

Effects of Organic Selenium on Growth Properties, Selenium Absorption and Utilization, Antioxidant Activity and Immunity in Weaning Piglets

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Abstract

This experiment was conducted to determine the influences of adding organic selenium (Se) on growth properties, Se absorption and utilization, immunity and antioxidant activity in diets of Duroc weaning piglets. This study was performed on 36 (average weight 7.6 kg) weaning piglets. The weaning piglets were randomly allocated to 1 of 4 homogeneous treatments (A, control treatment, no added Se; B, Sodium selenite, 0.3 mg Se/kg feed; C, yeast Se, 0.3 mg Se/kg; D, DL-methionine Se 0.3 mg Se/kg). Every treatment had 3 replicates, every replicate had 3 piglets. The experiment lasted for 35 d, with the first 7 d for adaptation. Feed intake, residual and contaminated feed were recorded every day. Every piglet weight was weighted respectively at beginning and end of experiment. Daily intake, gain weight and feed conversion rates of every replicate were calculated finally. Se concentrations of serum, blood antioxidant and immunity index were analyzed in the 36th d of experiment. The results showed average daily gain of treatment C was significant higher ($P < 0.05$) and D had higher trend than that of treatment A and B ($P = 0.06$) respectively. And feed and gain ratio of C and D had trends to lower than them of A and B ($P = 0.14$). However, all the intake of every week and whole period had no significant differences among treatments ($P > 0.05$). At same time, except for the Immunoglobulin M of treatment D and C was higher than that of treatment A and B significantly ($P < 0.05$), all the other Se contents of serum, immunity indexes, blood cell parameters and enzymatic activities had no significant differences among treatments ($P > 0.05$). But they

took on some obvious trends. For example, the Se contents and glutathione peroxidase activities increased successively in order of treatment A, B, C and D; the blood urea nitrogen and total bilirubin of treatment A had higher trend than that of other treatments ($P = 0.06$). All in all, adding to organic Se in basal diets could improve the animal's healthy levels, growth properties and Se utilization to some extent. Relatively speaking, the DL-methionine Se had more advantages compared to yeast Se.

Keywords

Se Absorption and Utilization, Growth Properties, Antioxidant Activity and Immunity, Weaning Piglets

1. Introduction

Selenium (Se) is an essential trace element for human and animal health, which has effects on anticancer, antioxidant, anti-cardiovascular diseases, strengthening antioxidant and immunity capacity, promoting growth and improving meat qualities, and so on [1] [2]. If the piglets are short of Se, they will appear muscular dystrophy, exudative diathesis, necrotic liver degeneration, and mulberry heart disease in farm animals [3]. To meet the Se need of piglets, the people often add a certain amount of inorganic Se in diets, for example, sodium selenite (SS). However, SS has lots of disadvantages such as potential toxic at high dietary level, low absorption rates, and potential pro-oxidation defects [4]. Thus, organic Se, for example DL-methionine Se and yeast Se, has received more attention in the additive industry during recent years [5]. However, the main Se compound present in yeast Se is L-selenomethionine based on previous research [6] [7]. Additionally, L-selenomethionine represents the exclusive form in natural selenomethionine compounds and the other stereoisomer is the D-selenomethionine form [8]. DL-selenomethionine is a synthetic product that has an equimolar mixture of D-selenomethionine and L-selenomethionine [9]. So, the DL-selenomethionine may be more effective for the Se additive than organic Se-enriched yeast. Some research results showed that selenomethionine could improve anti-oxidative capacity and meat quality of broilers or finishing pigs [10] [11]. But there's few study conducted to compare DL-methionine Se with yeast Se and SS, or to compare their effects on improving the antioxidant activity and Se status in weaning pig diets in China and elsewhere [12]. Hence, the objective of experiment is to compare the effects of DL-methionine Se, yeast Se and SS on growth properties, antioxidant and immunity capacities and Se contents of serum in weaning piglets.

