Nutritional strategies to increase the selenium and iron content in pork and promote human health

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Report prepared for the Co-operative Research Centre for an Internationally Competitive Pork Industry

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Executive Summary

Rationale:
Earlier studies have shown that dietary manipulation can enrich the specific nutrient component or composition in tissues of farm animals. The key objectives of this project were to examine whether the selenium and iron contents in fresh pork can be enriched by dietary manipulation and to evaluate the bioavailability and protective efficacy of Se-enriched pork against chemically-induced colorectal cancer in an animal model.

Outcomes of the project:

- Feeding of organic selenium supplement significantly increased selenium content of pork compared with the control pigs that were fed a wheat-based ration. Feeding of an organic iron supplement did not increase iron content in pork, however the female pigs had a higher level of muscle iron in pork than the male counterparts.
- When fed to rats, selenium enriched pork significantly reduced the number of putative preneoplastic lesions in colon tissues of rats induced with azoxymethane compared with rats fed the control and normal pork diets.
- The bioavailability and efficacy of selenium was greater in rats fed Se-enriched pork than those fed control or normal pork diets.
- The protective effect of Se-enriched pork against preneoplastic lesions in colon tissues might have been due to a significant increase in blood- and tissue-Se concentration, providing a greater protection against oxidative DNA damage.

Relevance of the project's outcomes to the Australian Pig Industry:

- Selenium content of pork can be increased via dietary manipulation.
- The marketing of pork can be improved locally and internationally using Se-enriched pork as a health enhancing product.
- There is an opportunity to develop high value differentiated pork products, which adds value to the existing pork product.
- The nutritional manipulation of Selenium in pork did not affect growth performance, efficiency of production or meat quality.

Further studies are needed to understand the effect of Se-enriched pork in preventing different stages of cancer progression and the mechanism by which these protective effects are mediated.

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1. Introduction

Minerals in diet are an essential factor for growth and development of individuals and maintenance of healthy life. The bioavailability and absorption of minerals from various food sources has been studied in animals (chickens, rats, sheep) and humans and is far from conclusive. The bioavailability and absorption of minerals such as selenium (Se) and iron can vary with the food source, composition of the diet, chemical forms, levels of other dietary components including vitamins, minerals, fibre, secondary plant compounds and the nutritional status of the individual. The impact on iron bioavailability and absorption by these factors, alone, or in combination, may be highly variable depending on the species studied. The role of Se-enriched diets as cancer preventing agents have been pursued in many human and animal studies (Hill 1997; Reddy, Rivenson et al. 1997; Rao, Wang et al. 2001; El-Bayoumy, Sinha et al. 2006; McIntosh, Royle et al. 2006) and are still far from conclusive.

Previous studies have indicated that dietary enrichment of Se in dairy cows increased Se bioavailability and had preventive effects against some forms of cancer and influenza. The dietary intake of Se in many countries including Australians is well below the recommended daily intake of 70 µg/day. The recommended daily intake of selenium from human diets for a protective effect against cancer is 200 µg/day. Improving the mineral content of Australian pork with Se and iron may potentially add value to the existing product, which is beneficial to general population and producers in terms of health maintenance and high valued product, respectively which may improve the marketability of pork.

The objectives of the study were:

1. To investigate the strategy to increase Se and iron content in pork through feeding systems;
2. To evaluate the effects of Se and iron enrichment in pork on meat quality
3. To determine the bioavailability of Se in rats fed with Se-enriched pork
4. To determine tumour progression and development in colon tissues of rats induced with azoxymethane, using molecular techniques and histological/ immunohistological examination

The report will be completed with the available data as Dhammika Jayasooriya is still conducting laboratory assays. These are expected to be completed by September 2009.

2. Methodology

Two experiments were conducted at the research facilities, Department of Primary Industries, Werribee 3030, Victoria. In Experiment 1, the effect of dietary manipulation of selenium (Se) and iron (Fe) in pork was investigated in finisher pigs using organic selenium and organic iron supplements. In Experiment 2, the bioavailability and efficacy of Se-enriched pork on the prevention of azoxymethane induced colon cancer in a rat model for humans was investigated. Another preliminary research project was funded by the Pork CRC to identify whether the iron content of pork can be manipulated by using inulin and organic iron supplementation and determine whether the gender effect observed on

muscle iron content in Experiment 1 was consistent. The final report of this study will be submitted in September 2009 as a separate document.

