

The Use of Selenium Enriched *Enterococcus faecium* as an Alternative Selenium Source for Growing-finishing Pigs

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Abstract

The aim of the study was to evaluate the effect of the feeding of selenium enriched *Enterococcus faecium* on selenium concentrations in muscle tissue and on meat quality indicators in finishing pigs. Groups of pigs fed Se-enriched *E. faecium* (n = 12), sodium selenite (n = 12) and Se-enriched yeast (n = 12) were used in the study. The tested feed mixtures were fed for a period of 3 months before the pigs were slaughtered. The selenium concentrations in muscle tissue achieved by the use of Se-enriched *E. faecium* were comparable to those achieved in sodium selenite diets and lower ($p \leq 0.05$) compared to Se-enriched yeast. Concentration of Se in serum and glutathione peroxidase (GSH-Px) activities did not differ in animals fed different Se forms. No differences in indices of meat quality were found among the groups. Se-enriched *E. faecium* bacteria can be recommended as an alternative selenium source for finisher pigs with the similar efficacy as sodium selenite.

Pork, meat quality, selenomethionine, selenocysteine

Selenium has an important antioxidant role as a part of enzyme glutathione peroxidase (Sunde 1994). Several studies in human medicine provided evidence for the efficacy of selenium as an anticancer agent (Duffield-Lillico et al. 2003). Selenium also enhances immune functions (Beck et al. 2001) and has an important role in the maintenance of fertility (Rayman 2000). It has been documented that pork can contribute significantly to the selenium intake in human population (Kadrabová et al. 1996; Fajt et al. 2009).

Selenium deficiency in pigs is manifested as mulberry heart disease, hepatosis dietetica and muscular dystrophy (Lindberg et al. 1972). Selenium requirements of growing-finishing pigs can be achieved by providing selenium either from an inorganic or an organic source. Sodium selenite represents the inorganic form. Selenium from Se-enriched yeast is the most commonly used organic selenium form (Schrauzer 2000).

Experiments in growing-finishing pigs revealed higher accumulation of organic Se compared to inorganic form in tissues (Mahan and Parrett 1996; Taylor et al. 2005). Also experiments in sows demonstrated higher Se content in newborn piglets tissues and in colostrum when the Se-enriched yeast was used (Mahan and Peters 2004).

Limited data are available so far regarding the use of other organic selenium sources in pigs. For instance Se from Se-enriched alga *Chlorella* spp. (Svoboda et al. 2009b) and soybean based selenium proteinate (Jang et al. 2006) were used as alternative selenium sources in finishing swine.

The aim of the present study was to evaluate the effects of Se from Se-enriched *Enterococcus faecium* on tissue selenium retention and meat quality indicators in growing-finishing pigs.

Materials and Methods

Experimental design

A total of 36 pigs were used in our experiment. Only female pigs were used. The experiment started at the beginning of the grower phase of production. Before the experiment, all pigs were fed an identical diet

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Table 1. Percentage composition of the feed mixture A1

Ingredient	Percentage
Zoofeed	1.0
Wheat	24.5
Corn	21.5
Barley	25.0
Soya bean pollard	20.0
Yeast Bitec Q	2.0
Nutrimilk feed	0.8
Soya bean oil	0.9
Limestone	1.7
Minfos MDCP20 (monocalciumphosphate)	0.7
NaCl	0.4
Trace mineral and vitamin premix	2.0

Supplied per kg of diet: 0.30mg of Se, 0.25mg of Co, 5.00mg of Cu, 40.00mg of Fe, 0.38mg of I, 15.00mg of Mn, 45.00mg of Zn, 5.00mg of calcium pantothenate, 3500.00 I.U. of vit. A, 625.00 I.U. of vit. D3, 20.00mg of vit. E, 0.75mg of vit. K3, 0.75mg of vit. B1, 2.10mg of vit. B2, 1.00mg of vit. B6, 0.013mg of vit. B12, 7.50mg of niacinamide, 0.025mg of biotin, 50.00mg of cholinchlorid, 0.10mg of folic acid, 25mg of Natuphos, 0.175g of L-lysine, 0.076g of L-threonine, 0.048g of D-L-methionine, 0.0045g of L-tryptophan

