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Effect of roasting and extrusion on the bioavailability of Selenium in soybean for young pigs

Jun-gang Li[1, 2], Hua Zhao[2],Ji-chang Zhou[2], Ge Gao[3] and Kang-ning Wang [1] *

[1]National Key Discipline, Animal Nutrition Institute, Sichuan Agricultural University, 625014, Ya`an, China; [2] International Center of Future Agriculture for Human Health, Sichuan Agricultural University, 611134, Chengdu, China; [3] Chengdu Municipal Center for Disease Control and Prevention, 610015, Chengdu, China.

*Corresponding author email: wkn8866@yahoo.cn

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ABSTRACT

Objective: To study the thermal-processing effect of roasting and extrusion on selenium bioavailability in soybean. *Methodology and results:* Twenty young piglets were fed a Se-deficient diet for 4 weeks when their body weight reached 10.36±0.96 kg. These pigs were randomly divided into 2 groups (n = 10) and fed on corn-extruded soybean and corn-roasted soybean, respectively, for 8 weeks. Results showed that extruded soybean significantly increased ($P < 0.05$) pig feed intake and daily gain, improved ($P < 0.05$) feed efficiency and Se apparent digestibility in comparison to the roasted soybean. Pigs fed on extruded soybean had higher ($P < 0.05$) tissue Se than pigs fed on roasted soybean. Relative to roasted soybean, extruded soybean increased ($P < 0.05$) tissue glutathione peroxidase activity. Plasma alanine aminotransferase activity in pigs fed on roasted soybean was 5 times higher ($P < 0.01$) than in the pigs fed on extruded soybean. *Conclusion and application of findings:* Results of this study suggested that Se was less bioavailable from roasted soybean than from extruded soybean. This finding is important for developing methods for processing Se-containing food and feed.

Key words: roasted soybean; extruded soybean; bioavailability; pigs

INTRODUCTION

Selenium (Se) is an essential micronutrient for both human and animals and its deficiency in diets causes various metabolic disorders in muscle (Brown et al., 1986), brain (Schweizer,Schomburg &Savaskan, 2004), endocrine tissues (Zhou et al., 2009) and immune response (Sheridan et al., 2007). Moreover, both epidemiology and animal models have indicated that Se could decrease cancer risk (Shamberger et al., 1977; Ganther, 1999; Sinha et al., 2001). Thus, higher Se intake has been proposed from the standpoints of both Se deficiency prevention and chemoprevention.

Roughly, half of the Se intake comes from animal-derived food and half from plant sources (Reeves et al., 2005). As a staple crop, soybean is an important food not only for providing energy and

protein, but also minerals (Tako et al., 2009). Moreover, legumes are important sources of selenium in the Mauritian vegetarian diet (Subratty et al., 2004). Soybean has a relatively high level of Se, and the major chemical form of Se is selenomethionine (Whanger, 2002).

The maximum utilization of Se in food is of great importance for human nutrition. Roasting and extrusion are two important thermal processing techniques commonly used in food industry (Marsman et al., 1997; Boge et al., 2009). The basic difference between extrusion and roasting is that extrusion has higher processing-temperature and less processing-time than roasting. These processing techniques are thought to differently affect Se bioavailability from buckwheat bran (Reeves et al., 2005).

Moreover, animal studies demonstrated that protein and energy are less bioavailable from roasted soybean in comparison to extruded soybean used for pig and poultry feed (Faber & Zimmerman, 1973; Marsman et al., 1997). However, the effect of roasting and extrusion on soybean Se bioavailability is not clear. Considering that pig and human are similar in anatomy, nutrition and physiology, pig was selected as an excellent model for human studies on mineral absorption and metabolism (Patterson et al., 2008).

The objective of this study was to investigate the processing effect of roasting and extrusion on Se bioavailability from soybean for young pigs.

MATERIALS AND METHODS

Soybean processing: Both corn and soybeans were produced in Hanyuan County, a Se-deficient area in Sichuan, China. A domestically-made roaster was used to dry-roast soybean at 120 °C for 30 min. Soybean was extruded with a commercial extruder (TPH200, Muyang, China) at 150 °C for 15 s. The processed soybeans were ground through a hammer mill equipped with a 2 mm screen and then mixed with other ingredients to form diets.