2.1 Experiment 1: Se and Fe enrichment in pork and analytical studies

Animals and Handling

Thirty six cross-bred pigs (Large White x Landrace) (initial weight, 56.6 ± 5.4 kg) were allocated to 12 pens (3 pigs/pen) based on body weight and sex. The pens were then randomly allocated to one of six dietary treatments consisting of: 1) Basal: 0.13 mg/kg Sodium selenite + 50 mg/kg $FeSO_4$; 2) 3 mg/ kg Diamond V Se; 3) 9 mg/kg Diamond V Se; 4) 100 mg/kg SQM iron; 5) 1000 mg/kg SQM iron and 6) 3 mg/kg Diamond V Se + 100 mg/kg SQM iron per kilogram of feed. A wheat-based diet was used as a basal diet for all animals. Pigs were given *ad libitum* access to experimental diets over a period of 28 days. The basal diet was formulated to meet National Research Council requirements for the grower/finisher pigs.The type and level of ingredients used for the formulation of experimental diets and the dietary protein, energy and mineral contents of the diets are given in Table 1. Dietary crude protein, energy and essential mineral contents in the diet were maintained according to the recommendation given for the growth and development of finisher pigs. Pigs were housed in a building with completely slated concrete floors and each pen was 1.52 x3.05 m, allocating around 0.77 m^2 per pig. Pigs were weighed and feed intakes were recorded weekly to calculate average daily gain (ADG) and average daily feed intake (ADFI). The experimental protocol was approved by the Animal Ethics Committee, Department of Primary Industries for all procedures involving animal care, slaughter procedure and tissue sampling.

Slaughter and Data Collection

At the end of the feeding period, pigs were transported (0.5 km) to the Meat Research & Training Centre slaughter facilities. After an overnight fasting (12 h) with unlimited access to water, pigs were exsanguinated using CO_2 stunning, hair removed and eviscerated. Samples (50 ± 5 g) of internal organs (liver, kidney, spleen, and pancreas) and muscle biopsies (50 ± 5 g) from *Longissimus thoracis* (Loin) and *Biceps femoris* (Ham) were collected and frozen at -20°C for further analysis. The carcasses were weighed and hung overnight at 4°C before being split in half. At 24 h post-mortem, loin muscle was collected from the left side of the carcass. Samples from loin collected at the same location were used for the following measurements described below.

Drip Loss

At 24-hr post mortem, duplicate 80 g samples were obtained, wrapped in netting square and suspended for 48 hours in a plastic bag at 4°C as described by Honikel (1998) and drip loss determined.

pH

The pH of the muscle was measured with an ionode electrode (Model No. IJ44 Tennyson, Qld, Australia) fitted to a digital pH meter (Jenco pH Vision Model 6007, Jenco Instruments, San Diego, CA).

Muscle Colour

A Minolta Chroma Meter CR-200 colorimeter (Minolta Corp., Ramsey, NJ) was used for colour measurements. Muscle samples maintained under retail display were allowed to bloom for at least 30 min at 4°C before the measurements were taken

in triplicates on different locations of the exposed meat surface. Measurements were recorded in the CIE colour convention, with outputs of *L** (lightness/darkness), *a** (red/green), *b**(yellow/blue) as reported (Young et al. 1999).

Warner Bratzler Shear Force and Cooking Loss

Meat tenderness was determined as Warner Bratzler shear (WBS) at day 1, 3 and 5 post-slaughter. Frozen samples (100 g) were thawed overnight and were cooked in a 80°C water bath until the internal temperature reached 71°C (35 minutes as determined from earlier studies), were allowed to cool for 30 min under running tap water, and lightly dried. After cooking and cooling, 6 strips (10 x 15 cm) were cut parallel to the muscle fibers from each sample. Each strip was sheared using a WBS blade fitted to a Lloyd LF*Plus* Texture Analyser (Lloyds Instruments Ltd., Fareham, Hants, England), with a cross head speed of 100 mm/min. The peak force (N) required to shear the muscle fibers was measured and the mean shear data from six measurements were used in the meat tenderness data analysis.

Retail Shelf-life

At 24 hour post mortem, 4 loin slices were placed on foam trays and then over - wrapped with polyethylene film and displayed for 0, 1, 3 and 5 days in a retail display unit (Shelly 120, A.J. Baker & Sons Pty. Ltd., WA, Australia). Subjective measures were made using standard templates (National Pork Producers Council 1991) for assessment of the visual parameters; colour, marbling, exudate and overall grade on a scale of 1-5. For marbling, 1, none; 2, traces; 3, slight; 4, moderate; 5 abundant. For colour; 1, pale pinkish grey; 2, greyish pink; 3, reddish pink; 4, purplish red; and 5, dark purplish red. For exudates; 1, none; 2, traces; 3, slight; 4, moderate; and 5, abundant. For grade; 1, unsatisfactory; 2, below average; 3, average; 4, above average and 5, premium.

Sample Analysis for Se and Iron

Samples of muscle (loin and ham) and liver were analysed for Se and iron content at the State Chemistry Laboratory, Department of Primary Industries, Werribee, Victoria. Selenium content was determined following digestion of sub-samples in a mixture of nitric and perchloric acid using test tubes fitted with reflux funnels in an electrically heated digestion block. At the final stage of the digestion process, hydrochloric acid was added to the tubes to reduce selenium (V) to selenium (III), with the final matrix being 30% hydrochloric acid. The digesta were diluted (to 20 mL final volume) prior to analysis using Inductively Coupled Plasma Mass Spectrometry/Vapour Generation (ICP-MS/VG). Both Standard Reference Material (SRM) and Laboratory Control Samples (LCS) were carried through all analyses. The SRM's were NIST standard 1549 (non-fat milk powder) and NIST standard 1577b (bovine liver). 'In-house'-prepared LCS were ovine kidney, chicken meat and non-fat milk powder. Three blanks were also included in the digestion and analysis process in each run that was performed on the ICP - MS/VG system to increase sensitivity.

