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Increasing selenium supplementation to a moderately-reduced energy and protein diet improves antioxidant status and meat quality without affecting growth performance in finishing pigs

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A R T I C L E I N F O

Keywords:
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Meat quality
Moderately-reduced energy and protein diet

A B S T R A C T

Background: Along with economic development and living standards' improvement, more and more attention has been converted from satisfying meat quantity to pursuing meat quality.

Research purpose: This study was conducted to evaluate the effects of increasing selenium (Se) supplementation to a moderately-reduced energy and protein diet (MREP) on growth performance, antioxidant status, meat quality in finishing pigs.

Basic procedures: A total of 144 "Duroc × Landrace × Yorkshire" pigs with the average body weight of 75 ± 1 kg were randomly allotted to 3 dietary treatments with six replicates per treatment and eight pigs per replicate. The 3 experimental diets were as follows: (1) Normal energy and protein (NEP) +0.2 mg/kg Se diet (14.02 MJ/kg DE, 14% CP and 0.2 mg/kg Se as selenite sodium), (2) MREP +0.2 mg/kg Se diet (13.60 MJ/kg DE, 13% CP and 0.2 mg/kg Se as selenite sodium), and (3) MREP +0.5 mg/kg Se diet (13.60 MJ/kg DE, 13% CP, 0.2 mg/kg Se as selenite sodium, and 0.3 mg/kg Se as Se-enriched yeast). The study lasted for 45 days.

Main findings: The results show that there were no differences for growth performance, antioxidant status and meat quality of finishing pigs between NEP +0.2 mg/kg Se group and MREP +0.2 mg/kg Se group ($P > 0.05$). However, compared to pigs from MREP +0.2 mg/kg Se group, pigs from MREP +0.5 mg/kg Se group had greater Se concentration, GSH-Px activity and GSH concentration, but lower MDA concentration in serum ($P < 0.05$). Also, pigs from MREP +0.5 mg/kg Se group had greater Se concentration, T-AOC, and SOD activity, but lower MDA concentration in loin compared with pigs from MREP +0.2 mg/kg Se group ($P < 0.05$). As for meat quality, pigs from MREP +0.5 mg/kg Se group had greater a* value (relative redness) at 45 min and 24 h in loin compared with pigs from MREP +0.2 mg/kg Se group ($P < 0.05$). Compared to pigs from MREP +0.2 mg/kg Se group, pigs from MREP +0.5 mg/kg Se group had lower MDA concentration of fresh pork during a simulated retail display at 0, 1, 2, 4, 6 and 7 day ($P < 0.05$).

Principal conclusions: In conclusion, increasing selenium supplementation to a moderately-reduced energy and protein diet improved antioxidant status and meat quality without affecting growth performance in finishing pigs. (New Aspects) The present study provided a nutritional strategy for reducing feed costs and improving pork quality without influencing growth performance in finishing pigs.

1. Introduction

During the past decades, the swine industry, especially in developing countries, excessively pursue growth performance, feed conversion efficiency, and lean pork rate regardless of pork quality [1,2]. Along with economic development and living standards' improvement, more and more attention has been converted from satisfying meat quantity to pursuing meat quality [3,4].

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It's demonstrated that moderately-reduced protein diet with supplementation of amino acids can reduce nitrogen excretion and feed costs without affecting growth performance and carcass trait of growing-finishing pigs [5–7]. Moderately-reduced energy diet also didn't have a discernible effect on pig performance and carcass composition during growing and finishing period [5]. It's reported that moderately reduced energy and protein diet improved the pork quality of finishing pigs [8,9].

There exists a significant link between oxidative stress and meat quality [10]. Selenium (Se) is an essential trace element for humans and animals and is known to have potent antioxidant, anti-inflammatory, antiviral, and anticancer functions [11–14]. Our previous studies demonstrated that maternal supplementation of Se-enriched yeast enhanced the antioxidant status of sows and their offspring piglets compared with sodium selenite [15,16]. Besides, our recent study also found that increasing selenium supply for sows improved the antioxidant status of sows, colostrum and milk, and their offspring piglets [17]. Recently, many studies have shown that increasing Se supplementation has a beneficial effect on meat quality of chicken [3,18–20], duck [21], and rabbit [22], which indicate that currently-used requirement and feeding recommendation of Se may be insufficient for livestock, especially for producing meat with high quality and Se enrichment. However, less is known about the effects of increasing Se supplementation at 0.5 mg/kg [the up-limit level of selenium in swine feed in EU (European Union), FDA (Food and Drug Administration in USA) and China standard] to a moderately-reduced energy and protein diet (MREP) on growth performance, antioxidant status, and meat quality of finishing pigs.

Therefore, the present study was conducted to investigate the effects of increasing Se supplementation at 0.5 mg/kg to a moderately-reduced energy and protein diet on growth performance, antioxidant status, and meat quality in finishing pigs (75–120 kg).

2. Materials and methods

All animal protocols used in this study were approved by the South China Agricultural University Institutional Animal Care and Use Committee (SCAU-AEC-2010-0416).

