AGRICULTURAL AND FOOD SCIENCE IN FINLAND

Vol. 11 (2002): 175–184.

Influence of inorganic Se + vitamin E and organic Se + vitamin E on reproductive performance of young boars

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The objective of the study was to compare the influence of the organic Se (Se-Yeast) + vitamin E and inorganic Se (Na_2SeO_3) + vitamin E on reproductive performance of young boars. The studies were carried on the 80 young boars. A feeding test was started on day 70 of their life. Inorganic Se group males received 0.2 mg inorganic Se + 30 mg vitamin E, those of organic Se group 0.2 mg organic Se + 60 mg vitamin E in 1 kg of the diet ration. The experiment was performed in two seasons: I – June–September, II – January–April. On day 180 of life (close of the test), the boars were subjected to live evaluation: testes volume, libido level, semen characteristics as well as Se content and glutathione peroxidase (GSH-Px) activity in seminal plasma and blood serum. The boars of group inorganic Se obtained higher ($P \leq 0.01$) selection index of live evaluation than those of the group organic Se. The boars of group organic Se were characterized by better libido level, higher ($P \leq 0.05$) concentration and total number of spermatozoa in an ejaculation, lower ($P \leq 0.01$) percentage of semen with morphological changes and higher ($P \leq 0.01$) value of osmotic resistance test of acrosome membranes in spite of the fact that GSH-Px activity in seminal plasma was lower when compared with those of the group inorganic Se. The findings show that organic Se + vitamin E has been of a more favourable influence on reproductive performance of young boars than that of inorganic Se + vitamin E. More favourable influence of organic Se + vitamin E was observed in winter-spring season than in summer.

Key words: selenium, vitamin E, boars, reproductive performance

Manuscript received January 2002

Introduction

Selenium is an integral component of the enzyme glutathione peroxidase (GSH-Px), which detoxifies lipid peroxides and provides protection of cellular and subcellular membranes against peroxide damage. Thus, the mutual sparing effect of selenium and vitamin E stems from their shared antiperoxidant roles. GSH-Px is present both in a number of tissues and in the body fluids. Its high activity has been observed both in the organs of the reproductive system and in the fluids secreted thereby (Saaranen et al. 1989).

Besides many other functions, selenium and vitamin E are of great importance in reproductive processes. Female hyposelenosis is accompanied by retained placenta, disturbances in uterus involution and in ovarian functions, ovarian cysts, decreased fertilization rates, abortions, stillbirths, reduced litter size and decreased piglet survivability, inflammatory states of the uterus and Mastitis Metritis Agalactia syndrome in sows (Segerson and Ganapathy 1980, Segerson et al. 1981a, Chavez and Patton 1986, Wandurski 1990, Dembiński et al. 1992).

Selenium also regulates male reproductive processes. High concentration of Se in testes and epididymides of the boars implies that this element is essential for the process of production and maturation of spermatozoa (Heimann et al. 1984, Saaranen et al. 1989, Marin-Guzman et al. 1997). Selenium is a component of mitochondrial capsule selenoprotein, which maintains the stability of spermatozoa mitochondria (Calvin et al. 1981, Kleene 1993, Marin-Guzman et al. 1997). Studies carried out on males belonging to various species of the farm animals have shown the positive influence of selenium on semen quality, especially on the concentration, vitality, mobility and morphological defects of spermatozoa (Siegel et al. 1980, Pratt et al. 1980, Liu et al. 1982, Udała et al. 1995, Marin-Guzman et al. 1997, 2000).

On the contrary, other studies demonstrated that an addition of selenium to diet did not improve the semen quality of rams (Buchanan-Smith et al. 1969), bulls (Bartle et al. 1980, Segerson and Johnson 1980) or boars (Segerson et al. 1981b, Henson et al. 1983). Probably, selenium was not deficient in the animals in those studies and therefore no influence was observed of the element on the reproductive processes of the males. This suggestion has a confirmation in the studies by Heimann et al. (1984).