2. Materials and Methods

2.1. Animal, Diets, and Experimental Design

The 36 (average weight 7.6 kg) weaning piglets (weaned on d 28) were randomly

allocated to 1 of 4 homogeneous treatments (A, control treatment, no added Se; B, SS, 0.3 mg Se/kg feed; yeast Se, 0.3 mg Se/kg; DL-methionine Se 0.3 mg Se/kg). SS, yeast Se and DL-methionine Se were added to the basal diets at the expense of premix. Every treatment had 3 replicates, every replicate had 3 piglets

The experiment lasted for 35 d, with the first 7 d for adaptation. The SS was bought from Guangxi Nanning Jun Wei: Feed Co., LTD, and its content is 1000 mg/kg in product. The yeast Se and DL-methionine Se were bought respectively from Le Fu yeast companies in the Unites States and Puno (Chengdu) biological technology co., LTD, and their contents are 2000 mg/kg in product. The piglets of every group was closed in a house with access to water and feed ad libitum. During the course of experiment, all the experiment houses were well ventilated and dried, and kept temperature 25°C - 28°C and relative humidity 55% - 70%. Furthermore, the experiment houses were swept and fumigated twice at am 8:00 and pm 14:00 every day.

The formula and nutrient levels of the experiment diet are presented in **Table 1**.

Table 1. Ingredients and nutrient content of the basal diet (DM basis).

Ingredients	%
Corn	66.3
Peeled soybean meal	21.6
Whey power	4.9
Imported fish meal	2.9
CaHPO ₄	0.8
Limestone	0.8
Soybean oil	0.7
Lysine	0.4
Acidifiers	0.3
NaCl	0.2
DL-Methionine	0.1
Threonine	0.1
Premix	0.9
In total ¹	100
Nutrient levels	%
Digestive energy (MJ/kg, DM)	14.0
Crude protein ²	18.5
Calcium ²	0.75
Phosphorus ²	0.60
Lysine	1.30
L-Methionine	0.45
Threonine	0.80

Note: ¹Provided per kilogram of diet: vitamin A 11,000 IU, vitamin D₃ 1500 IU, vitamin E 44.1 IU, vitamin K₃ 4.0 mg, vitamin B₁ 1.4 mg, vitamin B₂ 5.22 mg, vitamin B₃ 20.0 mg, vitamin B₁₂ 0.01 mg, niacin 26.0 mg, pantothenic acid 14 mg, folic acid 0.8 mg, biotin 44 µg, Fe 100.0 mg, Cu 16.50 mg, Zn 90.0 mg, Mn 35.0 mg, I 0.30 mg. ²Crude proteins, Calcium, Phosphorus were measured. The Se content of basal diet was 0.04 mg/kg DM. Other values were calculated based on data from the diet supplier.

The basal diets and premix were all prepared according to the People's Republic of China feed standard in piglets (2004) by Henan Hyrum Feed Co., LED. And the feeds were offered 4 times per day, respectively 8:00am, 12:00am, 16:00pm, 20:00pm. All ingredients were same except for Se sources in four diets.

2.2. Sampling, Measurement, and Analyses

Intake, residual and contaminated feed were recorded every day. Every piglet weight gain was weighted respectively at beginning and end of experiment. Daily intake, weight gain and feed and gain ratios of every replicate were calculated finally.

Before intake and water in the morning, the 5 ml blood samples (2 ml anti-clotting and 3ml no anti-clotting) were collected from the front cavity vein of piglets among 4 treatments (one piglet/treatment replicate) in the 36th d of experiment. The collected 2 ml anti-clotting blood then centrifuged at 4000 rpm for 10 min at 4°C to collect serum, which was frozen at -20°C until analyzed. Serum samples were later thawed and analyzed for quantification of glutathione peroxidase (GSH-px), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), blood urea nitrogen (BUN), creatinine (CREA), glucose (GLU), total bilirubin (TBIL), cholesterol (CHOL), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM) were determined according to method described in other studies [13] [14] [15].

Another 3 ml no anti-clotting blood was placed into a 10-mL tube for whole blood variable determination immediately. The whole blood samples were used to determine red blood cell count (RBC), haematocrit concentration (HCT), haemoglobin concentration (HGB), and white blood cell count (WBC), platelet (PLT) according to method reported previously [16].

Additionally, the Se concentration of serum was determined by a SpectraAA-30 atomic absorption spectrophotometer (Varian Techtron. Pty. Ltd., Mulgrave, Victoria, Australia).

2.3. Calculations and Statistical Analysis

Statistical analysis was conducted using SAS 9.1.3. All data were analyzed using the MIXED procedure. Piglets within treatment were subjected as random to test for main effects and interactions using the covariance type AR (1), and the residual error was used to test for week and week × treatment interaction. Mean comparisons across treatments were made when the interaction terms of the model were significant ($P < 0.05$) using LSMEANS and PDIF separation of all the treatments. Significant differences were declared at $P < 0.05$ and trends at $0.05 \leq P \leq 0.10$.

