed basal diet or diets containing Se of 0.05, 0.10, 0.15, or 0.20 mg·kg^{-1} diet provided with either Se yeast or sodium selenite for 10 days. The results showed the following: (1) Selenium yeast had better effects compared with sodium selenite on increasing serum superoxide dismutase activities ($P < 0.05$). Addition of Se yeast or sodium selenite increased the activities of serum gluthathione peroxidase ($P < 0.01$); (2) According to slope ratio assay, the bioavailability of Se from Se yeast was 132.1%, 205.7%, 140.0%, and 107.2% of that from sodium selenite when glutathione peroxidase activities and Se contents in serum, kidney, and liver were used as indicators. It is concluded that Se from Se yeast has higher bioavailability than Se from sodium selenite.

Keywords Wistar rats, selenium yeast, serum biochemical parameters, tissue selenium, bioavailability

1 Introduction

Selenium has been established as an essential trace element that is important in many biochemical and physiological processes (Burk et al., 2003). Research has shown that the organic and inorganic forms of Se vary in tissue retention and that organic Se is deposited in greater concentrations in many tissues compared with inorganic Se (Ku et al., 1973). Thus, the research and application of high efficient organic source of Se has become one of the focal points of animal nutrition home and abroad (Yang et al., 2000). Selenium yeast as a good source of organic Se has been explored as an alternative to inorganic supplementation (Payne and Southern, 2005). Since June 2000 when the FDA first permitted the use of Se yeast as an organic Se supplement for poultry diets, Se yeast products have become available from various sources. In China, however, there is no Se yeast product available for animal production in the market. Furthermore, the Se bioavailability of Se yeast from the literature is not consistent and needs to be further studied for better utilization of this new Se product. The purpose of our study was to investigate the effects of Se yeast on serum biochemical parameters, and tissue Se concentration in Wistar rats and quantify the bioavailability of Se yeast in comparison with sodium selenite.

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2 Methods

2.1 Experimental animals and design

The animal use and care protocol was approved by the Animal Care and Use Committee of Sichuan Agricultural University. A total of 54 female four-weeks-old Wistar rats (SPF, purchased from Institute of Laboratory Animals, Sichuan Academy of Medical Science, Chengdu, China) were fed with purified basal diet (Table 1) formulated according to AIN-93G (Reeves et al., 1993) for 13 days to delete the endogenous Se in the body. Then, the rats were allocated by weight into nine treatments with six replications each. One treatment was continued to feed on Se-deficient basal diet as negative control (NC). The other eight treatments were fed with experimental diets supplemented with Se of 0.05, 0.10, 0.15, or 0.20 mg·kg^{-1} diet from either sodium selenite premix (Se content at 10000 mg·kg^{-1} diet, provided by Institute of Animal Nutrition, Sichuan Agricultural University, Ya'an, China) or Se yeast (Se content at 1300 mg·kg^{-1} diet, prepared in our lab). The experiment lasted 10 days.

Table 1 Composition and nutrient level of basal diet (as feed basis, %)

ingredient	content	nutrient level	content
corn starch	48.30	DE /MJ·kg^{-1}	16.10
casein	21.00	CP	18.70
soybean oil	2.00	DLys	1.54
cellulose	1.00	DMet	0.57
cane sugar	23.60	Ca	0.83
$CaCO_3$	1.40	AP	0.31
NaCl	0.30		
$CaHPO_4$	0.80		
premix*	1.50		
choline	0.10		
total	100.00		

Note: * means Cu 10 mg, Zn 30 mg, Fe 100 mg, Mn 10 mg, I 0.5 mg, Mg 500 mg, K 3.5 g, VA 14000IU, VD_3 1500 IU, VE 120 IU, VK 5 mg, VB_1 12 mg, VB_2 12 mg, VB_6 12 mg, niacin 60 mg, biotin 0.2 mg, pantothenic acid 24 mg and folic acid 6 mg· kg^{-1} basal diet

The trial was carried out in the Research Base of Institute of Animal Nutrition. All the rats were individually housed in stainless cages at the size of 39 cm × 25 cm × 20 cm, with a stainless feeder and plastic waterbowl. Rats fed on the corresponding diets and double distilled water ad libitum. The room temperature and humidity were controlled at 20–25°C and 40%–60%, respectively. In the course of the trial, rats were given a constant 12 h day/12 h night diurnal cycle. Feed and water were replaced every day.

2.2 Sample collection and analytical methods

All rats were anesthetized by intramuscular injection of phenobarbital (0.1 mL·100 g^{-1} bodyweight) at the end of the experimental period. The serums of the same treatment were pooled uniformly and then divided into five EP tubes and stored at −20°C. Kidney, liver, and muscle samples were collected and washed clean with saline, then stored at −20°C for the determination of selenium.

Activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), and concentrations of malondialdehyde (MDA) in the serum were measured using an assay kit (Nanjing Jianchen Bioengineering institute, Nanjing, China). Manipulations were followed from the manufactures' instructions.