2.1. Animals and experimental design

A total of 144 crossbred pigs (Duroc × Landrace × Yorkshire) with an average body weight of 75 ± 1 kg were selected and randomly allotted to 3 dietary treatments with six replicates (pens) per treatment and eight pigs per replicate (male and female in half). The 3 experimental diets are as follows: (1) Normal energy and protein (NEP) +0.2 mg/kg Se diet (14.02 MJ/kg DE, 14% CP and 0.2 mg/kg Se as selenite sodium), (2) MREP +0.2 mg/kg Se diet (13.60 MJ/kg DE, 13% CP and 0.2 mg/kg Se as selenite sodium), and (3) MREP +0.5 mg/kg Se diet (13.60 MJ/kg DE, 13% CP, 0.2 mg/kg Se as selenite sodium, and 0.3 mg/kg Se as Se-enriched yeast). The limiting amino acids for pigs (Lys, Met + Cys, Thr, and Trp) were adjusted to the same level in the 3 experimental diets. The Se was Se-enriched yeast (Se-yeast with 2000 mg/kg Se; Selenosource-AF 2000, Diamond V Mills Inc., Cedar Rapids, IA). The feeding trial lasted for 45 days.

2.2. Diets and management

The diets were corn-soybean meal diets, which meet or exceed nutritional requirements of finishing pigs according to the Chinese feeding standards of local pigs. And the ingredient composition and nutrient composition are shown in Table 1. During the experimental period, all pigs had *ad libitum* access to feed and fresh water.

Table 1
Ingredient composition and nutrient level of experimental diets (as-fed basis).

Ingredient, %	NEP +0.2 mg/kg Se	MREP +0.2 mg/kg Se	MREP +0.5 mg/kg Se
Corn	773.50	741.34	741.19
Soybean meal, 46.0% CP	156.00	108.00	108.00
Wheat flour	40.00	40.00	40.00
Wheat bran	0.00	78.00	78.00
Limestone	8.80	9.30	9.30
Dicalcium phosphate	7.10	6.60	6.60
Salt	2.50	2.40	2.40
Choline chloride (60%)	0.80	0.80	0.80
Mold inhibitor	0.40	0.40	0.40
Soybean oil	0.70	0.00	0.00
Premix[1]	6.00	6.00	6.00
L-Lys·sulfate (70%)	2.99	4.85	4.85
L-Thr (98%)	0.76	1.35	1.35
DL-Met (98%)	0.36	0.73	0.73
L-Trp (98%)	0.09	0.23	0.23
Se-enriched yeast[2]	0.00	0.00	0.15
Nutrient level, unit			
DE[3], MJ/kg	14.02	13.60	13.60
CP[4], %	14.00	13.00	13.00
CF[4], %	1.9	2.4	2.4
Ash[4], %	0.3	0.3	0.3
Fat[4], %	3.0	3.1	3.1
Ca[4], %	0.55	0.55	0.55
Se[4], mg/kg	0.25	0.25	0.55
Total P[4], %	0.44	0.46	0.46
Available P[3], %	0.18	0.18	0.18
Digestible Lys[3, 5], %	0.73	0.73	0.73
Digestible Met[3, 5], %	0.26	0.26	0.26
Digestible Met + Cys[3, 5], %	0.45	0.45	0.45
Digestible Thr[3, 5], %	0.51	0.51	0.51
Digestible Trp[3, 5], %	0.14	0.14	0.14

Note: NEP, normal energy and protein; MREP, moderately-reduced energy and protein. [1]Premix supplied per kilogram of complete diet: 60 mg Zn ($ZnSO_4·H_2O$), 60 mg Fe ($FeSO_4·H_2O$), 35 mg Mn ($MnSO_4·H_2O$), 8 mg Cu ($CuSO_4·5H_2O$), 0.35 mg I (CaI_2O_6), 0.2 mg Se ($NaSeO_3$), 1300 IU vitamin A, 150 IU vitamin D_3, 25 IU vitamin E, 2.5 mg vitamin K_3, 1.5 mg vitamin B_1, 5 mg vitamin B_2, 0.02 mg vitamin B_{12}, 20 mg niacin, 7.5 mg D-pantothenate, 0.5 mg folic acid and 0.04 mg D-biotin. [2]The Se-enriched yeast included 2000 mg/kg Se (Selenosource-AF 2000, Diamond V Mills Inc., Cedar Rapids, IA). [3]Calculated values. [4]Analyzed values. [5]The limiting amino acids for pigs (Lys, Met + Cys, Thr, and Trp) were adjusted to the same level in the 3 experimental diets.

2.3. Data and sample collection

2.3.1. Growth performance

Pigs were weighed at the beginning and the end of the feeding trial with the pen as replicate, respectively. Daily feed intake was recorded. And average daily gain, average daily feed intake, and feed/gain ratio were calculated.

2.3.2. Serum sample collection

At the end of the feeding trial, six pigs per treatment were selected according to gender and the average body weight of pen (1 pig per pen, three female and three male), fasted for 12 h (overnight: from 7 pm to 7 a.m.) and then sampled for blood. Blood was collected from anterior superior vena cava using sterile syringe and then remained at room temperature for 1 h to facilitate clot formation. The blood samples were centrifuged in a clinical centrifuge at 4℃, 1000 × *g* for 10 min, and the serum was pipetted into micro-tubes. Serum samples were immediately frozen in liquid nitrogen and stored at -80℃ until analysis.