Table 2. Percentage composition of the feed mixture A2

Ingredient	Percentage
Zoofeed	1.0
Wheat	28.0
Corn	21.4
Barley	24.0
Soya bean pollard	19.5
Soya bean oil	0.9
Minfos MDCP20 (monocalciumphosphate)	0.7
Limestone	1.6
NaCl	0.4
Kalama (benzoic acid)	0.5
Trace mineral and vitamin premix	2.0

Supplied per kilogram diet: 0.30mg of Se, 0.25mg of Co, 5.00mg of Cu, 35.00mg of Fe, 0.38mg of I, 15.00mg of Mn, 45.00mg of Zn, 4.50mg of calcium pantothenate, 3000.00 I.U. of vit. A, 495.00 I.U. of vit. D3, 17.60mg of vit. E, 0.60mg of vit. K3, 0.65mg of vit. B1, 2.10mg of vit. B2, 0.90mg of vit. B6, 0.013mg of vit. B12, 6.50mg of niacinamide, 0.026mg of biotin, 50.00mg of cholinchlorid, 25mg of Natuphos, 0.149g of L-lysine, 0.064g of L-threonine, 0.03g of D-L-methionine, 0.004g of L-tryptophan

supplemented with 0.3 mg·kg⁻¹ Se in the form of sodium selenite. The compositions of experimental mixtures are presented in Table 1 and Table 2. The feed mixtures were composed of a basal diet and a mineral supplement. The pigs received experimental mixtures for the period of 3 months before the slaughter. The feed mixture A1 was administered during the first 30 days. The feed mixture A2 was given to pigs for the rest 60 days.

The pigs were divided into 3 equal groups of 12 animals.

In group A (n = 12) pigs were fed feed mixtures A1 and A2 supplemented with organic selenium at the dose of 0.3 mg·kg⁻¹ in the form of Se-enriched *Enterococcus faecium*.

The group B (n = 12) had feed mixtures A1 and A2 supplemented with inorganic sodium selenite at the dose of 0.3 mg·kg⁻¹.

In group C (n = 12) the pigs received feed mixtures A1 and A2 with the addition of organic selenium in the form of Se-enriched yeast (0.3 mg·kg⁻¹).

The experiment was approved by the Ethics Committee of the University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.

Sampling

The blood samples were taken from all pigs at the beginning of the trial and one day before the slaughter. The pigs were bled from vena cava jugularis. Serum was collected and frozen for further analysis.

Tissue samples for selenium determination (heart, musculus biceps femoris, liver and ovaries) were taken after the slaughter and frozen for further analysis. Muscle samples for the evaluation of the meat quality indicators were taken from musculus biceps femoris (300 g).

Determination of Se in tissues (heart, liver, muscle, ovaries)

The samples for selenium determination were mineralized in a closed system using a microwave (MLS-1200, Milestone, Italy) digestion technique with HNO₃ and H₂O₂. The samples were evaporated and the mineral residue was dissolved in water to which 20% HCl was added. Selenium content was then determined with Solar 939 AA Spectrometer (Unicam, UK) using a hydride AAS technique.

Determination of GSH-Px activities in serum

The method of Paglia and Valentin (1967) was used for GSH-Px activity measurements in serum. The test kit RANSEL (Randox Laboratories, Ltd., United Kingdom) was employed for this purpose.

Meat quality indicators

Meat colour was measured 24 h after slaughter. The colour was determined objectively by a Konica Minolta spectrophotometer CM-2600d with 8-mm-diameter measurement area, using the D65 illuminant and the 10° standard observer. The instrument was calibrated against a white standard plate. The results were expressed in terms of lightness L*, redness a*, yellowness b*.

