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QUALITY OF MEAT AND SELENIUM CONTENT IN TISSUES OF RABBITS FED DIETS SUPPLEMENTED WITH SODIUM SELENITE, SELENIZED YEAST AND SELENIZED ALGAE

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Abstract: Forty rabbits weaned at 35 d of age were randomly assigned to 4 groups of 10 rabbits each. The rabbits were fed a basal granulated diet containing 0.08 mg Se/kg, or diets obtained from basal diet, supplemented with sodium selenite, selenized yeast (Se-yeast) or selenized algae Chlorella (Se-algae) to increase the Se concentration to 0.40 mg/kg. After 6 weeks rabbits were slaughtered at 77 d of age. Samples of meat, liver and hair were analyzed. The Se supplements did not influence rabbit growth, feed intake and weight loss during cooling. The nutrient composition of meat (dry matter, protein and fat concentration) was only marginally influenced. The Se concentration in the loin and hindleg meat increased by 23.0% and 19.2%, respectively in rabbits receiving the selenite supplement. In rabbits fed Se-yeast and Se-algae, the Se content in meat doubled from 0.11-0.15 mg/kg to 0.24-0.29 mg/kg. High Se concentrations (=1 mg/kg) were observed in hair and liver of rabbits which had received the Se-supplements. In all supplemented groups, the activity of glutathione peroxidase in the loin meat was higher than in control rabbits by 51.9-72.8% (P<0.001). The oxidative stability of rabbit meat, however, was not influenced. It follows from our results that (i) the enrichment of meat with Se is the main benefit of supranutritional Se supply in rabbits, and (ii) Se-yeast and Se-algae are more effective in increasing Se content in tissues than selenite.

Key Words: rabbit, selenium, selenite, selenized yeast, selenized algae, meat.

INTRODUCTION

Selenium (Se) is a trace element, necessary for human and animal health. The dietary Se intake in humans varies considerably between countries, largely due to uneven geographical distribution of Se in soils. Vast land areas do not supply enough of this element for optimum animal and human nutrition (Rayman, 2008). Though plants are an important source of Se in the human diet, animal products seem to be a more reliable source of this essential nutrient. It has been shown that animals readily incorporate supplemental Se into edible tissues, so that it is possible to produce Se-enriched meat and eggs (reviewed by Surai, 2006). Unlike other animal species, information on the effect of Se supplements in rabbits is limited. Erdélyi *et al*. (2000) observed that dietary Se supplementation with selenized yeast increased the activity of glutathione peroxidase (GSH-Px) in the blood and Se concentration in blood and liver. In the experiment

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by Dokoupilová *et al*. (2007), the loin and hindleg meat of rabbits fed a diet supplemented with Se-enriched yeast contained four times more Se than the meat of control rabbits. The purpose of this study was to evaluate the influence of different inorganic and organic forms of dietary Se on performance, Se deposition in meat, and meat oxidative stability in fattening rabbits. Rabbits were fed diets supplemented with sodium selenite, Se-enriched yeast (Se-yeast), and Se-enriched algae Chlorella (Se-Chlorella; a novel Se source).

MATERIALS AND METHODS

Animals and diets

20 male and 20 female Hyplus rabbits from different litters were weaned at 35 d of age. Male and female rabbits were randomly assigned to 4 groups, one of which received a granulated basal diet containing 0.08 mg Se/kg (Table 1), and the remaining diets supplemented with Se to increase the Se concentration to 0.40 mg/kg. Three sources of supplemental Se were used: sodium selenite (Sigma-Aldrich), selenized yeast (Sel-Plex, Alltech) and selenized algae Chlorella (Institute of Microbiology, Třeboň, Czech Republic). The profiles of organic compounds of Se in the Se-yeast and Se-Chlorella differ. Se-methionine is the principal Se-containing compound in Se-yeast, but dimethylselenonium propionate in Se-Chlorella. Other Se compounds in Chlorella include Se-ethionine and Se-allyl-Se-cysteine at very low concentrations (Larsen *et al*., 2001). Sodium selenite, Se-yeast and Se-algae contained Se at 447, 1 and 0.25 mg per g, respectively. Rabbits were housed individually in stainless steel mesh cages. The environmental temperature was about 18°C and the humidity about 65%. The animals had *ad libitum* access to feed and water. The consumption of feeds was recorded individually. Animals were weighed once a week. No rabbit died in the course of the experiment. Rabbits were slaughtered at 77 d of age. Hot carcass weight including head, liver, kidney and perirenal fat was measured 15-30 min after slaughter (Blasco and Ouhayoun, 1996). The carcass was chilled at 4°C and the weight loss during cooling was measured in

Table 1: Ingredients and determined chemical composition of the basal rabbit diet.

