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The importance of pyridoxine for the impact of the dietary selenium sources on redox balance, embryo development, and reproductive performance in gilts

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ABSTRACT

This study aimed to determine the effects of dietary pyridoxine and selenium (Se) on embryo development, reproductive performance and redox system in gilts. Eighty-four gilts were fed one of five diets: CONT) basal diet; MSe$B_6$0) CONT + 0.3 mg/kg of Na-selenite; MSe$B_6$10) diet 2 + 10 mg/kg of HCl-pyridoxine; OSe$B_6$0) CONT + 0.3 mg/kg of Se-enriched yeast; and OSe$B_6$10) diet 4 + 10 mg/kg of HCl-pyridoxine. Blood samples were collected for long-term (each estrus and slaughter) and peri-estrus (fourth estrus d −4 to d +3) profiles. At slaughter (gestation d 30), organs and embryos were collected. For long-term and peri-estrus profiles, Se level and source affected ($P < 0.01$) blood Se concentration whereas B_6 level increased ($P < 0.01$) erythrocyte pyridoxal-5-phosphate concentration. A B_6 level ($P < 0.05$) effect was observed on long-term plasma Se-dependent glutathione peroxidase (Se-GPX) activity whereas peri-estrus Se-GPX was minimum on d −1 ($P < 0.01$). Selenium level increased sows' organs and embryo Se concentration ($P < 0.01$). Selenium source tended to enhance embryo Se content ($P = 0.06$). Within-litter embryo Se content was increased by B_6 level ($P < 0.01$). Selenium level tended to affect Se-GPX and total GPX activities in organs mitochondria ($P = 0.09$ and 0.07, respectively). Selenium source affected kidney ATP synthesis ($P = 0.05$). In conclusion, B_6 level affected the Se-GPX activity on a long-term basis, whereas the basal level of Se was adequate during the peri-estrus period. Embryo quality was not improved by dietary Se, and B_6 impaired within-litter homogeneity.

1. Introduction

During the peri-estrus period, intensified ovarian metabolism generates reactive oxygen species (ROS) [1] that, in excess, has been linked to ovulatory dysfunction [2]. These detrimental metabolic by-products must be neutralized by antioxidants such as glutathione peroxidases (GPX). This is particularly important for polytocous species such as pigs where poor quality of ovulation is increasingly observed in modern hyperprolific sows [3].

Dalto et al. [4] have recently shown beneficial effects of selenium (Se) and vitamin B_6 (B_6) on the metabolic control of GPX in gilts during the peri-estrus period. Such regulation was more apparent in gilts fed organic Se (OSe) plus B_6 than inorganic Se (MSe), possibly because OSe is dependent on B_6 to direct Se toward the GPX system via the transsulfuration pathway [5,6], whereas MSe short-circuits this process and the regulation of GPX by redox changes. In early gestation, provisions of dietary OSe and B_6 considerably stimulated the transcriptome of 5-d porcine embryos, but not GPX expression probably because of the hypoxic uterine conditions [7].

Later in gestation, however, O_2 tension rises with placentation. This is associated with higher expression of ROS markers [8] which needs to be modulated by an efficient antioxidant system to prevent perturbations of embryo development [9] and higher risk of embryo losses [10]. In this way, it has been demonstrated that enhanced maternal Se transfer to 30-d pig conceptuses induced by dietary OSe compared to MSe in early pregnancy was associated to improved embryonic development [11]. The physiological mechanisms related to those effects are, however, poorly understood.

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Table 1
Composition of the basal diet[a,b].

Ingredients	Amount,%
Corn	52.6
Wheat shorts	20.0
Distillers dried grain with solubles	10.0
Canola meal	9.7
Soybean hulls	4.0
Limestone	2.0
Salt	0.6
Monocalcium phosphate	0.5
L-Lysine *P*	0.1
Chlorure choline	0.1
Feed curb	0.1
Mineral and vitamin premix[c]	0.3

[a] The calculated compositions for ME, CP, lysine, Ca, and P of the basal diet were (as-fed basis) 2,702 kcal/kg, 14.0, 0.6, 1.0, and 0.6%, respectively.
[b] The basal Se and pyridoxine content of the diet were 0.3 mg/kg and 2.4 mg/kg, respectively (analytical values determined according to Giguère et al. [16] and Matte et al. [30], respectively).
[c] Provided per kilogram of diet: Mn as manganous oxide, 40 mg; Zn as zinc oxide, 150 mg; Fe as ferrous sulfate, 140 mg; Cu as copper sulfate, 21 mg; I as calcium iodate, 2.0 mg; vitamin A, 14,580 IU; vitamin D_3, 1,500 IU; vitamin E, 44 IU; vitamin K, 2.6 mg; thiamine, 2.7 mg; riboflavin, 4.9 mg; niacin, 31 mg; pantothenic acid, 21 mg; folic acid, 10 mg; biotin, 400 μg; and vitamin B_{12}, 25 μ.

The present experiment aimed to confirm the importance of B_6 for an adequate flow of Se toward the GPX system during the peri-estrus period, and determine their effects on maternal and embryonic antioxidant status, maternal Se transfer to embryos, and litter development at 30-d gestation gilts.