3. Results

3.1. Growth Properties

The effects of organic Se on growth properties were shown in **Table 2**. We could

Table 2. Effects of organic selenium on intake and daily gain of piglets.

Controls	The first week intake (g)	The second week intake (g)	The third week intake (g)	The fourth week intake (g)	The whole period intake (g)	Average daily gain (g)	Feed and gain ratio (F/G)
A (n = 3)	464 ± 59	631 ± 46	772 ± 30	876 ± 41	686 ± 163	321 ^b ± 40	2.14 ± 0.12
B (n = 3)	456 ± 34	636 ± 55	769 ± 28	870 ± 49	683 ± 162	314 ^b ± 63	2.20 ± 0.28
C (n = 3)	476 ± 32	645 ± 49	770 ± 27	870 ± 46	690 ± 154	419 ^a ± 128	1.72 ± 0.34
D (n = 3)	492 ± 34	652 ± 38	761 ± 27	877 ± 51	695 ± 149	385 ^{ab} ± 116	1.82 ± 0.27

Note: In the same column, values with different capital and small letters mean very significant difference at $P < 0.01$ and significant difference at $P < 0.05$.

know that average daily gain of treatment C was significant higher ($P < 0.05$) and D had higher trend than that of treatment A and B ($P = 0.06$) respectively. And feed and gain ratio of C and D had trends to lower than them of A and B ($P = 0.14$). However, all the intake of every week and whole period had no significant differences among treatments ($P > 0.05$).

3.2. Se Contents in Serum, Immunity Indexes, Blood Cell and Biochemistry Parameters

The effects of organic Se on Se contents in serum, immunity indexes, blood cell and biochemistry parameters were shown in **Tables 3-6**. Except for the IgM of treatment D and C was higher than them of treatment A and B significant ($P < 0.05$), all the other Se contents of serum, immunity indexes, blood cell parameters and enzymatic activities had no significant differences among treatments ($P > 0.05$). But they took on some obvious trends. For example, the Se contents and GSH-Px activities increased successively in order of treatment A, B, C and D; the BUN and TBIL of treatment A had higher trend than them of other treatments ($P = 0.06$).

4. Discussion

The effects of Se on growth performance of animals are somewhat variable. It was reported that different Se sources and levels did not effect on the average daily gain weight, intake and feed and meat ratio among the groups [17] [18] [19]. However, Jiang *et al.* (2009) suggested that 0.225 mg/kg of selenomethionine could highly improve the growth performance of broilers [10]. Zhan *et al.* (2011) reported that maternal selenomethionine intake of 0.3 mg/kg significantly increased the average weight gain of piglets from birth to weaning compared with the 0.3 mg/kg SS group [20]. In this experiment, all the intake of every week and whole period had no significant differences among groups ($P > 0.05$), but average daily gain of treatment C was significant higher ($P < 0.05$) and D had higher trend than that of treatment A and B ($P = 0.06$) respectively, which led to lower feed and gain rates of treatment C and D compared to them of treatment A and B ($P = 0.14$). It was consistent with their reports. Wang *et al.* (1997) suggested Se from organic sources was more available than inorganic Se for growth [21]. The present study indicated that organic Se had a positive effect on the

Table 3. Effects of organic selenium on Se contents in serum and immunity of piglets.

Controls	Se in serum (mg/kg)	IgA (g/L)	IgG (g/L)	IgM (g/L)
A (n = 3)	0.12 ± 0.02	0.06 ± 0.02	3.69 ± 0.81	0.74 ^b ± 0.26
B (n = 3)	0.13 ± 0.03	0.03 ± 0.01	4.11 ± 0.45	0.62 ^b ± 0.25
C (n = 3)	0.13 ± 0.01	0.04 ± 0.02	4.58 ± 1.05	1.04 ^a ± 0.31
D (n = 3)	0.17 ± 0.06	0.10 ± 0.14	4.44 ± 0.91	1.30 ^a ± 0.20

1) In the same column, values with different capital and small letters mean very significant difference at $P < 0.01$ and significant difference at $P < 0.05$. 2) IgA, Immunoglobulin A; IgG, Immunoglobulin G; IgM, Immunoglobulin M.

Table 4. Effects of organic selenium on blood cell parameters of piglets.