The Se contents in serum and tissue samples were determined by the diaminonaphthalene (DAN) fluorometric method (Whetter and Ullrey, 1978). First, the sample was digested using the mixture of nitric acid and perchloric acid at a molar ratio of 3∶1, then reacted with DAN at 100°C for 5 min, and finally extracted with hexamethylene. The fluorescence intensity excited at 376 nm and recorded at 520 nm was used for calculating Se content based on the fluorescence intensity of standard concentrations of Se.

2.3 Statistical analysis

Statistical analysis was performed using the GLM procedure of SAS 8.0. Differences among treatment means were evaluated using the PDIFF option of SAS software. Regression analysis (PROC REG) was done to determine the relationship between the GPX activity, tissue Se contents, and added dietary Se concentrations. A slope ratio assay (Kim and Easter, 2001) was derived for the effects of added dietary Se concentrations by source on GPX activity and Se concentrations in tissues. The relative bioavailability was then determined by comparing the slopes of the regression lines.

3 Results

3.1 Serum biochemical parameters

Table 2 Serum biochemical parameters of Wistar rats fed with different forms of selenium

Se source and level/(mg·kg^{-1})	SOD/ (U·mL^{-1})	MDA/ (nmol·mL^{-1})	GPX/(U)
sodium selenite (SS)			
0	301.67	5.85	873.68
0.05	303.87	5.19	976.60
0.10	302.25	5.56	1000.79
0.15	303.77	5.80	923.32
0.20	306.07	5.00	1049.80
selenium yeast (SY)			
0	301.67	5.85	873.68
0.05	312.56	5.25	1026.88
0.10	311.51	5.61	984.19
0.15	320.97	5.12	1034.78
0.20	326.99	5.01	1067.19
pooled SE	10.51	0.47	40.51
	probability		
ANOVA			
Se sources (S)	0.045*	0.689	0.215
Se concentration (C)	0.501	0.364	0.002**
S×C	0.776	0.894	0.554

Note: * means $P < 0.05$, ** means $P < 0.01$. The same in Table 3.

Table 2 shows that selenium source has a significant effect on SOD activity with Se yeast higher than sodium selenite ($P < 0.05$). There is no significant difference in the SOD activity among different dietary Se levels, but SOD activity tended to increase as Se level increased. Se source and level had no significant effect on serum MDA contents ($P > 0.05$). GPX activity increased linearly as dietary Se

level rose, and the linear equations for Se yeast (SY) and sodium selenite (SS) are

GPX activity = 789.8 × SY + 918.4 (R^2 = 0.69, P = 0.003), and

GPX activity = 597.9 × SS + 905.0 (R^2 = 0.69, P = 0.035), respectively.

Se sources had no significant influence on GPX activity.

3.2 Tissue Se concentration

Table 3 shows that Se yeast extremely increased the content of serum Se compared with sodium selenite ($P < 0.01$). Different levels of additional Se also had an extreme influence on the content of serum Se ($P < 0.01$). Serum Se content was increased linearly as Se level increased with the equations of Serum Se content = 0.323 × SY + 0.186 ($R^2 = 0.89$, $P = 0.001$) and Serum Se content = 0.157 × SS + 0.187 ($R^2 = 0.86$, $P = 0.001$) for Se yeast and sodium selenite, respectively.

Se levels had an extremely significant influence on the kidney ($P < 0.01$) and liver ($P < 0.05$) Se concentrations. Linear relationships existed for both Se sources. The equations are

Kidney Se concentration = 0.308 × SY + 0.643 (R^2 = 0.90, P = 0.009),

Kidney Se concentration = 0.220 × SS + 0.644 (R^2 = 0.83, P = 0.020),

Liver Se concentration = 0.328 × SY + 0.467 (R^2 = 0.71, P = 0.046), and

Liver Se concentration = 0.306 × SS + 0.464 (R^2 = 0.73, P = 0.042).

Muscle Se concentration was not influenced significantly by Se sources and levels.

Table 3 Serum, kidney, liver, and muscle Se concentrations of Wistar rats fed with diets with different dietary concentrations and sources of Se

Se source and added Se/ ($mg \cdot kg^{-1}$)	serum/ ($\mu g \cdot mL^{-1}$)	kidney/ ($mg \cdot kg^{-1}$)	liver/ ($mg \cdot kg^{-1}$)	muscle/ ($mg \cdot kg^{-1}$)
sodium selenite (SS)				
0	0.189	0.638	0.446	0.222
0.05	0.197	0.656	0.494	0.223
0.10	0.197	0.676	0.510	0.225
0.15	0.210	0.678	0.509	0.224
0.20	0.222	0.682	0.515	0.225
selenium yeast (SY)				
0	0.189	0.638	0.446	0.222
0.05	0.197	0.665	0.505	0.223
0.10	0.225	0.680	0.511	0.226
0.15	0.222	0.681	0.511	0.225
0.20	0.258	0.707	0.525	0.231
pooled SE	0.003	0.014	0.024	0.021
	probability			
ANOVA				
Se sources (S)	0.001**	0.329	0.762	0.909
Se concentration (C)	0.001**	0.005**	0.029*	0.999
S×C	0.001**	0.886	0.999	1.000