2.2 Experiment 2: Bioavailability of selenium enriched pork and colon cancer protection in rats: used as a model for humans

Animals

Sprague Dawley rats were used in the study because of their known sensitivity to the carcinogen, azoxymethane (AOM). The animals were obtained from the Monash University breeding facility in Clayton, Victoria, from a specific pathogen

free environment to an ISO 9002 level of quality assurance. Ninety eight of 28-day old male rats were housed in wire cages at Werribee Small Animal House facility, Department of Primary Industries. The animal house facility provides a clean air-conditioned environment with a 12 hour light: dark cycle and temperature maintained at 23 ± 1^{o}C throughout the experimental period from Nov. 2007- April 2008. Animals were allocated randomly into 4 experimental groups of 24 rats and housed in wire cages of 3 per cage, with appropriate bedding material provided. Approval was granted by the Animal Ethics Committee, Department of Primary Industries to undertake this study.

Diets

Experimental diets for the animals were formulated according to the guidelines recommended by American Institute of Nutrition (AIN-93 G) and prepared by Specialty Feeds (Glen Forrest, Western Australia). Selenium enriched pork produced in the previous feeding study (AEC protocol 2752) conducted in July - September 2006 was used to prepare the pork-based diets for this study. The pork cuts from loin and ham were minced, patties made with 70-80 g mince (approximately 1 cm thick) and cooked on a hot plate to an internal temperature of 75 °C. The cooked patties were freeze dried, ground and the dried homogenized meat components were used to prepare the experimental diets. The control diet was prepared using casein as the protein source. The protein content of the rat diets was balanced across the treatments to 18%, either with pork, casein or a combination.

The treatments consist of:

1. Control, standard AIN 93G diet
2. Standard AIN 93G ingredients supplemented with non-Se enriched pork
3. Standard AIN 93G ingredients supplemented with Se enriched pork
4. Standard AIN 93G diet supplemented with selenised yeast (Diamond V, Feedworks Pty. Ltd.).

Feed was offered at *ad libitum* level over the 21 weeks and purified water was available at all times.

Experimental Procedure

At the commencement of experimental study, two rats were killed to assess initial blood and tissue Se status. Blood samples were collected via tail bleeding approximately at 1% body weight for blood/plasma parameters. Body weight of the rats was recorded weekly. After feeding experimental diets for 4 weeks, 3 rats from each treatment were killed using carbon dioxide anaesthesia and exsanguinated. Blood was collected by cardiac puncture procedure into lithium heparinized vacutainer tubes. Blood collected in vacutainer tubes were centrifuged at 3000 g for 10 min and plasma was separated and stored at -80°C for subsequent analysis. Liver and skeletal muscle were collected, frozen in liquid nitrogen and stored at -80 °C. The remaining animals from all dietary treatments (21 rats/treatment) were subcutaneously injected with azoxymethane (AOM, St Louis MO) at a low (15 mg/kg) dose rate, once weekly for 2 weeks (week 4 & week 5) to induce colon cancer. The dietary treatments were continued for the following 15 weeks. Blood samples were collected from the rats by tail venipuncture into heparinized eppendorf tubes at the start, 2 weeks, 4 weeks, 10 weeks and 18 weeks of feeding. Blood samples were centrifuged at 3000g for 10 min; plasma was collected and stored at -80 °C until further analysis. At the end of the study i.e., after 21 weeks on experimental diets, rats were killed by using carbon dioxide anaesthesia and exsanguinations. Blood was collected by cardiac

puncture procedure into EDTA treated vacutainer tubes for hematology, and into lithium heparinized vacutainer tubes for other measures. Blood collected in lithium heparinized tubes were centrifuged at 3000 g for 10 min; plasma was collected and stored at -80 C for subsequent analysis of plasma Se and glutathione peroxidase assays. Four centimetre long sections of colon (distal to the Peyer's patch) were removed and immediately frozen in liquid nitrogen and stored at -80°C for subsequent DNA extraction and other enzyme assays.

Aberrant crypt foci (ACF) assay

The remaining sections of colon were used to examine the aberrant crypt formation according to the method established by Bird (1987), Bird (1995) and McLellan and Bird (1988). The colons were removed from the caecal end, slit opened longitudinally, laid flat on Hybond-C Nitrocellulose membrane (Amersham Corporation, Arlington Heights, IL) and were carefully washed with phosphate buffered saline (PBS; pH 7.4). They were then fixed in 10% phosphate buffered formalin for 24 hours, and were transferred into 70% ethanol solution until further use for histological evaluation. The colons were stained with 0.2% methylene blue for 3-5 min and the mucosal surfaces were examined under a light microscope at 40x magnification for lesions or crypts formation. Aberrant crypts were identified by alterations in the size, shape and the intensity of the stain. Aberrant crypts were scored blind in duplicates and recorded as small for ACF with one crypt, medium for ACF with 2 crypts and large for ACF with 3 or more crypts.