2.3.3. Relative organ weight

After blood sampling, those pigs were killed via exsanguination after electrical stunning. After sacrifice, heart, liver, spleen, lung, and kidney were weighed. The relative organ weight was calculated using the method of Barea et al. (2011): Organ weight was divided by alive body weight [23].

2.3.4. Loin samples collection

Fresh loin samples anterior to the 13th rib from the left side carcass were collected for the analysis of Se concentration, antioxidant status and meat quality [24].

2.3.5. Carcass performance

Backfat thickness was determined between the 3rd and 4th last ribs on the midline of the carcass [25]. Skin thickness was measured between the 6th and 7th rib on the dorsal line [26]. Loin eye area was measured between the 10th and 11th ribs [26]. Carcass straight length was determined between the 1st cervical vertebra cavity and the public symphysis midline, while carcass oblique length was measured from the joint of the first rib and sternum to the pubic symphysis midline [26].

2.4. Laboratory analysis

2.4.1. Se concentration

The Se concentration in serum and loin samples were analyzed using the fluorometric method as described in our previous studies [15–17], and this method was also used for porcine samples by Dalto et al. (2015, 2016) [27,28]. The Se standard used for Se quantification was purchased from SCP Science with the Se concentration of 1000 μg/mL (Cat#: 140-051-340, SCP Science, Baie D'Urfe, Canada). Briefly, the elemental Se standard and samples were digested using complex acids of nitric acid and perchloric acid so that Se complex in sample was oxidized to be Se^{4+}, and under acidic medium conditions, Se^{4+} act with 2,3-diaminonaphthalene (DAN) to form 4,5-benzo piaselenol, and then was extracted by cyclohexane. The organic phase (cyclohexane) was analyzed for fluorescence degree in a spectro-fluorometer (RF-5301, shimadzu corporation, Kyoto, Japan) using an excitation wavelength of 376 nm and an emission wavelength of 520 nm, and fluorescence degree has linear relationship with the Se concentration when sample Se concentration is less than 0.5 μg. When the sample weight is 1 g (or 1 mL), the detection limit of the method is 0.01 μg/g (or 0.01 μg/mL). All samples were analyzed in duplicate. The absolute difference between two independent determinations obtained under repeatability conditions shall not exceed 20% of the arithmetic mean.

2.4.2. Antioxidant status

The antioxidant status, including total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-Px) activity, glutathione (GSH) concentration and malonaldehyde (MDA) concentration in serum and loin were measured as described in our previous studies [15,16,29] using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Those methods were also used for analyzing porcine samples by Zhan et al. (2007) [30] and Wan et al. (2017) [31].

Briefly, the T-AOC was analyzed using spectrometric method, which measured the ferric reducing capacity of the supernatant with Trolox as reference material. Ferric ion was reduced by antioxidant reducing agents, and blue complex 2,4,6-tri(2-pyridyl)-s-triazine was formed. The optical density was measure at 520 nm. One unit of T-AOC was defined as the amount that increased the absorbance by 0.01 at 37 ℃. Data were expressed as U/mL or U/mg protein.

The SOD assay utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine to form a red formazan dye. The optical density was measure at 550 nm. The SOD activity was calculated as the degree of inhibition of this reaction.

The GSH-Px activity was measured by testing the catalyzed reaction rate of GSH per minute. The optical density was tested at 412 nm. The result was expressed as a decrease of 1.0 μM GSH per 5 min at 37 ℃ after the nonenzymatic reaction is subtracted, and data were expressed as U/mL or U/mg protein.

The GSH concentration was tested based on the reaction, in which reduced GSH reacts with 5,5dithiobis-(2-nitrobenzoic acid) to produce a compound, the optical density was measured at 412 nm. Data were expressed as mg/L or mg/g protein.

The MDA concentration was analyzed according to the reaction, in which MDA reacts with thiobarbituric acid in an acidic medium condition at 95 ℃ for 30 min. The optical density was tested at 532 nm. Data was expressed as nmol/mL or nmol/mg protein.

2.4.3. Meat quality

The loin shear force was measured using the method of Jin et al. (2018). Briefly, the loin samples were packaged and incubated in a water bath at 70 °C for 30 min, and then were dried using tissue paper and stored at 4 °C for 24 h. At last, four cores per loin sample (diameter: 1 cm; long: 3 cm) were collected and measured using a digital muscle tenderness tester (C-LM3B, TENOVO, BeiJin, China) [4].

The drip loss of loin was measured according to the method of Hu et al. (2017) [32]. Briefly, approximately 50 g of fresh loin were weighed, and placed in a Whirl-Pak bag and reweighed after being hung in a 4 °C cooler for 48 h. Results are expressed as the percentage of weight loss.

The loin color was measured at 45 min, 24 h, and 48 h after-slaughter, respectively. Briefly, the meat color was measured using a colorimeter (CR-410, KONICA MINOLTA, Japan) with 50 mm aperture according to the method of Jin et al. (2018) [4], setting on the L* (relative lightness), a*(relative redness), b* (relative yellowness) system.

The loin pH was measured at 45 min, 24 h, and 48 h post-slaughter according to the method of Jin et al. (2018), respectively [4]. Briefly, the pH was measured on the same location of loin using a digital pH meter (HI99161, HANNA, Italy), and the pH meter was adjusted using standard liquids before usage (pH = 4.01, 6.86, and 9.18).