2. Materials and methods

2.1. Animals and treatments

Eighty-four Yorshire–Landrace gilts were selected for this study at 91.4 ± 1.0 kg BW and 135–170 d of age. They were grouped in pens (1.5 × 2.5 m, half-slatted concrete flooring) of 6–7 animals until the first estrus was detected. For at least 14 d, they were fed ad libitum a basal breeding/gestation diet (Table 1) without Se and pyridoxine supplements but with levels higher than the NRC [12] recommendation for all other nutrients. From the first estrus (116.2 ± 1.8 kg BW), gilts were placed into individual stalls (0.6 × 2.2 m, half-slatted concrete flooring), the daily feed allowance was controlled and limited to 2.8 kg, and they were randomly assigned (according to their BW and blood concentration of Se) to one of the 5 experimental diets: (1) basal diet containing 0.3 mg/kg and 2.4 mg/kg of native Se and pyridoxine, respectively (Table 1), top-dressed with 50 g of ground corn without supplemental Se or pyridoxine (CONT, $n = 16$); (2) basal diet top-dressed with 50 g of ground corn providing supplemental 0.3 mg/kg of feed of MSe as sodium selenite (214485 Sigma–Aldrich, St-Louis, MO, USA), and without pyridoxine ($MSeB_60$, $n = 17$); (3) basal diet top-dressed with 50 g of ground corn providing supplemental 0.3 mg/kg of feed of MSe as sodium selenite, and 10 mg/kg of feed of pyridoxine, as hydro-chloride pyridoxine (P9755 Sigma–Aldrich, St-Louis, MO, USA) ($MSeB_610$, $n = 18$); (4) basal diet top-dressed with 50 g of ground corn providing supplemental 0.3 mg/kg of feed of OSe as Se-enriched yeast (Alltech, Lexington, KY,USA), and without pyridoxine ($OSeB_60$, $n = 17$); and (5) basal diet top-dressed with 50 g of ground corn providing supplemental 0.3 mg/kg of feed of OSe as Se-enriched yeast, and 10 mg/kg of feed of pyridoxine, as hydro-chloride pyridoxine ($OSeB_610$, $n = 16$). Total dietary levels (native + supplements) of Se and pyridoxine per kg of diets were, respectively, 0.3 mg and 2.4 mg for CONT, 0.6 mg and 2.4 mg for $MSeB_60$ and $OSeB_60$, and 0.6 mg and 12.4 mg for $MSeB_610$ and $OSeB_610$. Estrus detection was performed by introducing a young boar (8–12 months of age) into the pen once daily for the first 4 estruses (10 min; between 08:00 and 09:00 h) and twice daily for the fifth estrus (10 min each; between 08:00 and 09:00 h and between 16:00 and 17:00 h). When estrus was detected in the morning, gilts were inseminated 8 and 24 h later. If estrus was detected in the afternoon, they were inseminated 16 and 24 h later. The inseminations were performed using 85 mL of semen (3×10^9 live sperm cells from pooled semen of 3 Duroc boars) provided by a local AI center (CIPQ Inc., St-Lambert, Quebec, Canada). Out of the initial group of 84 gilts, seven did not get pregnant and were eliminated of the experiment. The remaining 77 gilts were sacrificed 30 d after the first insemination (184.3 ± 1.3 kg BW).

Throughout the experimental protocol, animals were cared for according to the recommended code of practice of Agriculture Canada [13] and the procedures were approved by the local Animal Care Committee following the guidelines of the Canadian Council on Animal Care [14].

2.2. Sampling

Blood samples were collected into EDTA-containing tubes (10 mL; Becton Dickinson and Co., Rutherford, NJ) from the jugular vein by venipuncture on all gilts at arrival to the research centre, on the d after each onset of estrus, and at slaughter (long-term profiles). On d 14 after the third estrus, 5 gilts from treatments CONT, $MSeB_60$ and $MSeB_610$, and 6 gilts from treatments $OSeB_60$ and $OSeB_610$ were cannulated at the jugular vein by a nonsurgical technique described by Matte [15]. Blood samples were then collected daily from d −4 to d +3 of the fourth onset of estrus (peri-estrus profiles). For repeated measurements according to estrus, data from the first, second, third, and fifth estrus, and for slaughter were used to assess the long-term effects of treatments. Those from the fourth estrus were excluded from the long-term responses and were used exclusively for peri-estrus effects of treatments. This strategy is due to the fact that different blood sampling procedures through either a jugular vein catheter or a vacuum puncture after snare restraint may alter metabolites concentrations as described by Dalto et al. [4] and interfere with the interpretation of values collected for long-term (by venipuncture) and peri-estrus (through a jugular vein catheter) profiles. For storage, part of the whole blood samples was frozen at −20 °C. The remaining blood was centrifuged at 1800 × *g* for 12 min at 4 °C, plasma was separated into aliquots and stored at −80 °C and at −20 °C, and erythrocytes were stored at −20 °C.

At slaughter, the reproductive tract, liver, and kidneys were collected from the 77 pregnant gilts at 30 d of gestation (15 gilts for CONT, $MSeB_60$ and $MSeB_610$, and 16 gilts for $OSeB_60$ and $OSeB_610$). Liver weight was recorded. Corpora lutea (CL) were dissected from the ovaries and embryos harvested from the uterus. Ovulation rates (number of CL) and embryo survival (percentage of CL represented by viable embryos) were recorded. Embryos length and CL diameter were measured, and their weight assessed. Samples of liver and kidney tissue, as well as, entire individual embryos and the CL pooled by gilt were immediately frozen in liquid nitrogen and further stored at −80 °C.

2.3. Laboratory analysis

Concentrations of Se were measured in samples of blood, liver, kidney, pooled CL, and individual embryos. Concentrations of pyridoxal-5-phosphate (P-5-P) were measured in samples of erythrocytes (long-term profile) and plasma (peri-estrus profile). The activity of total (GPX) and Se-dependent GPX (Se-GPX) was measured in plasma, liver, liver mitochondria, kidney, kidney mitochondria, and only Se-GPX activity in embryos. The concentration of LH was measured in plasma. Protein and DNA concentration