Journal of Animal and Veterinary Advances 11 (5): 603-608, 2012
ISSN: 1680-5593

Blood and Semen Selenium Concentrations and Semen Quality in Boars Fed Diets Supplemented with Organic or Inorganic Selenium

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Abstract: Effect of dietary supplementation of organic or inorganic selenium on blood and semen selenium concentrations and semen quality was determined in 10 boars. During the 4 weeks of pre-experimental period, all boars were fed a basal diet containing 0.15 mg kg^{-1} of inorganic selenium. Thereafter, all cows were randomly allocated into 2 groups of five boars which were fed a basal diet supplemented with either 0.3 mg kg^{-1} of inorganic selenium or 0.3 mg kg^{-1} of organic selenium for 84 days. Blood samples were collected from all boars to determine selenium concentrations at the end of pre-experimental period and at days 49 and 84 after supplementation. Semen samples were collected at the end of pre-experimental period and at days 35, 49, 63 and 84 to determine selenium concentrations and semen evaluation. For both inorganic and organic selenium groups, blood selenium concentrations at days 49 and 84 were higher than the concentration at day 0 and the concentrations did not differ between the two groups at all sampling periods. Semen selenium concentrations at days 35, 49, 63 and 84 were higher than the concentration at day 0 for both inorganic and organic selenium groups and the concentrations did not differ between the 2 groups at days 35, 49, 63 and 84. Sperm motility parameters including motility (%), progressive motility (%), Average Path velocity (VAP, μm sec^{-1}), Straight-line velocity (VSL, μm sec^{-1}) and Curvilinear velocity (VCL, μm sec^{-1}) did not differ between the 2 groups and among sampling periods. Results revealed that 0.3 mg kg^{-1} supplementation of either inorganic or organic selenium form in the basal diet containing 0.15 mg of selenium per kg could increase blood and semen selenium levels in the boars. With normally-fertile boars, both inorganic and organic form of selenium supplemented in the diet had similar effect on sperm motility characteristics in the boars.

Key words: Boar, computer assisted semen analysis, selenium, semen, sperm, Thailand

INTRODUCTION

Selenium, a trace mineral plays a crucial role as an antioxidant by incorporating into a molecule of Glutathione Peroxidase (GSH-Px), an enzyme that helps protect the cells against free radicals (Hansen and Deguchi, 1996). In boars, supplementation of selenium increases GSH-Px activities and selenium concentrations in semen, blood and other organs such as kidney, liver, heart and muscle, etc. (Marin-Guzman *et al*., 1997). Several studies have been conducted to evaluate the effect of selenium supplementation on reproductive characteristics in breeding boars. Injection of selenium increased number of spermatozoa per ejaculate but did not have any effects on percentages of viable or morphologically normal spermatozoa (Segerson *et al*., 1981). Both dietary selenium and vitamin E enhanced boar semen quality but the positive effects of selenium supplementation on semen characteristics were more pronounced than were the effects of vitamin E supplementation (Marin-Guzman *et al*., 1997, 2000a, b). The concentration of spermatozoa and total spermatozoa were higher in boars fed the diet containing the higher concentration of selenium and vitamin E (Kolodziej and Jacyno, 2005). Selenium may have several roles in development of spermatozoal midpiece, development of sertoli cell and component of sperm glutathione perioxidase (Marin-Guzman *et al*., 2000a).

In animal feed, selenium is presented in either inorganic or organic form. Inorganic form such as sodium selenite is widely used due to its relatively lower cost than organic form such as selenomethionine. Selenium

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concentraions in seminal plasma of boars supplemented with either organic selenium or inorganic selenium were similar however, glutathionine peroxidase activity was higher in boars fed a diet containing inorganic selenium (Jacyno *et al*., 2002). Oragnic selenium is more effectively retained than inorganic selenium (Mahan and Parrett, 1996). The concentration of spermatozoa and total spermatozoa were higher in boars fed the diet containing the organic source of selenium (Jacyno *et al*., 2002). The boars fed the diet containing organic selenium had lower percentages of spermatozoa with minor or major morphological abnormalities (Jacyno *et al*., 2002). Although, supplementation of diets with an organic source of selenium is superior to supplementation with inorganic selenium, effects of selenium in this study were confounded with concentration of vitamin E. The study therefore, aimed at investigating blood and semen selenium concentrations and semen quality in boars fed diets supplemented with either inorganic or organic selenium.