Samples were placed in a polyethylene bag, and cooked in water bath. Internal temperature of samples was maintained at 70 °C for 60 min. After being cooled to room temperature, samples were weighed. Cooking loss was expressed as percentage weight difference between uncooked and cooked samples.

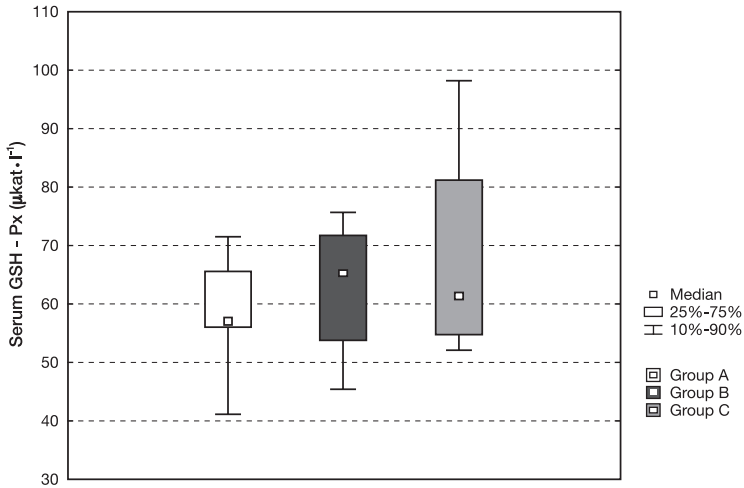
Drip loss percentage was determined as weight loss relative to 24 h.

The pH value was measured with a pH-meter 340i WTW (WTW, Germany). The needle probe SenTix Sp was used.

Lipid oxidation-thiobarbituric number (TBARS) was determined spectrophotometrically at 532 nm after distillation of the sample with vapour and reaction of distillate with 2-thiobarbituric acid in hot water bath (Castellini et al. 2002).

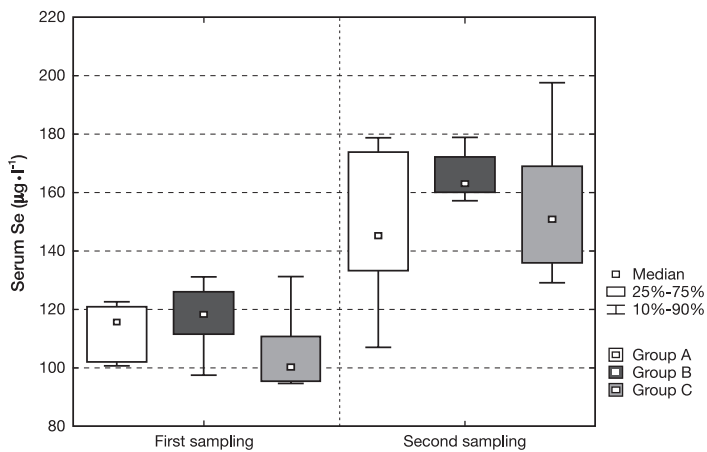
Results

The results of selenium concentrations in serum and of GSH-Px activities in serum are presented in Figs 1 and 2. No differences in serum selenium concentrations were found among the groups at the beginning of the trial. Before the slaughter, the two groups with organic form of selenium (Se-enriched yeast and Se-enriched *E. faecium*) did not differ



A - selenium enriched *Enterococcus faecium*, B - sodium selenite, C - selenium enriched yeast