2.4.4. Oxidative stability of fresh pork during simulated retail display

Loin samples from slaughtered pigs were placed on individual styrofoam trays and overwrapped with transparent, oxygen-permeable, polyvinyl chloride film. And samples were placed in a refrigerated display cabinet at 4℃ under continuous fluorescent light (1600 lx) for 7 days to simulate common retail display condition [33]. On day 0, 1, 2, 3, 4, 5, 6, and 7 of retail display, approximately 10 g of loins were removed for MDA concentration analysis. MDA concentration was analyzed as described in our previous study [16] using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Statistical analysis

Statistical analyses were performed using SPSS 22.0 (SPSS, INC., Chicago, IL, USA). All data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test. Results are presented as means and a pooled SEM. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Growth performance

Effect of increasing Se supplementation to a moderately-reduced energy and protein diet on growth performance in finishing pigs is shown in Table 2. The final body weight, average daily feed intake, average daily gain, and feed to gain ratio were not affected by dietary treatments ($P > 0.05$).

Table 2
Effect of increasing Se supplementation to a moderately-reduced energy and protein diet on growth performance in finishing pigs (n = 6).

Item	NEP +0.2mg/kg Se	MREP +0.2mg/kg Se	MREP +0.5mg/kg Se	SEM	*P*-value
Initial body weight, kg	75.0	75.1	75.2	0.1	0.900
Final body weight, kg	119.9	118.7	119.8	0.6	0.231
Average daily feed intake, g pig^{-1} day^{-1}	2776	2728	2808	37	0.252
Average daily gain, g pig^{-1} day^{-1}	995.6	968.2	993.0	13.6	0.264
Feed/gain ratio	2.79	2.82	2.83	0.03	0.981

Note: NEP, normal energy and protein; MREP, moderately-reduced energy and protein.

Table 3
Effect of increasing Se supplementation to a moderately-reduced energy and protein diet on carcass performance in finishing pigs (n = 6).

Item	NEP +0.2mg/kg Se	MREP +0.2mg/kg Se	MREP +0.5mg/kg Se	SEM	*P*-value
Backfat thickness, cm	1.92	2.34	2.25	0.06	0.132
Skin thickness, cm	0.29	0.33	0.31	0.01	0.551
Loin eye area, cm^2	56.94	52.78	54.75	1.68	0.843
Carcass straight length, cm	94.60	94.28	95.00	0.65	0.952
Carcass oblique length, cm	76.46	75.82	77.23	0.65	0.901

Note: NEP, normal energy and protein; MREP, moderately-reduced energy and protein.

3.2. Carcass performance

Effect of increasing Se supplementation to a moderately-reduced energy and protein diet on carcass performance in finishing pigs is described in Table 3. The backfat thickness, skin thickness, loin eye area, carcass straight length, and carcass oblique length were not impacted by dietary treatments ($P > 0.05$).

3.3. Relative organ weight

Effect of increasing Se supplementation to a moderately-reduced energy and protein diet on relative organ weight in finishing pigs is summarized in Table 4. The relative organ weight of heat, liver, spleen, lung, and kidney was unaffected by dietary treatments ($P > 0.05$).

3.4. Se status and Se deposit

Effect of increasing Se supplementation to a moderately-reduced energy and protein diet on Se status and Se deposit of finishing pigs is displayed in Fig. 1. There was no difference for Se concentration in serum and loin of pigs between NEP +0.2 mg/kg Se group and MREP +0.2 mg/kg Se group ($P > 0.05$). However, pigs from MREP +0.5 mg/kg Se group had greater Se concentration in serum and loin of finishing pigs compared to pigs from MREP +0.2 mg/kg Se group ($P < 0.05$).

3.5. Antioxidant status in serum

As shown in Table 5, there was no difference for antioxidant status in serum of pigs between NEP +0.2 mg/kg Se group and MREP +0.2 mg/kg Se group ($P > 0.05$). However, pigs from MREP +0.5 mg/kg Se group had greater GSH-Px activity and GSH concentration, but lower MDA concentration in serum compared with pigs from MREP +0.2 mg/kg Se group ($P < 0.05$).

Table 4
Effect of increasing Se supplementation to a moderately-reduced energy and protein diet on relative organ weight in finishing pigs (n = 6).

Item	NEP +0.2mg/kg Se	MREP +0.2mg/kg Se	MREP +0.5mg/kg Se	SEM	*P*-value
Heart, g/kg	3.83	3.85	3.72	0.05	0.605
Liver, g/kg	13.45	14.99	14.83	0.44	0.596
Spleen, g/kg	1.93	2.18	2.06	0.07	0.392
Lung, g/kg	10.53	9.19	8.78	0.57	0.674
Kidney, g/kg	3.28	3.14	2.85	0.08	0.201

Note: NEP, normal energy and protein; MREP, moderately-reduced energy and protein.

3.6. Antioxidant status in loin

As depicted in Table 6, there was no difference for antioxidant status in the loin of pigs between NEP +0.2 mg/kg Se group and MREP +0.2 mg/kg Se group ($P > 0.05$). However, pigs from MREP +0.5 mg/kg Se group had greater T-AOC and SOD activity, but lower MDA concentration in loin compared with pigs from MREP +0.2 mg/kg Se group ($P < 0.05$).