MATERIALS AND METHODS

Boars and dietary treatment: The experiment was conducted in a commercial pig farm including grandparent and parent stock and about 2,200 sows for producing piglets. The resaerchers selected 10 mature boars aged 2-4 years. The boars were kept in individual pens and were offered basal diet at a rate of 2 kg day^{-1} for 4 weeks. As fed basis, the basal diet contained 450 g kg^{-1} of broken-milled rice, 130 g kg^{-1} of rice bran, 100 g kg^{-1} of wheat bran, 125 g kg^{-1} of soybean meal (44% CP), 100 g kg^{-1} of full-fat soybean, 40 g kg^{-1} of fish meal (58% CP), 25 g kg^{-1} of lard, 0.8 g kg^{-1} of L-lysine, 0.4 g kg^{-1} of D, L methionine, 0.5 g kg^{-1} of L-treonine, 21 g kg^{-1} of dicalcium phosphate (18%), 5 g kg^{-1} of sodium salt and 2.5 g kg^{-1} of premixes. The premixes contained 8,000,000 IU kg^{-1} of vitamin A, 1,200,000 IU of vitamin D_3, 18 g kg^{-1} of vitamin E, 1.6 g kg^{-1} of vitamin K, 0.8 g kg^{-1} of vitamin B_1, 2.4 mg kg^{-1} of vitamin B_2, 0.8 g kg^{-1} of vitamin B_6, 8 mg kg^{-1} of vitamin B_{12}, 7.2 g kg^{-1} of calcium pantothenate, 10 g kg^{-1} of nicotinic acid, 0.4 g kg^{-1} of folic acid, 80 mg kg^{-1} of biotin, 80 g kg^{-1} of choline, 48 g kg^{-1} of iron, 40 g kg^{-1} of zinc, 24 g kg^{-1} of manganese, 0.24 g kg^{-1} of cobalt, 0.4 g kg^{-1} of iodine, 60 mg kg^{-1} of selenium. Thereafter, the boars were randomly allocated into 2 groups; 5 boars were fed a basal diet supplemented with 0.3 mg of inorganic selenium (sodium selenite) per kg and 5 boars with 0.3 mg of organic selenium (selenomethionine) per kg.

Table 1: Nutrient composition of the diet offered to the boars before the beginning of the experiment and from day 0-84

Items	Before the experiment (4 weeks)	Inorganic group (day 0-84)	Organic group (day 0-84)
Net energy (Mcal kg^{-1})	3,200.00	3,200.00	3,200.00
Protein (%)	18.16	18.16	18.16
Fat (%)	7.62	7.62	7.62
Fiber (%)	4.17	4.17	4.17
Calcium (%)	1.03	1.03	1.03
Total phosphorus (%)	1.00	1.00	1.00
Available phosphorus (%)	0.45	0.45	0.45
Sodium chloride (%)	0.50	0.50	0.50
Lysine (%)	1.01	1.01	1.01
Methionine (%)	0.36	0.36	0.36
Methionine+cystein (%)	0.61	0.61	0.61
Treonine (%)	0.71	0.71	0.71
Tryptophan (%)	0.22	0.22	0.22
Choline (%)	0.13	0.13	0.13
Sodium chloride (%)	0.76	0.76	0.76
Sodium selenite (mg Se kg^{-1})	0.15	0.45	0.15
Selenomethionine (mg Se kg^{-1})	-	-	0.30

Supplementation period was 84 days. Nutrient compositions of the experimental diets are shown in Table 1. All boars had free access to tab water.