Fig. 1. Serum GSH-Px concentration

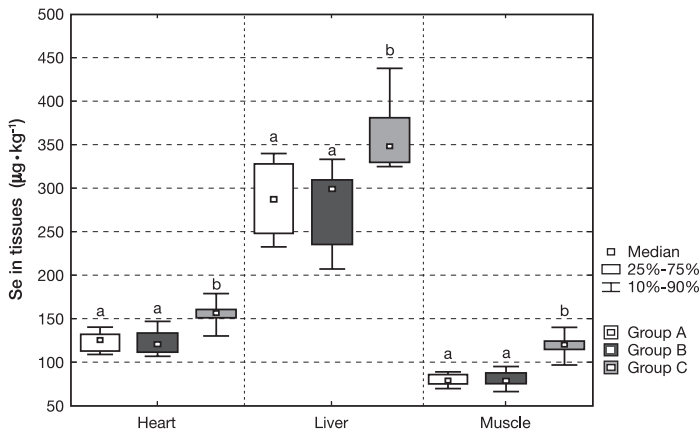


A - selenium enriched *Enterococcus faecium*, B - sodium selenite, C - selenium enriched yeast

Fig. 2. Serum Se concentration

in their serum Se concentrations and serum GSH-Px activities. The comparison between inorganic sodium selenite and two selenium in organic form groups did not reveal any significant difference either in Se serum concentrations or GSH-Px serum activities.

The group of pigs supplemented with inorganic sodium selenite had lower Se concentration in liver, heart and muscle tissues than the pigs fed with Se-enriched yeast. The use of Se-enriched *E. faecium* resulted in Se concentrations in liver, heart and muscle comparable to inorganic sodium selenite group. However they were significantly lower ($p \leq 0.05$) than in Se-enriched yeast supplemented group. In ovaries, the Se concentrations in pigs fed with inorganic sodium selenite were comparable to the both organic Se groups. The ovarian Se concentrations of *E. faecium* supplemented group were comparable to Se-enriched yeast group. The results are presented in Fig. 3.



A - selenium enriched *Enterococcus faecium*, B - sodium selenite, C - selenium enriched yeast

Fig. 3. Se concentration in tissues

Major carcass measurements and muscle characteristic are presented in Table 3. We did not find any differences among the tested groups.

Discussion

E. faecium is a nonpathogenic bacteria which can colonize both the human and swine intestine and exert beneficial probiotic effects (Mego et al. 2005). Moreover it positively influences both local and systemic immunity. It has been documented that *E. faecium* stimulates phagocytosis of peripheral neutrophils and production of antibodies and intestinal IgA secretion (Mikeš et al. 1995). The combination with selenium enhances immunostimulatory properties of the *E. faecium* preparations (Ferenčík et al. 2000). *E. faecium* enriched with organic selenium has been used for example for the elimination of pathogenic bacteria of inflammatory bowel disease and colon cancer (Mego et al. 2005) and for the treatment of experimental adjuvant arthritis (Rovenský et al. 2002).

It has been demonstrated that *E. faecium* can metabolize inorganic selenite and incorporate selenium in an organic form (Tilton et al. 1967). The form of selenium present in the Se-enriched *E. faecium* bacteria has not yet been characterized. There are so far no analytic methods available that can fully characterized the selenium kinds and their distribution in bacteria. As the utilization of Se by animals is influenced by the composition of seleno-amino acid analogues, the feeding experiments in animals can bring important information.