3.7. Meat quality

As displayed in Table 7, there was no difference for meat quality between NEP +0.2 mg/kg Se group and MREP +0.2 mg/kg Se group ($P > 0.05$). However, pigs from MREP +0.5 mg/kg Se group tended to have lower drop loss in loin after slaughter compared to pigs from MREP +0.2 mg/kg Se group ($P < 0.10$). Besides, pigs from MREP +0.5 mg/kg Se group had greater a* value (relative redness) at 45 min and 24 h in loin compared with pigs from MREP +0.2 mg/kg Se group ($P < 0.05$), which is consistent with the results of loin appearance at slaughter (Fig. 2).

3.8. Oxidative stability of fresh pork during simulated retail display

As summarized in Table 8, there was no difference for oxidative stability of fresh pork during simulated retail display between NEP +0.2 mg/kg Se group and MREP +0.2 mg/kg Se group ($P > 0.05$). However, compared to pigs from MREP +0.2 mg/kg Se group, pigs from MREP +0.5 mg/kg Se group had lower MDA concentration of fresh pork during the simulated retail display at 0, 1, 2, 4, 6 and 7 day ($P < 0.05$).

4. Discussion

The primary objective of this study was to investigate the effect of increasing Se supplementation to a moderately-reduced energy and protein diet on growth performance, antioxidant status and meat quality in finishing pigs (75–120 kg). It's demonstrated that organic Se has higher bioavailability and less toxic characteristic than inorganic Se [11,30], which might be explained by their different metabolic pathways [30]. Selenomethionine is either metabolized directly to reactive

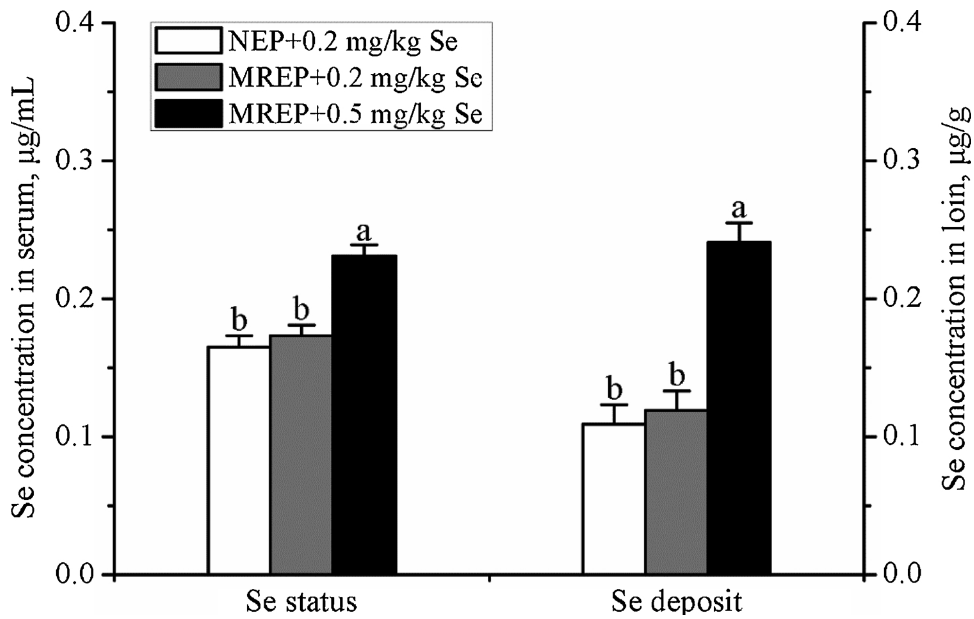

Fig. 1. Effect of increasing Se supplementation to a moderately-reduced energy and protein diet on Se status and Se deposit of finishing pigs (n = 6).
Note: NEP, normal energy and protein; MREP, moderately − reduced energy and protein. For each index, values with different letter superscripts indicate significant difference (*P* < 0.05).

forms of Se to synthesize selenoproteins or stored in place of methionine in body proteins [34], which indicates that Se-yeast can be efficiently used to participate in selenoproteins synthesis as well as Se deposit in meat-producing livestock animals. Furthermore, although Mahan and Parrett (1996) and Jiang et al. (2017) did not found side-effect of 0.5 mg/kg Se as Se-yeast or sodium selenite on growing or finishing pigs [35,36], Schwarz et al. (2017) found that pigs fed 0.5 mg/kg Se as sodium selenite had decreased average daily feed intake, but similar average daily gain and the ratio of feed to gain compared with pigs fed 0.2 mg/kg Se as sodium selenite during growing and finishing period [37]. Considering the above reasons, we did not include 0.5 mg/kg Se as sodium selenite in the present study.

In the present study, moderate-reduced energy and protein diet did not affect growth performance of finishing pigs. In agreement with our results, Zhou et al. (2016) found that growth performance of pig was unaffected when pigs were fed low protein vs. high protein diet (15% vs. 18% CP and 13% vs. 16% CP during growing period and finishing period, respectively) [6]. Consistent with our results, Kerr et al. (2003) reported that moderately-reduced energy diet also didn't have a discernible effect on pig performance during growing-finishing period [5]. Tang et al. (2010) found that pigs fed reduced energy and protein diet (12.55 MJ/kg DE and 11.08% CP) had similar ADG and ADFI, but higher F/G compared with normal energy and protein diet (13.81 MJ/kg DE and 14.19%) during finishing period [8]. In the present study, moderately-reduced energy and protein diet (13.60 MJ/kg DE and 13% CP) did not influence the growth performance compared to normal energy and protein diet (14.02 MJ/kg DE and 14% CP). The most likely explanation is that: (1) We reduced dietary energy and protein with supplementation of amino acids, whereas Tang et al. (2010) decreased dietary energy and protein without supplementation of amino acids; (2) We reduced energy and protein levels to a lesser extent, while Tang et al. (2010) reduced energy and protein levels to a greater extent. It is suggested that moderately reduction of energy and protein will decrease feed cost and relief environmental pressure without influencing growth performance of finishing pigs.