Biochemical assays

Plasma Se level was determined using the fluorometric assay described by Sheehan and Gao (1990). The Se levels in diets, and tissues of colon, muscles and liver were analysed using wet acid digestion (perchloric/nitric acid) at the State Chemistry Laboratory, Werribee Victoria as reported above in this section. Total iron content in blood, muscle and liver were also determined at State Chemistry Laboratory, Werribee, Victoria as reported. For the determination of Glutathione peroxidise activity in plasma, the glutathione peroxidise assay kit (Cayman, 703102, Australia) was used. The enzyme activity was determined by monitoring the disappearance of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm at 25°C and activity expressed as nanomoles of NADPH oxidised per millilitre per minute.

3. Outcomes

Enrichment of pork with selenium and iron through dietary manipulation

Table 1 - Ingredients and chemical composition of rations used for finisher pigs[a]

Ingredients	[b]*Basal (g/kg)*	[b]*Diet2 (g/kg)*	[b]*Diet3 (g/kg)*	[b]*Diet4 (g/kg)*	[b]*Diet5 (g/kg)*	[b]*Diet6 (g/kg)*
Wheat 10.8 % CP	500	500	500	500	500	500
Barley 12.5 % CP	274	274	274	274	274	274
Meat and Bone Meal	100	100	100	100	100	100
Canola Meal	62.5	62.5	62.5	62.	62.5	62.5
Blood Meal	31.3	31.3	31.3	31.3	31.3	31.3
Tallow	20.0	20.0	20.0	20.0	20.0	20.0
Methionine	0.28	0.28	0.28	0.28	0.28	0.28
Di-Calcium Phosphate	7.82	7.82	7.82	7.82	7.82	7.82
Limestone	10.4	10.4	10.4	10.4	10.4	10.4
Salt	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin Mineral Premix (P210M)[c]	1.5	1.5	1.5	1.5	1.5	1.5
Diamond V (2000 ppm Se) (g)	-	1.5	4.5	-	-	1.5
SQM iron (14.6 % Fe) (g)	-	1.5	-	0.68	6.8	0.68
Analysed Values						
Dry Matter *(%)*	90.5	90.7	90.8	90.7	90.6	90.7
Crude Protein (N x 6.25) *(%)*	20.4	21.0	21.3	20.9	20.4	20.4
Acid Detergent Fibre *(%)*	5.5	5.6	5.8	5.9	5.9	5.1
Digestible energy (MJ/Kg)#	13.6	13.6	13.6	13.6	13.6	13.6
Ash *(%)*	5.1	5.4	5.3	5.3	5.5	5.9
Se (μgkg^{-1})	290	3300	9300	475	350	3400
Iron ($mgkg^{-1}$)	440	380	425	475	1430	530
Diamond V (2000 ppm Se)	1.9 ppm					
SQM iron (14.6 % Fe)	12 % Fe					

[a]Diets were offered at *ad libitum* level at all times.

[b]Diet 1: Wheat based basal diet (Basal); [b]Diet 2, Basal + Diamond V selenised yeast (3ppm Se); Diet 3, Basal + Diamond V selenised yeast (9ppm Se); Diet 4, Basal + SQM iron (14.6 %) (100 ppm iron); Diet 4, Basal + SQM iron (14.6 %) (1000 ppm iron); and Diet 6, Basal + Diamond V selenised yeast (2000 ppm) and SQM iron (14.6 %) (100ppm iron and 3 ppm Se).

[c]0.13 mg/kg Sodium selenite and 50 mg/kg $FeSO_4$. #Calculated values.

Feeding of Se and iron supplemented diets was conducted during October to November 2006 and the pigs were slaughtered at the Meat Research and Training Centre, Werribee facilities. Dietary Se or iron supplements did not affect (P>0.05) growth performance of pigs or the meat quality parameters such as tenderness (Warner Bratzler [WB] shear force), cook loss or surface colour (Table 2). However, WB shear force values of pork from pigs fed a combined Se/Fe supplement (Diet 6) tended to be higher than for pigs fed diets 1-5.

Table 2 - The effect of dietary selenium and iron supplementation on growth performance of pigs, and pork tenderness, cook loss and colour in finisher pigs fed a wheat-based diet

	Dietary treatments						*Statistics*	
	[b]Basal	[c]Diet2	[c]Diet3	[c]Diet4	[c]Diet5	[c]Diet6	SE	P-value
Final live weight, kg	101	101	97.9	104	100	104	2.9	0.62
ADG, kg	1.03	1.04	1.01	1.07	0.97	1.05	0.06	0.84
WB shear force, kg	4.9	4.7	4.9	4.7	4.8	5.2	0.14	0.06
Cooking loss, %	23.7	24.3	23.7	23.9	24.7	23.2	0.72	0.77
Meat colour, *L**	48.1	48.1	49.1	48.0	47.9	49.1	0.44	0.15
Meat colour, *a**	6.5	6.3	6.1	6.1	6.0	6.6	0.17	0.09
Meat colour, *b**	4.9	4.7	4.9	4.7	4.8	5.2	0.14	0.06

[a]Values are means of six observations.