Blood and semen samples: At least 2 mL of blood of all boars were collected from jugular vein before selenium supplementation (day 0) and at day 49 and 84 after supplementation. All blood samples were transported to the laboratory in an ice box. In the laboratory, each 0.75 mL of blood was placed in 2 digestive tubes (25×200 mm) and 10 mL of mixed acid (HNO_3:$HClO_4$, 4:1) were added to each tube. The tubes were kept at room temperature until determination of selenium concentration.

Semen of all boars was collected by the gloved-hand technique as described by Shipley before selenium supplementation (day 0) and at day 35, 49, 63 and 84. Immediately after collection, the ejaculates were measured for volume and sperm concentration and later were divided into 2 parts. The first part, 1 mL was subdivided into 2 subparts of 0.5 mL, each subpart was placed in a digestive tube and 10 mL of a mixed acid were added to each tube. The tubes were kept at room temperature until analysis. The fresh semen samples from the second part were diluted using a commercially available semen extender (Bio Pig: Megapor®, Pornchai Intertrade Ltd., Partnership, Ratchaburi, Thailand) and were transported to the laboratory for determination of sperm motility characteristics using a Computer Assisted Semen Analysis (CASA) system (IVOS model 12.0, Hamilton-Thorne Biosciences, Beverly, MA, USA).

Determination of selenium: Selenium concentrations in blood and semen samples were determined using spectrofluorometric method as described by Paimer and Thiex (1997). In brief, the samples predigested with mixed

acid ($3H_2NO_3$:$1HClO_4$) were digested in the block digester at 180°C. Thereafter, 0.1 M of Ethylene Diamine Tetraacetate (EDTA), 1% of 2,3-Diaminonaphthalene (DAN) and cyclohexane were used to extract selenium. The cyclohexane portion was harvested from each tube to measure selenium concentration using spectrofluorometer (Shimadzu RF-5301 PC, Shimadzu Corporation, Tokyo, Japan) at an excitation wavelength of 365 nm and an emission wavelength of 525 nm.

Determination of sperm motility characteristics: In the laboratory, different sperm motility characteristics including motility (%), progressive motility (%), Average Path velocity (VAP, μm sec^{-1}), Straight-Line velocity (VSL, μm sec^{-1}) and Curvilinear velocity (VCL, μm sec^{-1}) were measured with a CASA system (IVOS model 12.0, Hamilton-Throne Biosciences, Beverly, MA, USA). Prior to CASA analysis, extended semen samples were introduced and warmed at 37°C in an incubator for 20 min. Semen samples were then submitted to the CASA analysis in a random order and by only one person during the entire experiment. The software settlings were followed by the manufacturer for analysis of the boar sperm. The settings of CASA used for boar semen analysis are shown in Table 2.

Statistical analysis: Data were tested for normal distribution using Shapiro-Wilk W test (Petrie and Watson, 1999). Normally distributed data were subjected to ANOVA using type of selenium for supplementation as a fixed main effect and sampling period as a repeated measure (Petrie and Watson, 1999). Parameters between the two treatments were compared using student's t-test or when data were not normally distributed with the Mann-Whitney U-test (Petrie and Watson, 1999). Parameters within group were compared between sampling periods using Paired Student's t-test.