Bioavailability of selenium depends on the species of animals, the source, level and on which chemical form of the element is present in the diet. In monogastric animals, organic Se (seleno-amino acids) is more effectively retained than inorganic Se (sodium selenite – Na_2SeO_3 and sodium selenate – Na_2SeO_4) that is commonly used in animal diets. Mahan and Parrett (1996) found that Se retention in the body of pigs receiving 0.3 ppm organic Se was by 85% higher than that in pigs receiving 0.3 ppm inorganic Se. The results obtained by the authors show that the amount of Se retained in the body of pigs receiving 0.3 ppm inorganic Se was equivalent to that at 0.13 ppm organic Se in the diet. Organic Se, deposited in the muscular tissue, may be launched as the need arises. According to Pehrson (1994), when supplementing the feeding rations with organic Se one should take into account the higher level of vitamin E, which is synergically active with that element.

The studies on biological activity of organic versus inorganic form of selenium did not bring clear results. There is also no assessment, as to which chemical form of selenium is more efficient in the feeding of males. The aim of the present paper is to compare the influence of the organic Se (Se-Yeast) + vitamin E and inorganic Se (Na_2SeO_3) + vitamin E, utilised in rearing young boars, on the their reproductive performance.

Material and methods

The studies were carried out at the State Center of Pig Hybridization in Poland on the 80 young boars of the 990 synthetic line. A feeding test

Vol. 11 (2002): 175–184.

Table 1. Formulation of diets.

	Control (Inorganic Se)	Experimental (Organic Se + vitamin E)
Ingredients, g kg^{-1}		
Wheat	450.0	450.0
Barley	100.0	100.0
Triticale	200.0	200.0
Wheat bran	8.0	7.74
Soybean oilmeal	180.0	180.0
Fish meal	20.0	20.0
Dicalcium phosphate	11.0	11.0
Limestone	12.0	12.0
Sodium chloride	3.0	3.0
Lysine	2.0	2.0
Methionine	0.5	0.5
Threonine	0.5	0.5
Premix (of inorganic Se)[1]	10.0	–
Premix (without Se)[2]	–	10.0
Seleno-Yeast[3]	–	0.2
Vitamin E 50[4]	–	0.06
Binder pellets	3.0	3.0

[1] The premix supplied the following per kg diet:
7700 IU vit. A, 2100 IU vit. D_3, 30 mg vit. E, 1.5 mg vit. K_3, 1.05 mg vit. B_1, 3.6 mg vit. B_2, 2.1 mg vit. B_6, 0.021 mg vit. B_{12}, 15 mg nicotynic acid, 1.05 mg calcium pantothenate, 0.45 mg folic acid, 0.021 mg biotin, 300 mg cholin chloride, 100.5 mg Zn, 30 mg Mn, 21 mg Cu, 75 mg Fe, 0.6 mg J_2, 0.204 mg Se (Na_2SeO_3).

[2] Premix has no selenium, the other components as above.

[3] Se-Yeast (0.1% Se in forms of SeMet and Se Cys) provided 0.2 mg Se-organic / kg diet.

[4] Provided 30 mg vitamin E / kg diet.

was carried out from 70 to 180 days of age in two seasons: I June – September and II January – April.

The boars were fed on the same diet until 70 days of age. At 70 days, the boars were distributed into two groups (inorganic Se and organic Se). In each group there were 20 boars. The allocation to the groups (in each season) was made with analogues method, i.e. from one litter one boar was assigned to a group. During the feeding test the boars of the inorganic Se group were fed on standard diet, which contained 0.2 mg inorganic Se (Na_2SeO_3) and 30 mg vitamin E per 1 kg. The organic Se group received 0.2 mg organic Se (Se-Yeast) and 60 mg vitamin E per 1 kg of the ration. Considering almost twice as large retention of organic Se in comparison with that of inorganic Se in pig body (Mahan and Parrett 1996), a double level of vitamine E in experimental diet (organic Se + vitamin E) than in the control (inorganic Se) one. The feeds in granulate form were prepared for each season of the study. The composition of the diets in both seasons was identical (Table 1). Chemical composition of the diets, which were applied in individual seasons of the experiment, was very similar, and the average values are given in Table 2.