[b]Basal diet was offered at *ad libitum* to control and all other treatment groups

[c]Diet 2, Basal + Diamond V selenised yeast (3ppm Se); Diet 3, Basal + Diamond V selenised yeast (9ppm Se); Diet 4, Basal + SQM iron (14.6 %) (100ppm iron); Diet 4, Basal + SQM iron (14.6 %) (1000 ppm iron); and Diet 6, Basal + Diamond V selenised yeast (2000 ppm) and SQM iron (14.6 %) (100 ppm iron and 3 ppm Se).

Dietary Se supplementation significantly (P<0.0001) increased the Se concentration of pork muscles and organ tissues in a linear manner (Table 3). Conversely, the iron supplements had no effect on the iron content in both loin (P=0.88) and ham (P=0.14). The iron content of loin and ham muscles were higher (P=0.0004 and P=.056 respectively) in gilts than boars but levels were similar in other tissues such as kidney, spleen and pancreas (Table 4). The results indicate that there is potential for nutritional fortification of Se in pork with organic Se supplementation, whereas there are no beneficial effects of feeding higher levels of organic iron. Higher iron levels observed in gilts over the boars warrant further investigation. Further research is currently being undertaken to identify the influence of other forms of dietary iron supplementation on muscle iron content. The final report will be submitted to APL by September 2009.

Table 3 - Effect of dietary selenium and iron supplements on Se and iron content in muscle tissues (loin, ham) and other organs (Liver, Kidney, pancreas and spleen)

	Dietary treatments						*Statistics*	
	[b]Basal	[c]Diet2	[c]Diet3	[c]Diet4	[c]Diet5	[c]Diet6	SE	P-value
LM- Se, µg/kg	133	903	2433	125	132	980	57.5	0.0001
BF- Se µg/kg	138	935	2533	138	138	977	54.3	0.0001
Liver- Se, µg/kg	531	2283	6633	603	528	2317	91.5	0.0001
Kidney- Se µg/kg	1683	3133	4967	1667	1800	3000	89.5	0.0001
Pancreas- Se, µg/kg	382	1351	3567	357	362	1517	92.6	0.0001
Spleen- Se µg/kg	330	1183	3083	330	332	1250	40.3	0.0001
LM- Iron, mg/kg	12.3	12.5	12.3	12.2	11.7	13.2	0.71	0.79
BF- Iron, mg/kg	11.7	14.8	9.3	10.0	10.8	12.3	0.72	0.15
Liver-Iron, mg/kg	217	313	217	307	347	297	26.4	0.03
Kidney-Iron, mg/kg	39.5	46.5	36.3	44.2	42.3	43.3	3.6	0.41
Pancreas-Iron, mg/kg	16.5	19.0	17.7	21.2	18.5	17.2	1.8	0.55
Spleen-Iron, mg/kg	182	247	220	240	248	220	27.2	0.53

[a]Values are expressed in mg/kg and average of six observations.
[b]Basal diet was offered at *ad libitum* to control and all other treatment groups
[c]Diet 2, Basal + Diamond V selenised yeast (3ppm Se); Diet 3, Basal + Diamond V selenised yeast (9ppm Se); Diet 4, Basal + SQM iron (14.6 %) (100 ppm iron); Diet 4, Basal + SQM iron (14.6 %) (1000 ppm iron); and Diet 6, Basal + Diamond V selenised yeast (2000 ppm) and SQM iron (14.6 %) (100 ppm iron and 3 ppm Se).

Table 4 - Effects of dietary organic iron supplementation on muscle tissue (loin, ham) and liver tissue iron concentration[a]

Tissue Type	*Finisher Pig Diets*						*Statistics*	
	[b]Basal	[c]Diet2	[c]Diet3	[c]Diet4	[c]Diet5	[c]Diet6	*SE*	*P value*
Loin								
Gilts	13.3	13.6	13.0	13.3	14.0	14.0	1.0	0.001[x]
Boars	11.3	11.3	11.3	10.3	10.3	12.3		0.79 [y]
								0.94 [z]
Ham								
Gilts	13. 0	18.8	9.3	13.7	9.7	12.3	1.9	0.05 [x]
Boars	10.3	11.7	9.3	8.0	10.3	12.3		0.15 [y]
								0.38 [z]
Liver								
Gilts	280	377	233	407	313	303	37.3	0.01 [x]
Boars	243	250	200	287	300	290		0.03 [y]
								0.45 [z]

[a]Values are expressed in mg/kg and average of three observations.
[b]Basal diet was offered at *ad libitum* to control and all other treatment groups
[c]Diet 2, Basal + Diamond V selenised yeast (3ppm Se); Diet 3, Basal + Diamond V selenised yeast (9ppm Se); Diet 4, Basal + SQM iron (14.6 %) (100 ppm iron); Diet 4, Basal + SQM iron (14.6 %) (1000 ppm iron); and Diet 6, Basal + Diamond V selenised yeast (2000 ppm) and SQM iron (14.6 %) (100 ppm iron and 3 ppm Se).
[x]=gender; [y]=diet; [z]=gender x diet.