Controls	RBC (quantities × 10 ¹² /L)	WBC (quantities × 10 ¹⁰ /L)	PLT (quantities × 10 ¹¹ /L)	HGB (g/L)	HCT (%)
A (n = 3)	6.91 ± 0.79	2.84 ± 0.59	1.38 ± 0.53	111 ± 11	41.4 ± 3.7
B (n = 3)	6.72 ± 0.76	3.57 ± 0.64	3.00 ± 0.97	102 ± 14	38.2 ± 5.3
C (n = 3)	6.54 ± 0.59	2.27 ± 0.35	3.34 ± 1.76	107 ± 13	39.6 ± 4.5
D (n = 3)	6.27 ± 0.88	3.16 ± 0.82	2.59 ± 0.51	100 ± 12	37.6 ± 3.8

RBC, red blood cell count; WBC, white blood cell count; PLT, platelet; HGB, haemoglobin concentration; HCT, haematocrit concentration.

Table 5. Effects of organic selenium on blood biochemistry parameters of piglets.

Controls	TP (g/L)	ALB (g/L)	BUN (m mol/L)	CREA (u mol/l)	GLU (m mol/L)	CHOL (m mol/L)	TBIL (u mol/L)
A (n = 3)	53.9 ± 7.0	25.6 ± 2.9	5.52 ± 1.48	119 ± 22	2.42 ± 1.61	3.63 ± 0.97	3.20 ± 3.07
B (n = 3)	56.3 ± 4.4	24.0 ± 2.4	3.65 ± 0.60	96 ± 13	1.84 ± 1.25	2.41 ± 0.32	0.43 ± 0.17
C (n = 3)	50.5 ± 3.8	25.5 ± 5.9	4.24 ± 0.18	203 ± 198	2.27 ± 0.84	2.74 ± 0.40	0.35 ± 0.13
D (n = 3)	55.5 ± 8.4	22.9 ± 6.0	4.94 ± 0.39	99 ± 16	1.75 ± 0.72	3.70 ± 1.83	0.40 ± 0.19

TP, total protein; ALB, albumin; BUN, blood urea nitrogen, CREA, creatinine; GLU, glucose; CHOL, cholesterol; TBIL, total bilirubin.

Table 6. Effects of organic selenium on blood enzymatic activities of piglets.

Controls	GSH-PX Activities (U/ml)	ALT (U/L)	AST (U/L)	GGT (U/L)	ALP (U/L)
A (n = 3)	550 ± 105	59.8 ± 18.0	101 ± 21	150 ± 61	153 ± 62
B (n = 3)	588 ± 85	81.0 ± 15.3	117 ± 22	275 ± 38	239 ± 105
C (n = 3)	622 ± 76	69.3 ± 20.4	77 ± 25	246 ± 17	311 ± 120
D (n = 3)	683 ± 91	64.0 ± 15.3	114 ± 26	354 ± 48	176 ± 22

GSH-PX, glutathione peroxidase; ALT, alanine aminotransferase; AST, aspartate amino transferase; GGT, gamma-glutamyltransferase; ALP, alkaline phosphatase.

growth performance. The underlying mechanism might be they elevated health levels of animals and thus improved the growth performance of weaning piglets [4]. We suggested that DL-selenomethionine and yeast Se exhibited higher ab-

sorption and utilization efficiency than SS and could provide as an effective additive for weaning piglets to improve its growth performance.

Mahan and Peters (2004) reported that organic Se showed a higher potential to improve the Se status in the piglets [17]. Zhan *et al.* (2011) also reported that selenomethionine exhibited to be more effective to increase the Se content in all tissues of serum, liver, kidney, pancreas, muscle, and thymus and thyroid gland in weaned pigs at 28 days [20]. Kim and Mahan (2001) found that, compared with SS, the plasma Se concentration approximately doubled when Se-enriched yeast was fed. Additional, IgG, IgA, IgM are the immunoglobulin of human and animals [22]. Among them, the IgG is the most important one. Its main function is playing a role of protection in the immunization of bodies. Adequate intake of Se is required to ensure optimal immune function [18]. Peplowski *et al.* (1980) indicated that Se provided in diet or by injection increased the immune response of weaning pigs [23]. In this experiment, the Se contents of serum, IgA, IgG had no significant differences among treatments ($P > 0.05$), but the Se contents increased successively in order of treatment A, B, C and D, and the IgM of treatment D and C was higher than that of treatment A and B significantly ($P < 0.05$). They were consistent with the other's results. So it was showed that organic Se had higher absorption and utilization rates, and was benefit to improve immunity and resistance of weaning piglets. Especially, the DL-methionine Se had more advantages compared to yeast Se. The possible mechanism of absorption and utilization between inorganic and organic Se might be explained by the differences of metabolic pathways. Se in both forms can be incorporated into GSH-Px, but selenomethionine might also be incorporated in other proteins in substitution for methionine [24] [25]. Selenomethionine could be effectively metabolized to a Se analogue of S-adenosylmethionine, and was further metabolized in transmethylation reactions and in polyamine synthesis, similarly to the corresponding sulphur metabolites of methionine [26]. On the other hand, DL-methionine Se had more advantages compared to yeast Se in the way of Se absorption and utilization rates. The possible reasons were DL-methionine Se included D-methionine and L-methionine, and yeast Se only had L-methionine.