Animals and diets: The Animal Care Office of Sichuan Agricultural University approved the animal trial protocol. 20 PIC [(Landrace × Meishan × Duroc) X (Pretriain × Yorkshire) weanling male piglets were fed on a Se-deficient diet (Diet I, Table 1) for 4 weeks to adjust body Se status. Subsequently these pigs (10.36 ± 0.96 kg) were randomly divided into 2 groups (n = 10) and fed on corn-extruded soybean diet (Diet II, Table 1) or corn-roasted soybean diet (Diet III, Table 1), respectively. All nutrient requirements except for Se in diets were formulated with the reference to NRC (1998). Se in soybean and corn were 50.23 ± 4.21 μg kg-1 and 8.15±2.13 μg kg-1, and Se concentration in diet I, diet II and diet III were 30.25 ± 4.07 μg·kg-1, 40.09 ± 3.53 μg·kg-1 and 43.09 ± 4.13 μg·kg-1 (n = 3), respectively. The trial lasted for 8 weeks, and feed intake and animal body weight were recorded.

Sample collection: Blood was collected via the anterior vena cava in heparin-coated tube initially (0 wk) and at the end of the trial (8 wk). Plasma was separated by centrifuging blood (Eppendorf 5804R, Germany) at 1500 g for 10 min at 4°C. Pigs were housed individually, and the house temperature was controlled at 24 ± 4°C. Twelve pigs (n = 6 per group) with similar body weight were stunned and killed at the end of 8 weeks. The liver, muscle and pancreas were rinsed with ice-cold phosphate buffered saline (pH 7.2), frozen in liquid nitrogen and stored at -80°C until analysis. A 6-day digestion trial was conducted to test the apparent digestibility of dietary Se at the end of 7 weeks of the trial. All faeces from each pig were collected, mixed with 10% (v/v) HCl (1.2 mol L-1) and frozen at -20°C. Faeces were dried at 105°C and ground through 40-mesh screen before Se determination.

The apparent digestibility (AD) of dietary Se was

calculated using the equation: AD = (Se feed - Se faeces) × 100 / Se feed

Where Se feed and Se faeces refer to the amount of Se in feed and faeces, respectively

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Table 1: Composition of diets fed to pigs selenium trial.

Ingredients (g kg^{-1})	Diet I	Diet II	Diet III
Corn	552.4	688.0	688.0
Soybean	*Extruded*[1] 280.0	*Extruded*[1] 280.0	*Roasted*[1] 280.0
Whey Powder	120.0	-	-
Acidifier	2.0	-	-
Salt	3.0	3.0	3.0
Dicalcium Phosphate	16.0	7.0	7.0
Limestone	5.0	4.0	4.0
Amino acid premix[2]	10.6	6.5	6.5
Mineral Premix[3]	10.0	10.0	10.0
Vitamin Premix[4]	1.0	1.0	1.0
Nutrients composition			
Metabolic Energy (MJ kg^{-1})	13.4	13.4	13.4
Crude Protein (g kg^{-1})	177.0	155.0	155.0
Calcium (g kg^{-1})	8.3	6.0	6.0
Available Phosphorus (g kg^{-1})	5.5	3.1	3.1
True Digestible Lysine (g kg^{-1})	11.6	10.0	10.0

[1]*Extruded* and *roasted* in the table indicate the soybean was extruded or roasted, respectively; [2]Amino acid premix provided per kg of the diet: L-Lys, 4.0g; DL-Met, 0.5g; L-Trp, 0.5g; L-Thr, 1.5g; [3]Min premix provided per kg of diet:100.0 mg Fe ($FeSO_4$), 5.0 mg Cu ($CuSO_4.5H_2O$), 5.0 mg Mn ($MnSO_4.2H_2O$), 100.0 mg Zn ($ZnSO_4.7H_2O$) and 0.2mg I (KIO_3); [4]Vit premix (Xuke Corp, China) provided per kilogram of diet: 3000IU retinol acetate, 300IU cholecalciferol, 20IU tocopherol, 0.5mg menadione, 2mg thiamin, 5mg riboflavin, 20mg niacin, 3mg pyridoxine, 0.1mg biotin, 0.6mg folic acid and 0.04mg cobalamin.