The boars were housed in individual pens measuring 1×2 m, flooring was combination of solid concrete and slats (approximately 60:40). Water was provided for *ad libitum* and standardized feeding was applied. The daily feed ration was gradually increased along with the increasing body weight (from 1 kg per day at 70 days to 3.5 kg / day at 180 days of age and during the semen collection). The body weight

Table 2. Mean chemical composition of diets.

	Control (Inorganic Se)	Experimantal (Organic Se + vitamin E)
In 1 kg diet:		
Metabolizable energy[1], MJ	13.0	12.9
Dry matter, g	879	882
Crude ash, g	49.9	53.5
Crude protein, g	191	190
Ether extract, g	18.8	18.6
Crude fibre, g	27.0	27.1
N-free extractives, g	592	593
Lysine, g	10.1	10.1
Methionine, g	3.6	3.7
Methionine + cystine, g	6.4	6.3
Threonine, g	6.7	6.9
Calcium[2], g	8.7	8.7
Total phosphorus[3], g	6.7	6.6
Vitamin E, mg	30.0	60
Additive, mg/kg		
– inorganic Se	0.204	–
– organic Se		0.200

[1, 2, 3] Calculated from Polish Norm of Pigs Nutrition (1993)

of the boars, their daily gains, and feed conversion for the test period were determined at the age of 70 and 180 days.

Appraisal of the boars

At age of 70 and 180 days (the beginning and the end of the test) the testes were measured and their volume was established (Young et al. 1986). At 180 days of age, blood from jugular vein was collected and, after centrifugation, the serum was frozen at –20ºC. Selenium content and GSH-Px activity were determined in the serum. The selection index considering daily gain till 180 days of age was estimated for the boars, and the percentage carcass meatiness was ultrasonically probed at 180 day of age. Upon completion of the test (from 180 days of age on) the boars were trained to mount the phantom, and the collection of semen began, which was carried out with gloved hand technique. During the semen collection, the animal sexual activity was evaluated using the number of leaps and the time elapsed to effective mounting, as well as the time of ejaculation.

Evaluation of the semen

Immediately after the collection and filtration of ejaculate, its following characteristics were determined: ejaculate volume, percentage of motile spermatozoa, concentration of spermatozoa in 1 cm^3 (cytometric method in Bürker's chamber), and total number of spermatozoa in ejaculate. The minor and major morphological changes of the semen (according to Blom 1981) and the grade of acrosome defects (according to Pursel et al. 1972) were determined in the preparations. The osmotic resistance test (ORT) of acrosomal membranes was performed according to Schilling and Vengust (1987). The ORT was calculated according to the formula:

ORT = 1/2 [%NAR in 300 mOsm
(for 15 minutes)] + %NAR in 150 mOsm
(for 120 minutes)

Vol. 11 (2002): 175–184.

where: NAR = normal acrosome ridge.

In seminal plasma obtained by centrifugation of the fluid fraction of the semen, the content of selenium as well as the activity of GSH-Px and aspartate aminotransferase (AspAT) were determined. Prior to the analyses, the plasma was stored at –20˚C.

Chemical analyses

The basic nutrient concentrations in the feeds were determined with standard methods (AOAC 1990), and amino acids were measured using an automatic analyser (Beckman Instruments Inc.). The selenium concentrations in the premix, Se-Yeast, blood serum, and seminal plasma were determined with the fluorometric method according to Watkinson (1966).Vitamin E content was measured with the HPLC method, the activity of AspAT with the kinetic method, and GSH-Px activity according to Paglia and Valentine (1967). AspAT activity was converted as per $1 \cdot 10^9$ of spermatozoa.

The data were statistically analysed using STATISTICA PL software, by means of two-way analysis of variance, which included the effect of the feeding group, the season of the test, and the interactions between these factors.

Results and discussion

The results presented in Table 3 indicate that the boars receiving inorganic Se + vitamin E in diet (inorganic Se group) attained by 30 g higher ($P \leq 0.01$) average daily weight gain, and used by 0.1 kg less ($P \leq 0.05$) food per 1 kg of weight gain compared to the boars that received the organic Se + vitamin E in the diet (organic Se group). As a consequence, the boars of the group inorganic Se reached by 4.8 kg higher ($P \leq 0.01$) body weight at 180 days of age than the boars of the group organic Se. The selection index for the desired gains in this group was higher as well ($P \leq 0.01$).