3.3 The relative bioavailability of Se yeast

Serum GPX activity and Se concentrations in the serum, kidney, and liver were all increased linearly as dietary Se was enhanced. According to slope ratio assay, when in the GPX activity, Se concentrations in serum, kidney, and liver were used as assessment indicators, the relative bioavailability of Se from Se yeast was 132.1%, 205.7%, 140.0%, and 107.2% of that from sodium selenite, respectively (Table 4).

Table 4 The relative bioavailability values (RBV) for Se yeast relative to sodium selenite[(a)]

dependents	sodium selenite (SS)			Se yeast (SY)			RBV[(b)] /%
	intercept	SS slope	P-value	intercept	SY slope	P-value	
GPX/(U)	905.0	597.9	0.035	918.4	789.8	0.003	132.1
serum Se/ ($mg \cdot kg^{-1}$)	0.187	0.157	0.001	0.186	0.323	0.001	205.7
kidney Se/ ($mg \cdot kg^{-1}$)	0.644	0.220	0.020	0.643	0.308	0.009	140.0
liver Se/ ($mg \cdot kg^{-1}$)	0.464	0.306	0.042	0.467	0.328	0.046	107.2

Note: (a) Equation model: Dependent variable = slope × (dietary Se level) + intercept. (b) Relative bioavailability = (slope of SY) / (slope of SS).

4 Discussion

Thirteen days before the experiment, all the rats were fed with the basal diet without Se, and this period was called exhausted period. At the beginning and end of the exhausted period, six rats under the same situation were killed to determine the serum Se content, serum GPX activity, and Se concentrations in tissues to indicate Se status in the body. Values (data not shown) indicated that the Se-deficient rat model was established.

SOD is an important antioxidative enzyme in mammals. MDA is a product of oxidation of lipid. Both are important indexes reflecting the oxidation status of animals. Selenium, as a part of the enzyme GPX, works as an antioxidant by reducing hydrogen peroxide (H_2O_2) and organic hydroperoxides, providing protection to cellular and subcellular membranes against oxidative damage (Wang and Xu, 2006). In this experiment, Se yeast was

more efficient than sodium selenite in increasing the activity of SOD. Dietary supplemental Se with Se yeast or sodium selenite was tended to increase serum SOD activity and to decrease serum MDA content. The levels of dietary Se played an important role in the activity of GPX. Serum GPX activity increased along with the increase of the dietary Se levels. It indicated that dietary additional Se increased the oxidation resistance in Wistar rats. These were consistent with the results of Gao et al. (2006), Liu et al. (2007) and Yu et al. (2008). However, Payne and Southern (2005) reported that the GPX activity was not affected by Se source or concentration. It might be associated with the breed of broilers and experimental phase.

Serum Se concentrations increased along with the increase of dietary Se levels. Moreover, there was an Se source × Se level interaction. Serum Se concentrations were higher when rats were fed with Se yeast in comparison with sodium selenite. Dietary Se level affected the Se concentrations in kidney and liver but not significantly in muscle of rats. Kidney and liver Se increased linearly as the dietary Se level increased. These results indicated that both sources of Se had the consistent effect in kidney, liver, and muscle Se concentrations. Normally, Se is mainly deposited in the kidney, liver, and fur, with less Se deposition in muscle and blood. Selenium deposited in tissues usually exists in the forms of selenomethionine or selenocystine, and organic Se was more readily to be deposited than inorganic Se (Beilstein and Whanger, 1988; Wang et al., 2007). Guo and Huang (2006) found that the deposition of Se in broiler kidney, liver, and muscle increased along with the increasing of diets Se levels. In this experiment, Se yeast was more efficient than sodium selenite but having no statistical significance. It might be associated with the different experimental animals or feeding phase. However, larger studies are needed to fully explore this hypothesis.

Slope ratio analysis showed that the Se yeast was more available for the improvement of GPX activity and serum, kidney, and liver Se concentrations. It had been previously reported that feeding diets with selenomethionine, which was the predominant form in Se yeast (Rayman, 2004), as the source of Se resulted in a greater tissue accumulation of Se than sodium selenite (Wang et al., 2007). Our results showed that the Se yeast had a higher bioavailability than sodium selenite. Other reports also showed similar results (Mahan et al., 1999; Mateo et al., 2007; Wang and Xu, 2008).

5 Conclusions

Dietary supplemental Se can increase the oxidation resistance in Wistar rats. Compared with sodium selenite, Se yeast prepared in our lab has a higher bioavailability.

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