Chemical analysis: Tissue samples were homogenized in the buffer containing 20 mmol L^{-1} Tris-HCl (pH 7.4), 0.25 mol L^{-1} sucrose, 0.1% Triton X-100, 0.03 mmol L^{-1} EDTA, 1mmol L^{-1} PMSF and 5μg·mL^{-1} pepstatin A. The homogenate was centrifuged (Eppendorf 5804R, Germany) at 20,000 × g for 60 min at 4°C, and the supernatant was used for cytosolic glutathione peroxidase (GPX1) activity analysis. Activities of GPX1 and plasma glutathione peroxidase (GPX3) were measured in the coupled assay with NADPH oxidation using hydrogen peroxide as substrate (Lawrence,Sunde,Schwartz & Hoekstra, 1974). Enzyme activity was expressed as nmole of glutathione oxidized per min per mg protein. Plasma alanine aminotransferase (ALT) activity was determined with a kit (C009, Nanjing Jiancheng Bioengineering Institute, China). After digestion of tissues and faeces with concentrated acid mixture (HNO_3:$HClO_4$, 4:1), Se was determined with hydride generation-atomic fluorescence spectrometry (AFS-830, Titan Co., China) (Li &Guo, 2005).

Statistical analysis: Values were expressed as means ± SD, and T-test was used to compare means. The significance was set at $P \leq 0.05$, and all data was analyzed with SAS 8.2 (SAS Inst., Inc., Cary, NC).

RESULTS AND DISCUSSION

The final body weight, daily gain and feed efficiency of pigs fed on extruded soybean were all significantly higher ($P < 0.05$) than those of pigs fed on roasted soybean (Table 2). This was consistent with the report that soybean/soybean meal extrusion is more efficient than roasting in improving pigs (Faber & Zimmerman, 1973; Marty *et al*., 1994) or chick (Marsman *et al*., 1997) performances. This is because nutrients such as energy and amino acids are less bioavailable from roasted soybean than from extruded soybean (Faber & Zimmerman, 1973; Marty *et al*., 1994). This observation confirmed the animal model used in this study was appropriate

Table 2: Effect of extruded and roasted soybean on pig performance.

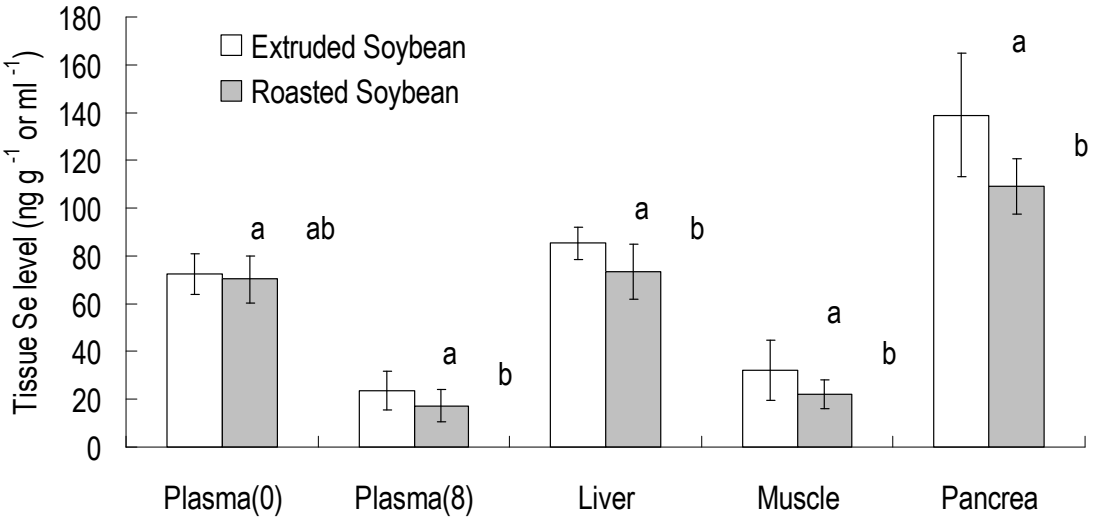
Parameter	Extruded soybean	Roasted soybean
Initial weight (kg)	10.30 ± 0.82	10.36 ±1.25
Final weight (kg)	47.30 ± 3.23[a]	44.73 ± 3.33[b]
Feed intake (g d^{-1})	1258.16 ± 89.38[a]	1173.35 ± 91.56[a]
Daily gain (g d^{-1})	665.38 ± 51.90 [a]	600.29 ± 46.27 [b]
Feed efficiency (G /F)	0.54 ± 0.02 [a]	0.50 ± 0.01 [b]

Values in a row followed by different superscript are significantly different at $P < 0.05$, n=10.