Bioavailability and protective efficacy of selenium-enriched pork against chemically (azoxymethane)-induced colorectal cancer in rats

Selenium bioavailability

The study was conducted during November 2007 to April 2008. The experimental diets used during the 21 week study are given in Table 5. The nutrient composition of the diet was maintained as stated in the standard AIN 93 G, which is recommended for the growth and development of young rats.

Table 5: Composition and nutrient analysis of Standard AIN 93 G diet and other diets supplemented with normal pork (Pork), selenium enriched pork (Se-pork) and selenised yeast (se-yeast) for Sprague Dawley rats[a]

Ingredients	[b]*Treatments*			
	Control	Pork	Se-pork	Se-yeast
Sucrose	10.0	10.0	10.0	10.0
Casein (Acid)	20.0	13.3	11.8	19.9
Dried Pork (Non Se enriched)	-	7.3	-	-
Dried Pork (Se enriched)	-	-	8.6	-
Canola oil	7.0	7.0	7.0	7.0
Cellulose	5.0	5.0	5.0	5.0
Starch	40.4	39.7	39.9	40.4
Dextinised starch	13.2	13.2	13.2	13.2
di Methionine	0.30	0.30	0.30	0.30
AIN 93G Trace Minerals	0.14	0.14	0.14	0.14
Lime (fine Calcium Carbonate)	1.31	1.31	1.31	1.31
Salt (fine sodium Chloride)	0.25	0.25	0.25	0.25
Pottasium Dihydrogen Phosphate	0.68	0.68	0.68	0.68
Pottasium Sulphate	0.16	0.16	0.16	0.16
Pottasium Citrate	0.25	0.25	0.25	0.25
AIN 93G Vitamins	1.00	1.00	1.00	1.00
Choline Chloride 60 % w/w	0.25	0.25	0.25	0.25
Selenised Yeast (Diamond V)	-	-	-	0.025
Analysed Values				
Dry Matter *(%)*	89.9	91.1	93	90.4
Crude Protein (N x 6.25) *(%)*	18.9	18.7	19	18.9
Energy (ME/Kg)				
Acid Detergent Fibre *(%)*	3.0	2.7	3.2	3.7
Ash *(%)*	2.7	2.8	2.9	2.6
Se (μgkg^{-1})	220	210	680	700

[a]Ingredients of the diet were reported in g/100 g of ration and experimental diets were offered at *ad libitum* levels.

[b]Diet 1 = AIN 93G; Diet 2 = AIN 93G modified with normal Pork; Diet 3 = 93G modified with Se-enriched Pork; and Diet 4 = 93G modified with Selenised yeast.

After feeding of experimental diets for 4 weeks, 3 rats per treatment, 12 rats in total were killed for the determination of Se and iron contents of muscle and liver tissues, respectively. Preliminary results indicate that feeding Se-yeast or Se-

enriched pork significantly increased the levels of selenium in muscle and liver tissues, but iron was significantly increased in the liver with both normal and Se-enriched pork compared with other treatments (Table 6). The colorectal cancer was induced by Azoxymethane (colon cancer initiation) at 4th week (14th of December 2007) and 5th week of feeding treatment diets (21st of December 2007), respectively. AOM is a carcinogenic substance, which induces colorectal cancers at high incidence in rats, while low dose of AOM (15mg/kg) applied in this study allow a slow development of the colorectal cancer. It has been widely used with benign and malignant tumours appearing between 4-6 months after two doses of AOM (Reeves et al., 1993).

Table 6 - Selenium and iron level in muscle (*longissimus dorsi*) and liver of Sprague Dawley rats after four weeks (before azoxymethane injection) of feeding control, selenium yeast (Se-yeast), normal pork and selenium enriched pork (Se-pork) diets[a]

Tissue type	[b]Treatments					P value
	Control	Pork	Se-pork	Se-yeast	SE	
Selenium (μg/kg)						
Muscle	126.7	116.7	276.7	270.0	12.3	P<0.001
Liver	763.3	726.7	1233.3	956.7	52.8	P<0.001
Iron (mg/kg)						
Muscle	14.3	12.6	14.7	12.7	2.4	P=0.75
Liver	72.3	136.7	120.0	76.0	16.5	P<0.02

[a]Values are the mean of 3 rats per each treatment group.

[b]Diet 1 = AIN 93G (Control); Diet 2 = AIN 93G modified with normal Pork; Diet 3 = 93G modified with Se enriched Pork (Se-pork); and Diet 4 = 93G modified with Selenised yeast (Se-yeast).

Changes in body weights

Weekly live weights and feed intakes were recorded over the 21 weeks of feeding. Although rats fed Se-pork diet had greater body weights from week 9 to week 21, there were no significant differences in body weights between treatments (Figure 1). Weight gain of rats was greater during the first 4 weeks of feeding (before AOM injection), with the highest gain in week 2. Azoxymethane injection continuously reduced live weight gain in all groups. For the entire feeding period, rats had the lowest weight gain at 14th and 19th week of feeding (figure 2).