Table 2: Computer assisted sperm analyzer set up for boar semen analysis adapted from Sontakke *et al.* (2004)

Parameter setup	Value
Temperature	37.0°C
Frames per second	60 Hz
Number of frames	45
Minimum contrast	46
Minimum cell size	7 pixels
Progressive minimum VAP	45.0 μm sec^{-1}
Straightness (STR), threshold	45.0%
VAP cutoff	20.0 μm sec^{-1}
VSL cutoff	5.0 μm sec^{-1}
Cell size	7 pixels
Cell intensity	50
Static head size	0.65-4.90
Static head intensity	0.50-2.50
Static elongation	0-87
Magnification	1.89
Video frequency	60
Bright field	No
Image type	Phase contrast
LED illumination intensity	2160
IDENT illumination intensity	3812
Chamber depth	20.0 μm
Chamber position A	3.90 mm
Chamber position B	21.00 mm
Chamber type	Leja4 (20 μm)
Field selection mode	SELECT

RESULTS AND DISCUSSION

Selenium concentrations in blood and semen: Blood selenium concentrations are shown in Fig. 1. During the pre-experimental period (day 0) when all boars were fed a basal diet containing 0.15 mg of inorganic selenium per kg, blood selenium concentrations were 0.588 (±0.029) ppm. Blood selenium concentrations at day 84 after supplementation were 0.662 (±0.029) ppm for the boars supplemented with inorganic selenium and were 0.737 (±0.034) ppm for the boars supplemented with organic selenium. At 49 and 84 days after selenium supplementation, blood selenium concentrations were higher than the concentrations at pre-experimental period in both inorganic and organic groups. However, during each sampling period, blood selenium concentrations did not differ between boars supplemented with inorganic and those with organic selenium. This result confirmed that addition of selenium in the diet could increase blood selenium levels (Marin-Guzman *et al.*, 1997) and the increase was similar between inorganic and organic form of selenium (Jacyno *et al.*, 2002; Lopez *et al.*, 2010).

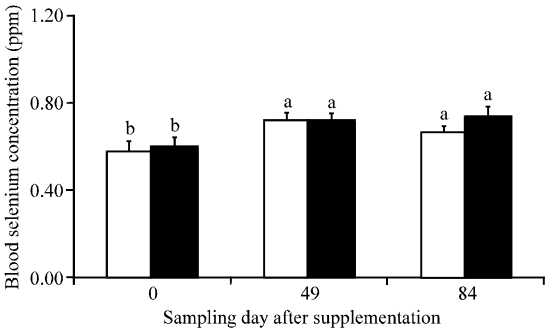

Fig. 1: Blood selenium concentrations (ppm) in boars fed a diet supplemented with 0.3 mg of inorganic selenium (□; n = 5) per kg and with 0.3 mg of organic selenium (■; n = 5) per kg, measured at 0, 49 and 84 days after selenium supplementation. Data represented mean and Standard Error (SE). Different letters demonstrated means significantly differed between groups and between sampling periods

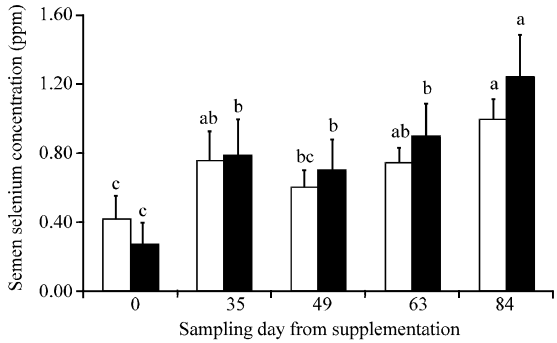

Fig. 2: Semen selenium concentrations (ppm) in boars fed a diet supplemented with 0.3 mg of inorganic selenium (□; n = 5) per kg and with 0.3 mg kg^{-1} of organic selenium (■; n = 5) per kg measured at 0, 35, 49, 63 and 84 days after selenium supplementation. Data represented mean and Standard Error (SE). Data represented mean and Standard Error (SE). Different letters demonstrated means significantly differed between groups and between sampling periods