Ingredients (g/kg)		Chemical composition (g/kg)	
Lucerne meal	300	Dry matter	882
Wheat bran	260	Crude protein	173
Barley	145	NDF	389
Oat	60	ADF	216
Sugar beet pulp	40	Starch	155
Sunflower meal (extracted)	130	Fat	45
Soybean meal (extracted)	20	Ash	66
Rapeseed oil	15	Se	8.10^{-5}
Limestone	10	Digestible energy[2] (MJ/kg)	11.0
Dicalcium phosphate	5		
Salt	5		
Vitamin-mineral supplement[1]	10		

[1]Supplied per kg of diet: 3.6 mg vitamin A, 50 µg vitamin D3, 50 mg α-tocopherol acetate, 2 mg vitamin K3, 3 mg vitamin B1, 7 mg vitamin B2, 4 mg vitamin B6, 20 µg vitamin B12, 50 mg niacin amide, 20 mg calcium pantothenate, 0.2 mg biotin, 1.7 mg folic acid, 0.6 g choline, 1 g methionine, 0.2 g lysine.HCl, 22 mg salinomycin, 1 mg Co, 20 mg Cu, 50 mg Fe, 1.2 mg I, 47 mg Mn, 50 mg Zn, 0.05 mg Se, 0.1 g antioxidant (BHT). [2]According to Maertens *et al*. (2002).

the period 0-24 h post-mortem. Dressing out percentage was calculated as a proportion of chilled carcass weight from live weight.

Sampling and analyses

The loin and hindleg meat, liver and hair of all rabbits were analyzed. Samples of meat and liver were taken 24 h *post mortem* and stored at −40°C. Meat samples for GSH-Px assay were stored at −70°C. Meat samples were ground before analyses. Meat dry matter (DM) was determined by oven drying at 105°C to constant weight (about 20 h), protein after the Kjeldahl destruction using the instrument Kjeltec 2460 Analyser (FOSS Tecator AB, Höganäs, Sweden), and free fat by extraction with petroleum ether without a prior hydrolysis in the Soxtec Avanti 2055 apparatus from the same company (ČSN-ISO 1444, 1977). The feed was analyzed as described previously (Dokoupilová *et al*., 2007): crude protein and fat employing the above mentioned instruments, fibre fractions according to Van Soest *et al*. (1991), and starch by the Ewers method (AOAC 1980; procedure 14.032). The digestible energy was calculated as the sum of DE content of the diet components (Maertens *et al*., 2002). To prepare samples for the Se analysis, feeds, tissues and hair were mineralized using the microwave digestion technique in a closed system, in the presence of nitric acid and hydrogen peroxide. Selenium in processed samples was measured by the atomic absorption spectrometry using the Solaar M-6 instrument (TJA Solutions, U.K.). The analytical procedure was validated by the analysis of a certified reference material RM 8414 Bovine Muscle (NIST).

The activity of GSH-Px in the loin meat was measured with tert-butyl hydroperoxide as a substrate by a coupled assay, recording the oxidation of NADPH by the decrease in absorbance at 340 nm. The activity was expressed as μmol NADPH oxidized/min per g meat (DeVore and Greene, 1982). Lipid oxidation in minced samples of hindleg meat was measured by the thiobarbituric acid method of Piette and Raymond (1999). Thiobarbituric acid-reactive substances (TBARS) were expressed in mg of malondialdehyde per kg muscle.

The effect of dietary treatment was statistically analyzed by one-way ANOVA using the GraphPad Instat (GraphPad Software, Inc., La Jolla, Ca, U.S.A.). Differences ($P<0.05$) were identified by Tukey's test. The rabbits were housed individually, thus a rabbit was the experimental unit.