Investigating the influence of the season on the obtained results, one has to ascertain that in the test performed in season II (January – April), body weight gain was better ($P \leq 0.01$), food conversion to 1 kg of body weight gain was more efficient ($P \leq 0.01$), and the selection index of the boars was higher ($P \leq 0.01$) compared to those in season I (June – September). The differences in favour of the control group and of the season II of the test are a consequence of the definitely best results obtained by the boars of group inorganic Se in season II of the test. The feeding group × season of the test interaction

Table 3. Growth rate, feed conversion, meatiness and selection index of young boars.

Season test, months	I June – September		II January – April		SEM	Differences		Interaction group × season
Groups	Inorganic Se	Organic Se	Inorganic Se	Organic Se		Organic Se – Inorganic Se	I–II	
Number of animals	20	20	20	20				
Body weight, kg								
– at 70th day of life	21.3	20.8	22.5	20.6	0.33	–1.2	–0.4	NS
– at 180th day of life	108	107	120	110	0.81	–4.8**	–7.8**	**
Daily gain (70–180 day of life), g	734	736	836	768	6.09	–30**	–66**	**
Feed conversion, kg/kg	2.99	3.05	2.76	2.91	0.024	0.11*	0.21**	**
Meatiness, %	59.3	58.3	58.1	58.5	0.21	–0.2	0.6	NS
Selection index, pts	120	117	127	121	1.1	–4.3**	–4.9**	**

Statistical significance: NS non – significant, * $P \leq 0.05$, ** $P \leq 0.01$
SEM – Standard error of means

Table 4. Testes volume, sexual activity, semen traits, selenium (Se) content and activity of glutathione peroxidase in the semen plasma and blood plasma of young boars.

Season test, months	I June – September		II January – April		SEM	Differences		Interaction group × season
Groups	Inorganic Se	Organic Se	Inorganic Se	Organic Se		Organic Se – Inorganic Se	I–II	
Number of animals	20	20	20	20				
Volume of both testes, cm^3								
– at 70th day of life	17.8	17.3	16.5	12.7	0.78	–2.2	3.0	NS
– at 180th day of life	577	595	564	594	16.8	24.5	8.3	NS
Time mounting upon phantom, s	331	372	281	170	19.0	–39.2	128**	**
Number of mounts	1.3	1.5	1.2	1.4	0.07	0.16	0.06	NS
Time of ejaculation, s	155	162	205	229	4.9	16.8	–58.7**	NS
Ejaculate volume, cm^3	104	103	105	109	1.0	1.1	–3.3	NS
Motile spermatozoa, %	71.9	74.0	72.1	73.3	0.61	1.7	0.2	NS
Concentration of spermatozoa, $n \times 10^6/cm^3$	175	202	191	246	12.4	41.7*	–31.2	NS
Total number of spermatozoa, $n \times 10^9$	18.1	20.6	19.8	26.8	1.29	4.8*	–4.2	*
Spermatozoa with major defects, %	16.1	13.1	28.2	10.0	1.79	–10.4**	–4.0*	**
Spermatozoa with minor defects, %	14.0	9.1	18.6	13.4	1.16	–4.9**	–4.2*	*
Spermatozoa with normal acrosome, %	71.7	74.8	85.7	85.0	1.47	1.4	–12.2**	**
Osmotic resistance test-ORT, %	58.9	70.7	63.4	77.3	2.20	12.8**	–5.5	**
AspAT, $U/10^9$ spermatozoa	0.112	0.068	0.157	0.083	0.010	–0.053*	–0.029	*
Se in the semen plasma, µg/mL	0.036	0.035	0.056	0.055	0.002	0.001	–0.002**	**
GSH-Px in the semen plasma, U/mL	0.605	0.490	0.457	0.368	0.061	–0.095*	0.132*	*
Se in the blood plasma, µg/mL	0.345	0.359	0.383	0.371	0.007	0.006	–0.027*	NS
GSH-Px in the blood plasma U/mL	8.98	10.10	6.52	6.49	0.404	0.700	3.02**	**

Statistical significance: NS non – significant, * $P \leq 0.05$, ** $P \leq 0.01$
SEM – Standard error of means

($P \leq 0.01$) was found for all the above discussed traits.