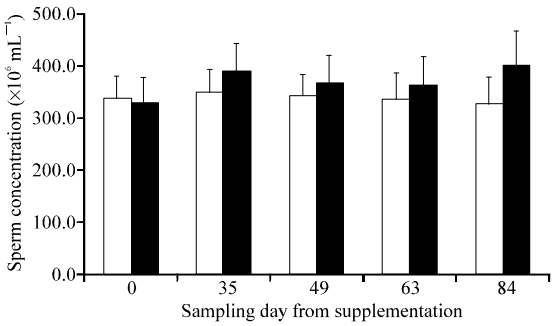

Fig. 3: Sperm concentrations (×10^6 mL^{-1}) in boars fed a diet supplemented with 0.3 ppm of inorganic selenium (□; n = 5) and with 0.3 ppm of organic selenium (■; n = 5) measured at 0, 35, 49, 63 and 84 days after selenium supplementation. Data represented mean and Standard Error (SE). Data represented mean and Standard Error (SE)

Marin-Guzman *et al*. (1997) also observed that growing boars fed a diet containing 0.5 ppm of inorganic selenium had higher serum glutathione peroxidase activity than did the boars fed a diet containing 0 ppm. Higher serum glutathione peroxidase activity was observed in grower and finisher pigs fed a diet supplemented with inorganic selenium as compared with organic selenium (Mahan and Parrett, 1996).

Semen selenium concentrations are shown in Fig. 2. During the pre-experimental period (day 0), semen selenium concentrations were 0.348 (±0.075) ppm for all boars fed a basal diet containing 0.15 ppm of inorganic selenium. Whole semen selenium concentrations at day 84 after supplementation were 0.982 (±0.116) ppm for the boars supplemented with inorganic selenium and were 1.226 (±0.246) ppm for the boars supplemented with organic selenium. At 35, 49, 63 and 84 days after supplementation, whole semen selenium concentrations were higher than the concentrations at day 0 in both boars supplemented with inorganic and those with organic selenium. Supplementation of selenium in the diet increased semen selenium concentrations (Kolodziej and Jacyno, 2005). Jacyno *et al*. (2002) reported that selenium concentrations in semen plasma did not differ between boars supplemented with inorganic and those with organic selenium. The results confirmed again that the forms of selenium had no effect on the concentrations of selenium in boars' semen. However, Jacyno *et al*. (2002) have observed that boars supplemented with inorganic selenium had higher glutathione peroxidase acitivity in the seminal plasma despite the lower semen quality. Although, it has been known that biological activity of selenium is through glutathione peroxidase, some selenoproteins may also play a crucial role in reproductive performance (Jacyno *et al*., 2002).

Semen parameters: At day 0, semen volumes per ejaculate were 273±36 and 291±64 mL for boars fed a diet supplemented with inorganic and with organic selenium, respectively. At day 35, 49, 63 and 84, semem volumes were respectively 304 (±50), 365 (±45), 329 (±35) and 345 (±47) for boars fed a diet supplemented with inorganic selenium and 347 (±54), 348 (±29), 348 (±43) and 352 (±45) for boars fed a diet with organic selenium. The mean volumes did not differ between two groups of boars. These results were in agreement with previous studies (Jacyno *et al*., 2002; Lopez *et al*., 2010), meaning that there was no significant effect of the form of selenium on semen volume per ejaculate. In addition, mean volumes did not differ among sampling periods in both groups of boars. Sperm concentrations in boars fed a diet supplemented with inorganic and organic selenium are shown in Fig. 3. At day 0, 35, 49, 63 and 84, sperm concentrations in boars supplemented with inorganic selenium did not differ between the two groups. Within each group, there was no significant difference of sperm concentrations between sampling periods. Lopez *et al*. (2010). When boars were fed with a low selenium diet and were injected with inorganic selenium every fortnight, the number of sperm per ejaculate was higher than those boars that were not injected (Segerson *et al*., 1981). The boars in this study

Table 3: Motility parameters of the sperm of the boars fed a diet supplemented with 0.3 mg of inorganic selenium (In-Se; n = 5) per kg and with 0.3 mg of organic selenium (Or-Se; n = 5) per kg. Data represented means and Standard Errors (SE). The sperm parameters are from Computer Assisted Analysis (CASA) using a Hamilton-Thorne at the start of the study (day 0) and 35, 49, 63 and 84 days after supplementation