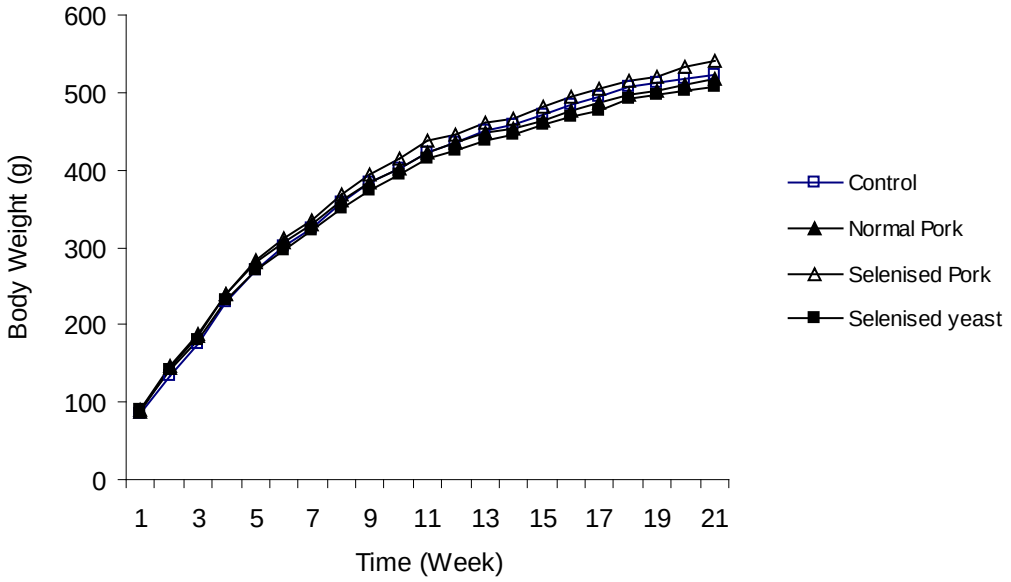

Figure 1 - Weekly body weights of rats fed control diet (CON), selenised yeast, normal pork diet (Pork) and Se-enriched pork diet (Se-Pork) over the 21 week feeding study.

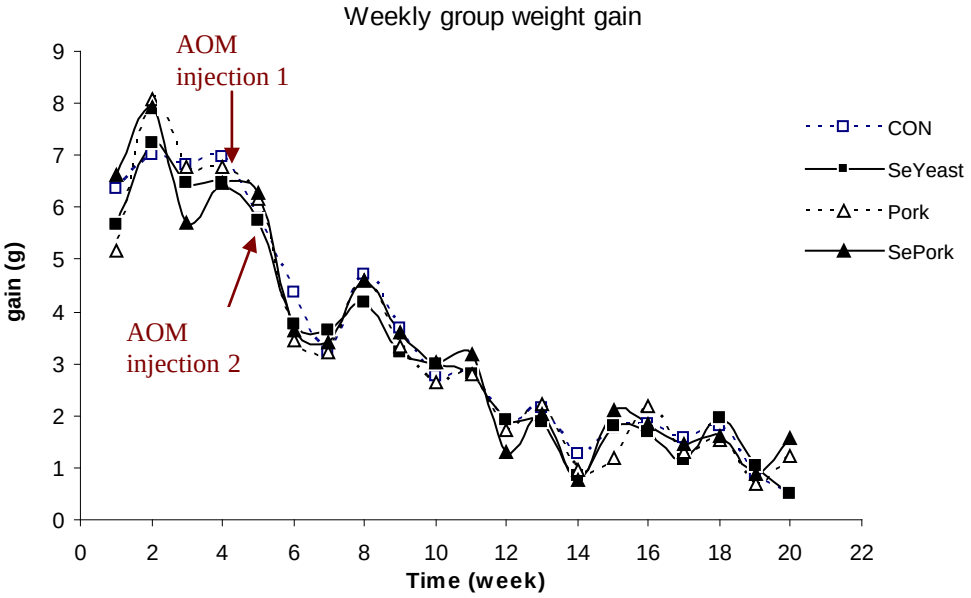

Figure 2 - Weekly weight gain of rats fed control diet (CON), selenium yeast diet (Se-Yeast), normal pork diet (Pork) and Se-enriched pork diet (Se-Pork) over the 21 week feeding study.

The selenium concentration in control, normal pork, Se-pork and Se-yeast diets were 0.07, 0.05, 0.5 and 0.5 ppm, respectively, for the entire feeding period. Rats fed the Se-pork and Se-yeast diets had higher levels of blood Se concentration than normal pork group, which in turn were higher than control group. Blood Se concentration continuously increased over the 21 week feeding period in rats in the control and Se-yeast treatments, but declined in rats in the normal and Se-

pork treatments from 14 to 21 week feeding period (Table 7). The Se concentration in muscle tissues of rats was greatest with Se-pork diet, moderate with Se-yeast diet and lowest with control and normal pork diet, respectively.