The apparent digestibility of dietary Se from extruded soybean was 18.49 ± 3.7%, which was significantly higher ($P < 0.05$) than that from roasted soybean, 13.56 ± 4.09%. This suggested that Se from extruded soybean was more easily absorbed than Se from roasted soybean. However, the two values were significantly lower than the reported absorption value of 80-90% in soy protein (Mason & Weaver, 1986). This is probably because some endogenic Se could be excreted from bile into the gut along with digestive enzymes. Se concentration in pig bile is more than 10 μg· g^{-1} even when pigs were fed on a Se-deficient diet (Kim & Mahan, 2001), and the high level Se in liver bile increase faeces Se level when Se is excreted into gut. So even with the trace Se excreted from bile at low Se level, the excreted Se will significantly decrease the apparent digestibility.

The standard way for assessing Se bioavailability is to compare the potential of different sources of Se in restoring tissue Se retention and Se-dependent enzyme activity (Cases *et al*., 2001; Shi & Spallholz, 1994). Initial plasma [plasma (0)] Se concentration was similar between pigs, while Se concentration after 8 weeks in [plasma (8)], muscle, liver and pancreas of pigs fed on roasted soybean were 60.60% ($P < 0.01$), 63.09% ($P < 0.01$), 81.30% ($P < 0.05$) and 78.54% ($P < 0.05$), respectively, of pigs fed on extruded soybean (Figure 1).

Consistent with tissue Se level, GPX3 activity in initial plasma was similar between the 2 groups (Figure 2), and the GPX3 activity in final plasma as well as GPX1 activity in liver, muscle and pancreas of pigs fed on extruded soybean were 222.04% ($P < 0.01$), 201.88% ($P < 0.01$), 180.70% ($P < 0.01$) and 177.94% ($P< 0.01$) of pigs fed on roasted soybean, respectively. Liver was the biggest Se pool in the body, and its Se level and GPX1 tightly fluctuated with dietary available Se (Cases *et al*., 2001; Kim & Mahan, 2001). The major chemical form of Se in soybean is selenomethionine, and Se concentration in plasma (Burk *et al*., 2006) and muscle (Reeves *et al*., 2005) significantly altered with dietary available selenomethionine



level.

Figure 1: Selenium (Se) concentration in different body tissues of pigs fed on roasted and extruded soybean. Values are means ±SD. For the same type of tissue, bars with different letters are significantly different (P<0.05), n=6.

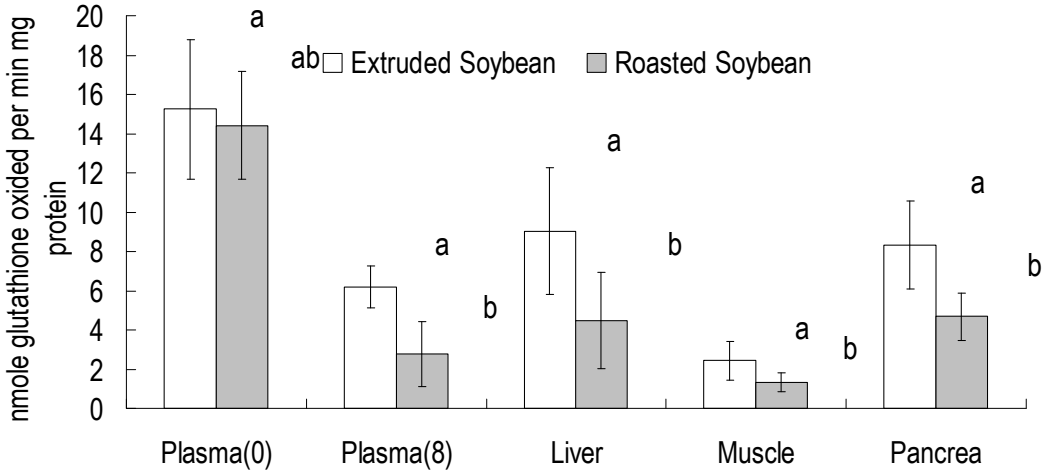


Figure 2: Glutathione peroxidase (GPX) activity in different tissues of pigs fed on extruded or roasted soybean. For the same type of tissue, bars with different letters are significantly different (P < 0.05), n = 6.