RESULTS

No significant differences on growth, feed intake, feed conversion, weight loss were observed with the selenium, supplemented diets during cooling, or dressing out percentage (Table 2). Se supplements had no

Table 2: Growth, feed intake, feed conversion and carcass yield in rabbits fed a basal diet (control) and diets supplemented with sodium selenite, Se-yeast and Se-algae.

	Control	Selenite	Se-yeast	Se-algae	R.M.S.E.[1]	*P*-value
Initial weight (g)	863	842	820	823	82	0.616
Final weight (g)	2700	2578	2728	2557	250	0.272
Weight gain (g/d)	43.7	41.3	45.4	41.3	5.7	0.273
Feed intake (kg)	5.76	5.51	5.81	5.43	0.57	0.387
Feed/gain (kg/kg)	3.14	3.17	3.05	3.13	0.30	0.358
Hot carcass weight (g)	1592	1534	1620	1530	160	0.521
Chilled carcass weight (g)	1493	1436	1520	1428	155	0.489
Dressing percentage (%)	55.3	55.7	55.7	55.8	2.0	0.867

[1]Root means square error; n=10.

Table 3: Dry matter, protein and fat concentration (g/kg) in loin and hindleg meat of rabbits fed a basal diet (control) and diets supplemented with sodium selenite, Se-yeast and Se-algae.

	Control	Selenite	Se-yeast	Se-algae	RMSE	*P*-value
Loin						
Dry matter	264	261	264	267	4.9	0.077
Protein	233	234	234	237	3.7	0.15
Fat	9.5	7.1	8.1	7.0	3.0	0.25
Hindleg						
Dry matter	284^{a}	285^{a}	276^{b}	278^{ab}	6.9	0.007
Protein	222	223	222	225	4.5	0.40
Fat	43.5^{a}	41.9^{ab}	34.6^{ab}	31.6^{b}	9.4	0.015

[1]Root means square error; Means in the same row with different superscripts differ significantly (P<0.05); n=10

effect on the dry matter, protein and fat concentration of loin meat (P≥0.077, Table 3). The hindleg meat of rabbits supplemented with Se-yeast contained less dry matter than meat of control rabbits and rabbits fed selenite (P=0.007), whereas concentration of protein was the same (P=0.40). The hindleg meat of rabbits fed Se-algae contained less fat than that of control rabbits (P=0.015). Se concentration in meat increased in supplemented groups (Table 4). A moderate increase of Se concentration in meat (statistically non-significant in hindleg meat and significant in loin) was observed in rabbits fed the selenite supplement. In rabbits fed diets supplemented with Se-yeast and Se-algae, the Se concentration in meat was doubled (P<0.001). Supplementation with Se increased the Se concentration in liver and hair (P<0.001). The effect of selenite was less pronounced than that of organic Se sources. In all supplemented groups, the activity of GSH-Px in the loin meat was significantly higher than in control rabbits (P<0.001). On the other hand, dietary Se did not significantly influence the formation of TBARS in the hindleg meat stored for 3 and 6 d at 4°C.

Table 4: Concentration of Se in meat, liver and hair, activity of GSH-Px in loin meat, and production of thiobarbituric acid-reactive substances (TBARS) in hindleg meat of rabbits fed a basal diet (control) and diets supplemented with sodium selenite, Se-yeast and Se-algae.

	Control	Selenite	Se-yeast	Se-algae	R.M.S.E.[1]	*P*-value
Se in loin meat (μg/kg)	113^{a}	139^{b}	286^{d}	243^{c}	14	<0.001
Se in hindleg meat (μg/kg)	146^{a}	174^{a}	276^{b}	257^{b}	42	<0.001
Se in liver (μg/kg)	888^{a}	1081^{b}	1370^{c}	1134^{b}	86	<0.001
Se in hair (μg/kg)	680^{a}	734^{a}	1096^{b}	979^{b}	90	<0.001
GSH-Px in meat[2]	0.81^{a}	1.40^{b}	1.23^{b}	1.27^{b}	0.11	<0.001
TBARS[3]						
Day 0	0.50	0.65	0.53	0.51	0.14	0.075
Day 3	1.37	1.36	0.90	1.32	0.44	0.075
Day 6	1.37	1.98	1.33	1.73	0.82	0.25

[1]Root means square error. [2]Expressed as μmol NADPH oxidized/min/g meat. [3]Expressed as mg malondialdehyde/kg meat; Means in the same row with different superscripts differ significantly (P<0.05); n=10.