The research showed the RBC increasing in quantities might promote the WBC phagocytic functions, improve the body's ability to deliver oxygen in blood, strengthen immunity and metabolic functions. However, the WBC, neutrophils and lymphocyte are the important indexes indicated immunity of animal bodies, and the changes of quantities are the stronger or weaker expression of cell [27]. In this experiment, the blood cell parameters had no significant differences among groups ($P > 0.05$), and did not appear obvious change. It was showed the selenium sources did not effect on immunity of weaning piglets obviously. It was consistent with the experimental results of laying hens. However, in our 84-d dairy experiment, it was found that the RBC and WBC of 0.3 mg/kg Se DL-methionine treatment are very higher than them of control and 0.3 mg/kg SS treatment significantly in the whole blood ($P < 0.01$) [28]. This maybe showed the change of blood cell parameters and improvement of immunologic functions

were relate to the Se sources and longer or shorter time of feeding. The specific reasons need to further discusses and study in depth.

The changes of blood biochemistry parameters are the reflex of permeability changes of tissue and cell and metabolism function changes of bodies. ALT exists in cytoplasm of dogs, cats and primates mainly and so on, and it is one of the most sensitive liver function test index of the animals. The AST contents are higher in liver cells, hearts and skeletal muscles and they can be used to explore the extensive organizational damages. Normally the AST increasing of cattle and goats often occurs in hepatic necrosis symptoms caused by all kinds of reasons [29]. ALP takes part in many biochemical metabolic pathways in animal's bodies, and comes from skeletal muscles and intestinal mucosa mainly. In normal, the ALP passes bile ducts and drains into with bile by liver cell activities. But when intestinal tract is blocked or liver suffer from disease, the ALP activities are improved greatly in serum [30]. GGT exists in the liver cell membrane and mitochondria mainly, and takes part in glutathione peptide metabolism. But the GGT comes from hepatobiliary system mainly in serum. When intrahepatic function hyper or stopping the bile exhaustion, the GGT contents increase in serum [31].

Se is active ingredient of GSH-PX. However the GSH-PX activity determination in plasma of piglets is the important indexes of measuring Se levels and antioxidant capacity of animal bodies. It is widely accepted that proper Se intake improved the antioxidant status of the body in any form of Se. In general, organic Se-enriched yeast showed more potential to improve the antioxidant status than inorganic Se [32] [33]. Zhan *et al.* (2007) reported that selenomethionine was superior to improve antioxidant status in finishing pigs when compared with SS [5].

In this experiment, the enzymatic activities had no significant differences among treatments ($P > 0.05$), but the GSH-Px activities increased successively in order of treatment A, B, C and D. It showed adding organic Se could improve the GSH-Px activities and body's antioxidant capacity. On the other hand, the GSH-Px activities of DL-selenomethionine were the highest among all treatments which showed DL-selenomethionine had more advantages compared to yeast Se. This was consistent with the other results.

5. Conclusion

In this experiment, adding to organic Se had improved the animal's healthy level, growth properties, Se utilization and antioxidant capacity to some extent. Relatively speaking, DL-methionine Se had more advantages in all kinds of ways. We suggested that organic Se, especially DL-selenomethionine, exhibited higher absorption and utilization efficiency, better antioxidant capacity than SS and could provide as an effective additive for weaning piglets to improve its growth performance. Hence, DL-selenomethionine, a pure organic Se source, would have bright implication in feed additives of the weaning piglets industry.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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