As an important endocrine gland, the pancreas has higher hierarchy of Se utilization than the liver and muscles (Schweizer *et al*., 2004). Our data also supported this tissue priority of Se utilization as evidenced by more Se and higher GPX1 activity in pancreas than in liver and muscle of pigs fed on extruded or roasted soybean. Even so, the pancreas of pigs fed on extruded soybean had higher Se retention and GPX activity than that of pigs fed on roasted soybean. These differences of tissue Se retention and Se-dependent GPX activity between pigs fed on roasted and extruded soybean suggested that more Se in extruded soybean than in roasted soybean could be available for tissue retention and GPX expression.

Alanine transarninase activity in initial plasma was similar in the pigs, but both roasted and extruded soybean significantly raised plasma ALT activity after 8 weeks (Figure 3**)**. However, plasma ALT activity in pigs fed on roasted soybean was about 5 fold higher (P < 0.01) of that in pigs fed on extruded soybean. As one sensitive indicator of liver cell membrane integrity, plasma ALT activity would increase when liver cell is exposed to oxidative damage (Cheng *et al*., 1999). In Se-deficient animals, liver cell is actually exposed to oxidative stress (Wu & Huang, 2004) because the bulk of hydrogen peroxide can not be scavenged owing to the decreased hepatic GPX1 activity (Lei *et al*., 2006). More liver Se retention as well as lower plasma ALT in pigs fed on extruded soybean suggested that more Se in extruded soybean was available for liver GPX expression.

In conclusion, we have provided 3 lines of solid evidence to demonstrate that more Se was available for young pigs from extruded soybean than from roasted soybean. This could probably be extrapolated to other foods of plant origin, especially for Se-enriched food. These results have given us insight into the implications of some of the methods used to properly process these foods.

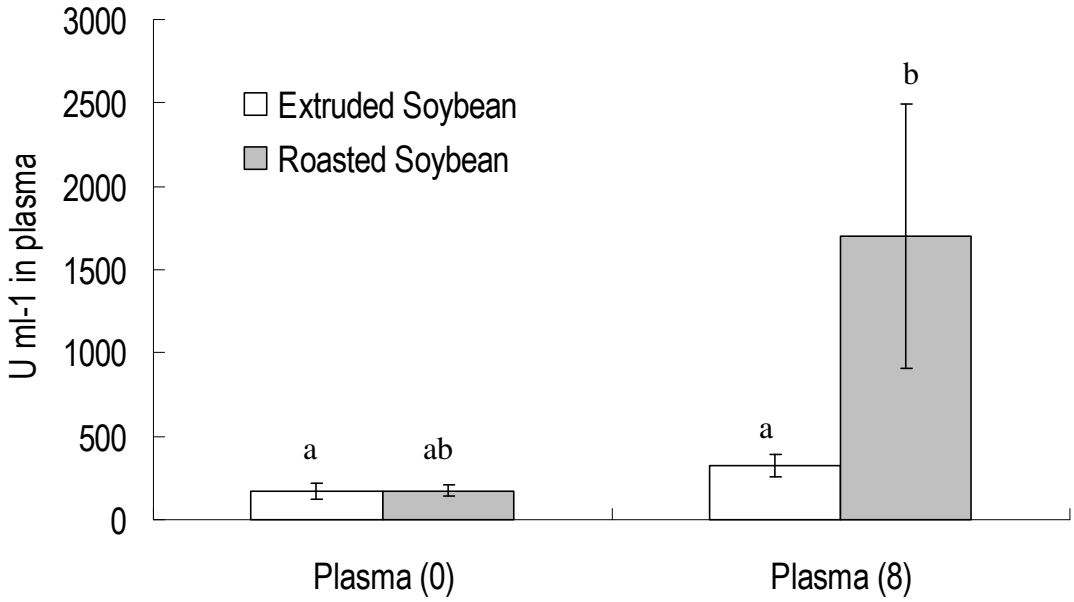


Figure 3: The Alanine transarninase activity in plasma of pigs fed on roasted and extruded soybean at different time. Bars with different letters are significantly different (P < 0.05), n = 6.

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