	Days after supplementation					
Motility parameters	0	35	49	63	84	Average (35-84)
Motility (%)						
In-Se	86.3±3.80	80.0±6.2	85.0±3.0	89.6±2.3	88.6±0.50	85.6±1.9
Or-Se	72.4±7.50	70.7±5.0	82.6±1.4	86.1±1.5	85.5±2.10	81.6±2.0
Progressive (%)						
In-Se	60.8±5.90	46.8±7.4	68.9±5.0	64.9±6.6	63.5±1.00	60.8±3.3
Or-Se	47.8±6.40	38.8±4.5	52.4±3.2	54.1±4.4	54.7±3.70	49.6±2.7
VAP (μm sec^{-1})						
In-Se	72.1±4.60	60.1±3.3	84.7±2.9	81.6±7.9	73.1±3.90	74.0±3.2
Or-Se	67.6±4.00	62.5±1.3	76.5±4.4	73.8±1.5	74.5±1.70	72.2±1.9
VSL[2] (μm sec^{-1})						
In-Se	55.7±3.90	43.4±2.4	62.3±2.6	57.9±6.0	51.1±1.60	53.2±2.4
Or-Se	49.0±2.30	38.6±1.8	50.1±9.0	46.7±2.2	48.3±2.20	48.2±2.9
VCL[3] (μm sec^{-1})						
In-Se	122.8±8.60	115.4±7.9	154.9±8.0	154.7±15.0	139.4±10.7	139.3±6.3
Or-Se	123.8±10.8	131.6±4.5	155.9±7.0	149.3±3.40	153.1±1.20	148.0±3.3

[1]Average path velocity; [2]Straight-line velocity; [3]Curvilinear velocity

were fed a diet with sufficient amounts of both inorganic and organic selenium in the diets therefore, semen volumes and sperm concentration were not affected by the sources of selenium.

Motility parameters of the sperm of the boars fed a diet supplemented with inorganic and with organic selenium are shown in Table 3. The motility parameters including motility, progressive motility, VAP, VSL and VCL did not differ between boars fed a diet supplemented with inorganic and those with organic selenium. Average motility (%), progressive motility (%), VAP, VSL and VCL (μm sec^{-1}) of the sperm, respectively ranged from 85-89.6, 46.8-68.9, 60.1-84.7, 43.4-62.3 and 115.4-154.9 for boars supplemented with inorganic selenium and ranged from 70.7-86.1, 38.8-54.7, 62.5-76.5, 38.6-50.1 and 123.8-155.9 for boars supplemented with organic selenium. Lopez *et al.* (2010) also reported similar results that there were no significantly differences of motility, progressive motility, VAP, VSL and VCL between boars fed a diet supplemented with inorganic and those fed organic selenium up to day 90. Due to sufficient amounts of both inorganic and organic selenium in the diets fed to the boars in this study, differences of motility parameters were not observed.

CONCLUSION

Feeding boars with a diet containing 0.45 ppm of inorganic or 0.15 ppm of inorganic and 0.3 ppm of organic selenium had no effects on blood and whole semen selenium concentrations. Semen volumes per ejaculate and sperm concentrations also did not differ between boars fed a diet supplemented with either inorganic or organic selenium. Furthermore, semen quality as evaluated by CASA was not affected by sources of selenium. Because the study selected the boars that were routinely used for semen collection at the farm, these boars might already have a good semen quality. Therefore, the effect of difference sources of selenium on boar semen quality was not overtly observed. Further study is required to investigate the effect of dietary organic or inorganic selenium in a group of boars with low fertility.

ACKNOWLEDGEMENTS

The researchers acknowledged financial support from Center for Agricultural Biotechnology, Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom 73140, Thailand; Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok 10900; and The Graduate School Kasetsart University. Agrifeed and Livestock Co. Ltd. for the use of experimental boars.

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