In China, the swine industry usually fortified 0.2 mg/kg Se as sodium selenite in the diet of finishing pigs, which was the Se level in the NEP +0.2 mg/kg Se group. While we increased Se supplementation at EU, FDA and Chinese standard upper-limit level (0.5 mg/kg) using Se-enriched yeast (0.2 mg/kg Se as sodium selenite and 0.3 mg/kg as Se-enriched yeast). Although the Se-yeast supplement is suggested not to exceed a maximum of 0.2 mg Se/kg complete feed in the EU [38], 0.3 mg/kg selenium as Se-yeast is commonly used in swine diet [11,19,30,36,39–42]. In most literature available for finishing pigs, the Se supplementation level was less than 0.3 mg/kg as inorganic or organic Se [30,40,43–47], and those authors found that dietary Se sources and levels did not affect growth performance of finishing pigs. Similar to our results, Mahan and Parrett (1996) found that growth performance was unaffected by dietary Se sources (sodium selenite and Se-enriched yeast) and levels (0.1, 0.3 and 0.5 mg/kg) [35]. Jiang et al. (2017) also found a similar result when finishing pigs were fed 0.5 mg/kg Se as Se-yeast vs. 0.3 mg/kg Se as sodium selenite or Se-yeast [36]. However, Schwarz et al. (2017) found that pigs fed 0.5 mg/kg Se as sodium selenite had decreased ADFI, but similar ADG and F/G

Table 5
Effect of increasing Se supplementation to a moderately-reduced energy and protein diet on antioxidant status in serum of finishing pigs (n = 6).

Item	NEP +0.2 mg/kg Se	MREP +0.2 mg/kg Se	MREP +0.5 mg/kg Se	SEM	*P*-value
T-AOC, U/mL	1.05	1.03	1.89	0.23	0.087
SOD, U/mL	81.88	80.12	83.55	1.17	0.817
GSH-Px, U/mL	1107.69^{ab}	1010.77^{b}	1269.23^{a}	47.97	0.040
GSH, mg/L	15.97^{ab}	15.24^{b}	17.74^{a}	0.45	0.041
MDA, nmol/mL	3.21^{a}	3.03^{a}	2.40^{b}	0.12	0.002

Note: NEP, normal energy and protein; MREP, moderately − reduced energy and protein; MDA, malondialdehyde; SOD, superoxide dismutase; T − AOC, total antioxidant capacity; GSH, glutathione; GSH − Px, glutathione peroxidase. In the same row, values with different letter superscripts indicate significant difference (*P* < 0.05).

Table 6
Effect of increasing Se supplementation to a moderately-reduced energy and protein diet on antioxidant status in the loin of finishing pigs (n = 6).

Item	NEP + 0.2 mg/kg Se	MREP + 0.2 mg/kg Se	MREP + 0.5 mg/kg Se	SEM	*P*-value
T-AOC, U/mg prot	0.16^{ab}	0.11^{b}	0.23^{a}	0.02	0.039
SOD, U/mg prot	18.66^{b}	18.18^{b}	22.29^{a}	0.59	0.006
GSH-Px, U/mg prot	2.07	2.29	4.08	0.40	0.082
GSH, mg/g prot	2.64	2.48	2.76	0.09	0.762
MDA, nmol/mg prot	0.13^{a}	0.14^{a}	0.11^{b}	0.01	0.004

Note: NEP, normal energy and protein; MREP, moderately − reduced energy and protein; MDA, malondialdehyde; SOD, superoxide dismutase; T − AOC, total antioxidant capacity; GSH, glutathione; GSH − Px, glutathione peroxidase. In the same row, values with different letter superscripts indicate significant difference ($P < 0.05$).

Table 7
Effect of increasing Se supplementation to a moderately-reduced energy and protein diet on meat quality of finishing pigs (n = 6).

Item	NEP + 0.2 mg/kg Se	MREP + 0.2 mg/kg Se	MREP + 0.5 mg/kg Se	SEM	*P*-value
Drip Loss, %	2.76	3.10	1.51	0.29	0.052
Shear Force, Kgf	5.27	5.81	4.41	0.24	0.137
pH					
45 min	6.29	6.37	6.54	0.05	0.095
24 h	5.59	5.55	5.53	0.02	0.790
48 h	5.52	5.53	5.50	0.02	0.945
L*					
45 min	37.65	37.23	36.45	0.43	0.326
24 h	38.57	37.97	36.34	0.89	0.765
48 h	42.57	41.16	40.18	1.04	0.812
a*					
45 min	12.38^{ab}	10.80^{b}	13.64^{a}	0.43	0.046
24 h	14.72^{b}	13.69^{b}	17.28^{a}	0.47	0.001
48 h	17.33	19.29	23.19	1.01	0.062
b*					
45 min	7.97	7.76	7.27	0.23	0.177
24 h	8.51	9.20	8.09	0.45	0.850
48 h	12.07	13.39	11.75	0.49	0.535