Suomi and Alaviuhkola (1992) and Mahan and Parrett (1996) did not observe any significant differences in body weight gain and feed conversion to 1 kg of weight gain of the growing pigs that received of either organic or inorganic Se in diet.

The values presented in Table 4, as well as their great variability, are characteristic for young (6–7 month-old) boars. The form of selenium in the diet, as well as the season of the feeding test, did not have a statistically significant influence on the volume of the boar testes at 180 days of age. The positive relationship between testis size and daily gain of the boars, which had been reported by other authors (Young et al. 1986), was not confirmed in this study.

A shorter (by 39 sec) time to effective phantom mounting and longer (by 17 sec) time of ejaculation of the boars received organic Se + vitamin E in the diet is an indication of their slightly higher level of libido in comparison with those receiving inorganic Se + vitamin E. The values of libido scores in individual seasons of the experiment demonstrate significantly higher ($P \leq 0.01$) sexual activity of the boars in season II (January – April). Organic Se + vitamin E had stronger influence on libido compared to inorganic Se + vitamin E during the same sea-

Vol. 11 (2002): 175–184.

son. The interaction: group × season of test was significant ($P \leq 0.01$) for the time of effective mounting upon the phantom. Sexual activity, as it is known, is characterized by a strong individual variability and, therefore, the results cannot be treated univocally. Also in this study the variability of the libido scores was high.

Organic Se, in comparison with inorganic Se, exerted a more stimulating influence on semen characteristics (Table 4). Liu et al. (1982) and Marin-Guzman et al. (1997) found that a deficit of selenium resulted in decreased motility of spermatozoa. The reason of that are disturbances in spermatozoa mitochondrial sphere of the connecting piece, which are a consequence of limited energy production indispensable for performing progressive motility (Pratt et al. 1980, Marin-Guzman et al. 2000 a). In the present study, the percentage of the spermatozoa showing progressive motility was a little higher (by 1.7%) in the ejaculates of the boars receiving organic Se + vitamin E in diet compared to those receiving inorganic Se + vitamin E. However, the influence of organic Se + vitamin E on the efficiency of seminiferous epithelium of the boar testes was stronger in comparison with inorganic Se + vitamin E. The differences in the concentration and total number of spermatozoa in an ejaculate were in favour of the boars receiving organic Se. These differences were statistically significant ($P \leq 0.05$) and amounted to, respectively: $41.7 \times 10^6/cm^3$ (23%) and 4.8×10^9 (25%).

Organic Se + vitamin E also more effectively prevented the spermatozoa both from changes in the morphological structure and from defects of acrosome compared to inorganic Se + vitamin E. In the ejaculates of the boars receiving organic Se + vitamin E, significantly ($P \leq 0.01$) lower percentage was found of the spermatozoa with either major morphological defects (by 10.4%) or minor defects (by 4.9%) than for the boars fed on the diet with inorganic Se + vitamin E. The number of spermatozoa without defects of acrosome was also slightly higher (by 1.4%) in the group with organic Se + vitamin E.

Marin-Guzman et al. (1997, 2000) found a significant positive influence of inorganic Se (0.5 ppm) on spermatozoa motility, concentration, morphology and fertilization rates, whereas vitamin E (220 IU/kg diet) had a little effect on those traits in boars. Mahan et al. (2000) did not note either any effect of vitamin E (30 and 60 IU/kg diet) on many reproductive characteristics in sows.

Thus, one should suppose that higher values of those characteristics of the semen obtained in the present study in the experimental group organic Se are mainly the result of more favourable influence of organic Se in comparison with that of inorganic Se, but not the larger amount of vitamin E in the diet. High levels of vitamin E do not completely eliminate the need for selenium (NRC 1998). In study with rats has confirmed that vitamin E and selenium act synergistically (Levander et al. 1995).

The evaluation of spermatozoa acrosomes state in different osmotic pressures was also more favourable for organic Se + vitamin E. The ORT values of the ejaculates of the boars from group organic Se were significantly ($P \leq 0.01$) higher than in the boars of group inorganic Se. In the studies by other authors, a strict positive relationship was demonstrated between ORT value and semen quality of the boars (Schilling and Vengust 1987).