Table 3. Major carcass and muscle characteristics in pigs

	Se-enriched <i>E. faecium</i> (A)			Sodium selenite (B)			Se-enriched yeast (C)		
	median	10% quantile	90% quantile	median	10% quantile	90% quantile	median	10% quantile	90% quantile
pH 24	5.49	5.34	6.24	5.49	5.37	5.91	5.63	5.43	6.11
Cooking loss (%)	32.58	26.70	37.04	31.63	28.53	34.57	30.05	27.05	32.85
Drip loss (%)	2.45	1.17	3.60	2.53	1.64	3.58	1.91	0.94	2.50
Dry matter (%)	24.80	23.47	25.49	25.05	23.81	25.46	25.24	24.57	26.14
Collagen (%)	0.61	0.45	0.83	0.53	0.38	0.64	0.50	0.39	0.81
Fat (%)	1.80	0.62	3.00	1.57	0.98	2.81	1.98	0.91	2.96
TBARS (meat) - value (mg·kg ⁻¹)	2.79	1.40	3.07	2.33	1.40	3.09	2.76	0.95	3.32
TBARS (fat) - value (mg·kg ⁻¹)	2.44	1.86	2.73	2.25	1.33	3.57	2.34	1.55	3.07
Pure protein (%)	20.28	17.05	21.97	20.37	19.50	22.20	20.80	19.92	21.58
Non collagen muscle protein (%)	19.71	16.22	21.36	19.76	18.90	21.81	20.26	19.22	21.07
Tenderness (N)	66.66	51.11	89.41	60.01	37.83	89.67	57.28	40.52	70.65
Cohesiveness (-)	1.29	1.28	1.31	1.29	1.27	1.30	1.29	1.24	1.31
L*24 (lightness)	50.47	45.47	55.30	49.09	45.09	55.98	48.75	46.61	52.87
a*24 (redness)	5.07	2.86	5.93	5.11	2.96	7.28	3.32	2.57	6.27
b*24 (yellowness)	11.78	9.48	14.18	11.65	9.13	13.29	11.36	9.35	12.67

produces selenium accumulation in muscle tissue comparable to inorganic sodium selenite and it does not influence meat quality traits.

Využití selenem obohacené bakterie *Enterococcus faecium* jako alternativního zdroje pro výkrmová prasata

Cílem experimentu bylo zhodnotit vliv krmení selenem obohacených bakterií *Enterococcus faecium* na koncentrace selenu ve svalovině a na ukazatele kvality masa. Byly použity

Selenomethionine is incorporated non-specifically into body proteins in place of methionine (McConnel and Hoffman 1972). Contrary to selenomethionine selenocystein is not incorporated non-specifically to proteins and it follows the same metabolic pathway as selenite (Schrauzer 2000). The use of Se-enriched *E. faecium* resulted in the Se tissue accumulation lower than in Se-enriched yeast group and in the accumulation comparable to sodium selenite group. Therefore it can be suggested that selenomethionine in the *E. faecium* represents smaller part and the selenocystein higher part of seleno-amino acid analogs.

No differences in GSH-Px serum activities were found among the groups supplemented with different form of selenium. This indicates that both inorganic and organic Se forms are an adequate source for GSH-Px synthesis.

No effects of different Se sources on meat-quality traits were found in our study. This is consistent with the findings of Wolter et al. (1999) and our previous studies (Svoboda et al. 2009a; Svoboda et al. 2009b) and contradictory to Mahan et al. (1999) who reported a negative effect of selenite on drip loss and meat color.

It can be concluded that the Se-enriched *E. faecium* represents an alternative selenium source for pigs. It

skupiny prasat, jejichž krmivo bylo suplementováno selenem obohacenými baktériemi *E. faecium* (n = 12), seleničitanem sodným (n = 12), selenem obohacenými kvasnicemi (n = 12). Testované krmné směsi byly krmeny po dobu 3 měsíců před odvezením prasat na jatky. Selen byl přidáván do krmiva v dávce 0,3 mg·kg⁻¹. Koncentrace selenu ve svalovině prasat po krmení selenem obohacené baktérie *E. faecium*, byly srovnatelné se seleničitanem sodným a byly nižší ($p \leq 0,05$) ve srovnání se selenem obohacenými kvasnicemi. Koncentrace selenu a aktivita GSH-Px v krevním séru jednotlivých skupin prasat byly srovnatelné. Mezi skupinami prasat nebyly zjištěny žádné rozdíly v kvalitě svaloviny. Selenem obohacené baktérie *E. faecium* lze doporučit jako alternativní zdroj selenu pro prasata ve výkrmu se srovnatelnou účinností jako při použití seleničitanu sodného.

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