Note: NEP, normal energy and protein; MREP, moderately − reduced energy and protein. In the same row, values with different letter superscripts indicate significant difference ($P < 0.05$).

compared with pigs fed 0.2 mg/kg Se as sodium selenite during growing and finishing period [37]. The most likely explanation is that inorganic Se supplementation at 0.5 mg/kg may have an adverse effect due to the fact that inorganic Se is more susceptible to selenosis than organic Se [48]. Therefore, our results indicate that increasing Se supplementation at 0.5 mg/kg using Se-enriched yeast to moderately-reduced energy and protein did not affect growth performance of finishing pigs.

Serum Se concentration is one of the most critical indicators of the animal body, while muscle Se concentration is not only the crucial indicator of Se deposit [49] but also a key maker of Se-enriched meat [50,51]. Therefore, we measured the Se concentration in both serum and loin, and we found that increasing Se supplementation distinctly enhanced the Se concentration in both serum and loin. Because Se

Table 8
Effect of increasing Se supplementation to a moderately-reduced energy and protein diet of finishing pigs on oxidative stability (MDA concentration, nmol/mg prot) of fresh pork during simulated retail display (n = 6).

Item	NEP + 0.2mg/kg Se	MREP + 0.2mg/kg Se	MREP + 0.5mg/kg Se	SEM	*P*-value
0-day	0.13^{a}	0.14^{a}	0.11^{b}	0.01	0.004
1-day	0.26^{a}	0.26^{a}	0.18^{b}	0.01	0.036
2-day	0.34^{a}	0.34^{a}	0.22^{b}	0.02	0.032
3-day	0.36	0.43	0.35	0.02	0.307
4-day	0.43^{a}	0.45^{a}	0.36^{b}	0.02	0.020
5-day	0.47	0.47	0.41	0.02	0.056
6-day	0.71^{a}	0.70^{a}	0.46^{b}	0.04	< 0.001
7-day	0.75^{a}	0.73^{a}	0.50^{b}	0.05	0.006

Note: NEP, normal energy and protein; MREP, moderately − reduced energy and protein. In the same row, values with different letter superscripts indicate significant difference ($P < 0.05$).

status and Se deposit are secure to be affected by dietary Se levels, increasing dietary Se supply for livestock is a common strategy to produce Se-enriched meat [18]. In agreement with our results, Mahan and Parrett (1996) found that loin Se concentrations were increased in growing or finishing pigs as dietary Se levels increased (0.1, 0.3 and 0.5 mg/kg) when pigs were fed Se-enriched yeast [35]. Jiang et al. (2017) also reported that loin Se concentrations were elevated when finishing pigs were fed 0.5 mg/kg Se as Se-yeast vs. 0.3 mg/kg Se as sodium selenite or Se-yeast [36], which confirmed our results. Hence, increasing Se supplementation at 0.5 mg/kg using Se-enriched yeast to moderately-reduced energy and protein improved Se status and Se deposit of finishing pigs. One question that must be raised is that what is the optimal Se dosage for producing Se-enriched meat without side-effect on animal health. Although it has been reported that dietary Se dosage above 0.5 mg/kg indeed improved Se status and meat Se deposit without adverse effect in broilers [3,18,20], rabbits [22] and ducks [21], to the best of our knowledge, no literature available about the effects of dietary Se level above 0.5 mg/kg on Se-enriched pork. To our understanding of our results, dietary Se dosage at 0.5 mg/kg Se using Se-enriched yeast is safe and efficient, but more research is needed to explore higher Se dosage for finishing pigs in the future.

As an essential trace element for humans and animals, Se plays a

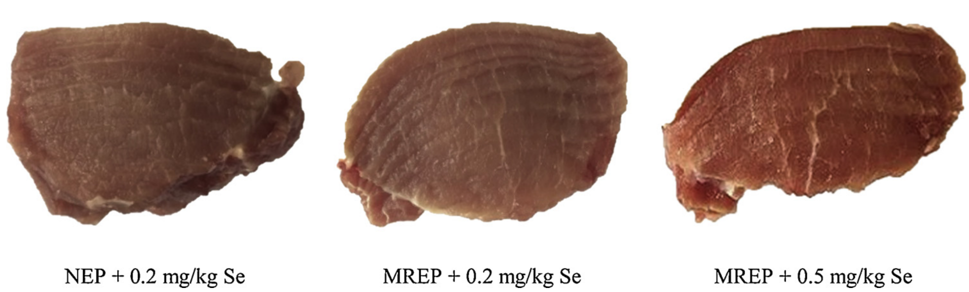

Fig. 2. Effect of increasing Se supplementation to a moderately-reduced energy and protein diet on loin appearance at slaughter of finishing pigs.
Note: NEP, normal energy and protein; MREP, moderately-reduced energy and protein.