DISCUSSION

Rabbits of the control group received Se at 0.08 mg per kg feed. At this concentration Se satisfies the nutritional need of rabbits for Se, which is 0.05 mg Se/kg (de Blas and Mateos, 1998). The Se supplementation was thus not expected to influence growth rate, feed intake and feed/gain ratio. No effect of Se supplementation on performance had been observed in previous experiments with pigs (Goehring *et al*., 1984), lambs (Molnár *et al*., 1998), calves (Skřivanová *et al*., 2007), rabbits (Dokoupilová *et al*., 2007), and chickens (Wang and Xu, 2008). In these studies the Se deposition in organs and tissues significantly increased in proportion to the dietary Se concentration. In the present study, the Se concentration in liver and hair of rabbits was several times higher than in meat. The Se supplementation in the organic form resulted in higher tissue Se concentrations than the Se supplementation in the form of selenite. This has also been shown in pigs (Goehring *et al*., 1984; Zhan *et al*., 2007), calves (Pavlata *et al*., 2001) and chickens (Payne and Southern, 2005). Se-yeast contains most Se in the form of selenomethionine, which is non-specifically incorporated into proteins in place of methionine (Rayman, 2004), so that its bioavailability is higher. The Se concentration in loin meat and liver was higher in rabbits fed the Se-yeast than in rabbits fed the Se-algae. A possible reason for this may be the different profile of organic compounds of Se in the Se-yeast and Se-Chlorella (Larsen *et al*., 2001). Se content in hair was shown to indicate the Se status in cattle (Kursa and Kroupová, 1975), and presumably also in other animals. Almost all sulphur of sulphur-containing amino acids in animal hair is present as cysteine (Kim and Mahan, 2001). It can be assumed that Se in hair is in the form of selenocysteine. Se content in hair may thus indicate how Se in Se-supplements is converted to selenocysteine, which is important for activity of selenoenzymes (Behne and Kyriakopoulos, 2001).

Se contributes to the protection of cells from oxidative damage via the activity of GSH-Px, which is an enzyme that catalyzes the reduction of hydrogen peroxide and organic peroxides (Behne and Kyriakopoulos, 2001). According to Lee *et al*. (1979) rabbits do not develop symptoms of Se deficiency when fed diets deficient in Se. Their liver and kidney contain a sufficient level of non Se-dependent GSH-Px, whereas lungs, heart, spleen, erythrocytes and plasma have Se-dependent GSH-Px activity. This may explain part of the lack of response by rabbits to dietary Se level. In the present study, the Se supplementation significantly increased the GSH-Px activity in meat without a corresponding effect on the oxidative stability of meat (TBARS). Erdélyi *et al*. (2000) and Dokoupilová *et al*. (2007), however, observed no significant effect of supplemental Se on the GSH-Px activity in meat. In our experiment it was necessary to assess the GSH-Px activity and TBARS production in different tissues because of the limited amount of meat for analyses, which reduced the correlation between both parameters. Also, the high dietary concentration of α-tocopherol (50 mg/kg), which is a strong antioxidant, could have influenced our results. TBARS production was measured in the hindleg meat, which is more susceptible to oxidative deterioration due to its higher fat content. Although the increase in GSH-Px activity did not affect the *post mortem* formation of TBARS in meat, it might still have an effect on *in vivo* protection of tissues against oxidative damage, which also involves oxidation of proteins and nucleic acids.

It can be concluded that (i) the enrichment of meat with Se is the main benefit of the supranutritional Se supply in rabbits, and (ii) organic Se sources are more effective in increasing Se content in tissues than sodium selenite. The effect of Se sources could be more pronounced if Se-supplements were fed for a longer period of time.

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