In order to determine the extent of damage to spermatozoa cell membrane, the activity of AspAT was assayed. As a consequence of such defects, AspAT leaks from the spermatozoa to the plasma. Higher ($P \leq 0.05$) AspAT activity was found in the seminal plasma of the boars receiving inorganic Se, which indicates that organic Se + vitamin E prevents the defects of spermatozoa cell membranes more effectively.

The analysis of the results by the season indicates that the concentration and total number of spermatozoa and the ORT value were higher, though statistically insignificantly, in season II (January – April) in comparison with the season I (June – September). A significant difference ($P \leq 0.01$), in favour of season II, appeared for the percentage of spermatozoa with normal acrosome. The significant interaction between the feeding group and the season of test was found

for the majority of the characteristics.

It has to be stressed, however, that the boars in the test during January – April had by 4.0% higher ($P \leq 0.05$) number of the spermatozoa with morphological defects and by 4.2% ($P \leq 0.05$) with minor defects compared to the boars in the test during season I (June – September). AspAT activity in season II of the test was also higher, which suggested a higher number of spermatozoa with cell membrane defects. The observed differences were a consequence of a considerably higher number of spermatozoa with morphological defects and a higher AspAT activity in season II in the ejaculate of the boars receiving inorganic Se in the diet. The interaction: feeding group × season of test was statistically significant.

The results obtained in this study found their confirmation in the studies by Glogowski et al. (1997). The authors reported that the concentration and total number of spermatozoa in boar ejaculates in spring were statistically significantly higher than those in summer or autumn. However, ejaculate volume and percentage of spermatozoa with progressive motility did not undergo seasonal changes. The results presented in this study suggest that the efficiency of organic Se + vitamin E is better in the winter–spring seasons than in the summer season.

The concentration of the selenium and GSH-Px activity in blood serum did not depend on the source of selenium in the diet of the boars. The differences, in favour of the boars receiving organic Se + vitamin E, were statistically non-significant. The amounts of selenium in seminal plasma of the boars receiving either organic Se or inorganic Se were very similar. However, GSH-Px activity in seminal plasma of the boars receiving inorganic Se + vitamin E was significantly ($P \leq 0.05$) higher, in spite of the fact that quality semen was worser. It has been assumed until quite lately that the whole biological activity of Se appears through GSH-Px. Discovery of new selenoproteins showed that greater part of Se is to be found in other proteins and some of them have, probably, an effect on animal reproduction.

In the studies by other authors, higher GSH-Px activity was reported in blood serum of the sows (Mahan 2000) and grower and finisher pigs (Mahan and Parrett 1996) if inorganic Se rather than organic Se was supplemented. Marin-Guzman et al. (1997) did not found the effect of vitamin E (220 IU/kg diet) on the amount of Se and the activity of GSH-Px in blood serum and seminal plasma in boars.

An influence of year season on Se concentration and GSH-Px activity was found. The boars subjected to the test in January – April (season II) demonstrated a higher concentration of selenium in both blood serum ($P \leq 0.05$) and seminal plasma ($P \leq 0.01$) than the boars tested in season I (June – September). However, the GSH-Px activity was lower in the season II of the test in blood serum ($P \leq 0.01$), as well as in seminal plasma ($P \leq 0.05$).

Conclusion

Inorganic selenium + vitamin E in the diet had a stronger influence on the growth rate and feed conversion to 1 kg of weight gain in growing boars than organic Se + vitamin E. On the other hand, organic Se had a more desirable influence on the reproductive performance of the young boars. The animals that received organic Se + vitamin E in diet were characterized by higher level of sexual activity and better semen quality, in spite of the fact that GSH-Px activity was significantly higher in the seminal plasma of the boars receiving inorganic Se + vitamin E in diet. Organic Se, if supplemented during the winter–spring season, had a considerably more positive influence on semen quality than in the summer season.

Acknowledgements. The study was conduced by grant no. 5 PO6E 01618 from State Commitetee for Scientific Research in Poland.

Vol. 11 (2002): 175–184.

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