crucial role in antioxidant defense [14]. It's demonstrated there is a significant relationship between GSH-Px activity and Se concentration in plasma in ducks [21]. As for pigs, it's also found that serum GSH-Px activity was increased linearly ($P < 0.01$) and quadratically ($P < 0.05$) with increasing supplemental selenium [52]. In the current study, dietary 0.5 mg/ kg Se supplementation increased GSH-Px activity and GSH concentration, and decreased MDA concentration in serum of finishing pigs. Consistent with our results, Baltić et al. (2015) found that the GSH-Px activity was increased as dietary Se increased when 1-day-old ducks were fed 0, 0.2, 0.4 and 0.6 mg/kg Se as Se-enriched yeast for 49 days [21]. In the present study, dietary 0.5 mg/kg Se supplementation improved the antioxidant status in loin of finishing pigs, which was indicated with greater T-AOC, SOD activity and lower MDA concentration. In agreement with our results, Papadomichelakis et al. (2017) found that dietary supplementation of 0.5 mg/kg Se reduced MDA concentration and oxygen radical absorbance capacity (ORAC) values in muscle of growing rabbits, however, 2.5 mg/kg Se diet increased pro-oxidant in muscle with increased MDA concentration and ORAC values [22]. Therefore, although there is still room to increase higher dietary Se supply to improve pork quality in finishing pigs, it must be pointed out that excessive Se intake may induce pro-oxidant effect.

In the present study, dietary 0.5 mg/kg Se supplementation to moderately-reduced energy and protein diet tended to decreased the drop loss in loin of finishing pigs. Indeed, organic Se has been reported to increase water-holding capacity compared with inorganic Se [30,53], and increasing dietary organic Se supply also decreased water loss in pork [41,54]. Similar to our results, Calvo et al. (2016) reported dietary supplementation of 0.4 mg/kg Se as Se-enriched yeast increased water-holding capacity and post-mortem muscle proteolytic activity, which indicated a possible relationship between water loss and muscle proteolysis [54]. It was also demonstrated that muscle drip loss had a negative correlation with the mRNA expression of *Sepw1* gene in the study of Li et al. (2011), who reported the effects of feeding 0, 0.3 and 3.0 mg/kg Se as Se-enriched Se to 10-kg piglets for 8 weeks, and found a markedly lower drip loss in muscle from piglets fed 3.0 mg/kg Se [41]. Meat color is one of the most critical factors affecting the purchase preference of meat consumers [55]. In the present study, we found that a* value (relatvie redness) was improved when 0.5 mg/kg Se was supplemented to moderately reduced energy and protein diet for finishing pigs. It is reported that feeding organic Se improved pork color in finishing pigs at the level under 0.3 mg/kg [39,53]. Calvo et al. (2017) found that dietary supplementation of 0.3 mg/kg Se as Se-enriched Se increased a* value of muscle at day 1 and 7 post-mortems in finishing pigs compared with 0.3 mg/kg inorganic Se [39]. Mahan et al. (1999) also found that, compared to organic Se, inorganic Se feeding led to paler color meat in growing-finishing pigs [53]. However, no literature is available about dietary supplementation at above 0.3 mg/kg Se as Se-enriched on pork color in finishing pigs. To our knowledge, the beneficial effect of increasing Se supplementation at 0.5 mg/kg may be attributed to the improved antioxidant characteristics because reduction of metmyoglobin is crucial to meat color life and dramatically depends on muscle's oxygen scavenging enzymes, reducing enzyme systems, and the nicotinamide adenine dinucleotide (NADH) pool [56]. Moreover, it's reported that dietary methionine supplementation increased pH_{24h} and decreased drip loss in the muscle of pigs [57], and the use of Se-yeast is an extra-supplementation of methionine as well. Can that be a factor for meat quality? After we calculated, we found that it may not be a factor affecting meat quality of pigs in the present study. Compared with the methionine in basal diet (0.26%), the amount of methionine from Se-yeast is negligible (0.75×10^{-4}%).

Lipid peroxidation is one of the leading causes of quality deterioration in raw meat products during storage [18]. In the present study, dietary increasing Se supplementation to moderately reduced energy and protein diet improved oxidative ability during simulated retail display. It's reported that dietary organic Se supplementation can enhance oxidative stability of fresh meat compared to inorganic Se [58], and increasing dietary Se supply as Se-enriched Se further improved oxidative stability of meat [18,22]. Lipid peroxidation is a common phenomenon involved in peroxidative loss of unsaturated lipids, which resulted in lipid degradation and membrane disordering. MDA is one of the most important metabolic products of lipid peroxides, which are generated by reaction of lipid oxidation induced by oxygen free radicals in tissues [30]. Dietary supplementation of Se increased the polyunsaturated fatty acids (PUFA) concentrations in meat, which indirectly indicated Se prevent meat from lipid oxidation and protected PUFA [18,30]. The protective effect of Se against meat quality deterioration at storage is mainly attributed to the prevention of lipid peroxidation and maintenance of muscle membrane integrity [18].

In conclusion, moderately-reduced energy and protein diet did not affect the growth performance of finishing pigs, while increasing Se supplementation to the moderately-reduced diet improved the antioxidant status and meat quality without affecting growth performance. The present study provided a nutritional strategy for reducing feed costs and improving pork quality without influencing growth performance in finishing pigs. There is still room to explore the effect of higher dietary Se supply on pork quality of finishing pigs in future research, but the side-effect of excessive Se intake must be considered as well.

Declaration of Competing Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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