Table 7 - Selenium content in plasma over the 21 weeks of feeding and skeletal muscle at kill from Sprague Dawley rats

Component	[x]Treatments					P value
	Control	Pork	Se-pork	Se-yeast	SE	
Muscle	146.5c	133.3 d	314.6 a	258.5 b	4.4	<0.001
Plasma- Initial[y]	273.9				11.1	
Week 2	403.5 b	405.8 b	499.4 a	456.2 ab	19.9	0.009
Week 4	428.3 c	527.2 bc	569.23 ab	612.8 a	26.3	0.013
Week 14	562.3 c	597.5 bc	652.9 a	647.3 ab	16.9	0.004
Week 21	595.5 b	558.3 b	615.0 ab	674.3 a	23.0	0.016

Values are the means of 21 observations per dietary group.
[a,b,c]Within rows, means with the same superscript are not significantly different (*p>0.05*).
[x]Diet 1 = AIN 93G (Control); Diet 2 = AIN 93G modified with normal Pork; Diet 3 = 93G modified with Se enriched Pork (Se-pork); and Diet 4 = 93G modified with Selenised yeast (Se-yeast).
[y]Initial plasma Se concentration was calculated from blood samples taken from 10 rats before the commencement of dietary treatments.

Development of colorectal cancer

The experimental study in rats was completed in April 2008 and samples of colon, muscle and other organs were collected for further analyses. Colon samples were stained with methylene blue and assessed for the presence of aberrant crypt foci (ACF) as a marker of cancer progression. Se-enriched pork significantly reduced the early colon neoplasia (ACF) development compared to normal pork and tended to be lower than control diet (standard AIN 93G diet). However, it did not differ from selenised yeast diet. Histology evaluation of colon show that there was a dietary effect on cancer development of pre-neoplasia lesions in colon tissues as indicated by the number of aberrant crypts per colon (Table 8) or number of ACF per each square centimetre of colon (Figure 3). Detailed crypt development and the crypt numbers (1 or 2 or more than 3) per each focus is shown in figure 4 below.

Table 8 - Number of crypt development per Aberrant Crypt Foci (ACF) in Sprague Dawley rats induced with Azoxymethane compound[a]

Crypts/ACF	***Treatment***					***P value***
	Control	***Pork***	***Se-pork***	***Se-yeast***	***SE***	
1	***8.29***	***8.74***	***6.94***	***7.52***	***0.56***	***P=0.05***
2	***6.45***	***6.46***	***5.34***	***6.31***	***0.42***	***P=0.1***
More than 3	***6.89***	***7.17***	***5.89***	***7.29***	***0.46***	***P=0.02***
Total	***21.65***	***22.37***	***18.2***	***21.1***	***1.29***	***P=0.03***

Values are the means of 21 rats/treatment.
[a]Rats from all treatments were sacrificed after 21 weeks and colons were assessed for the formation of crypts.

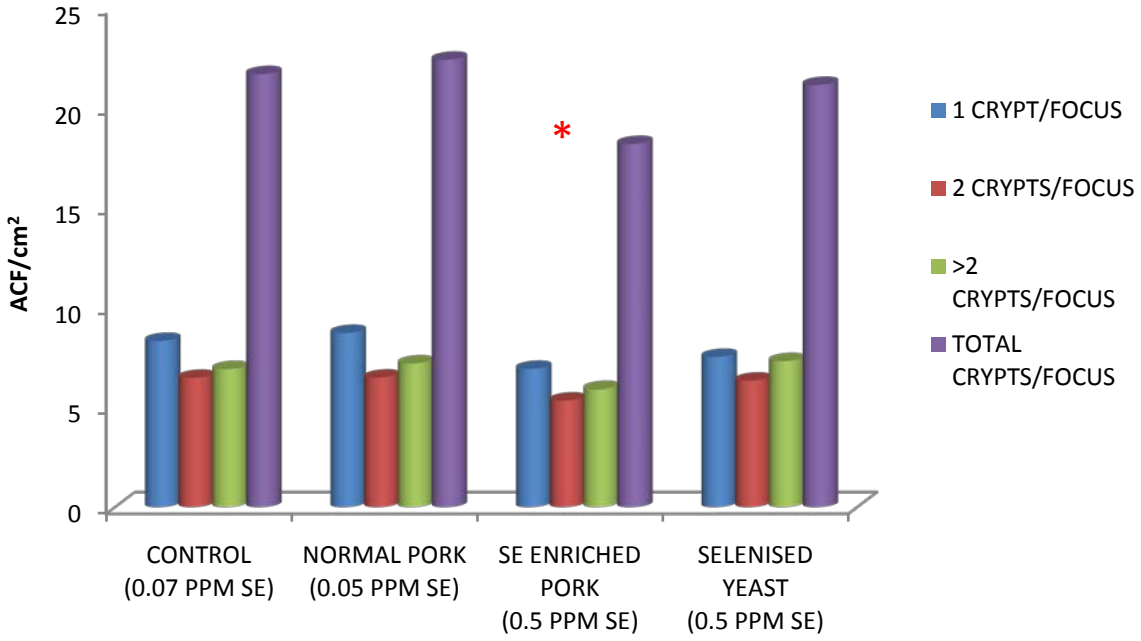

Figure 3 - Aberrant crypt Foci (ACF) development in Sprague Dawley rats induced with Azoxymethane compound

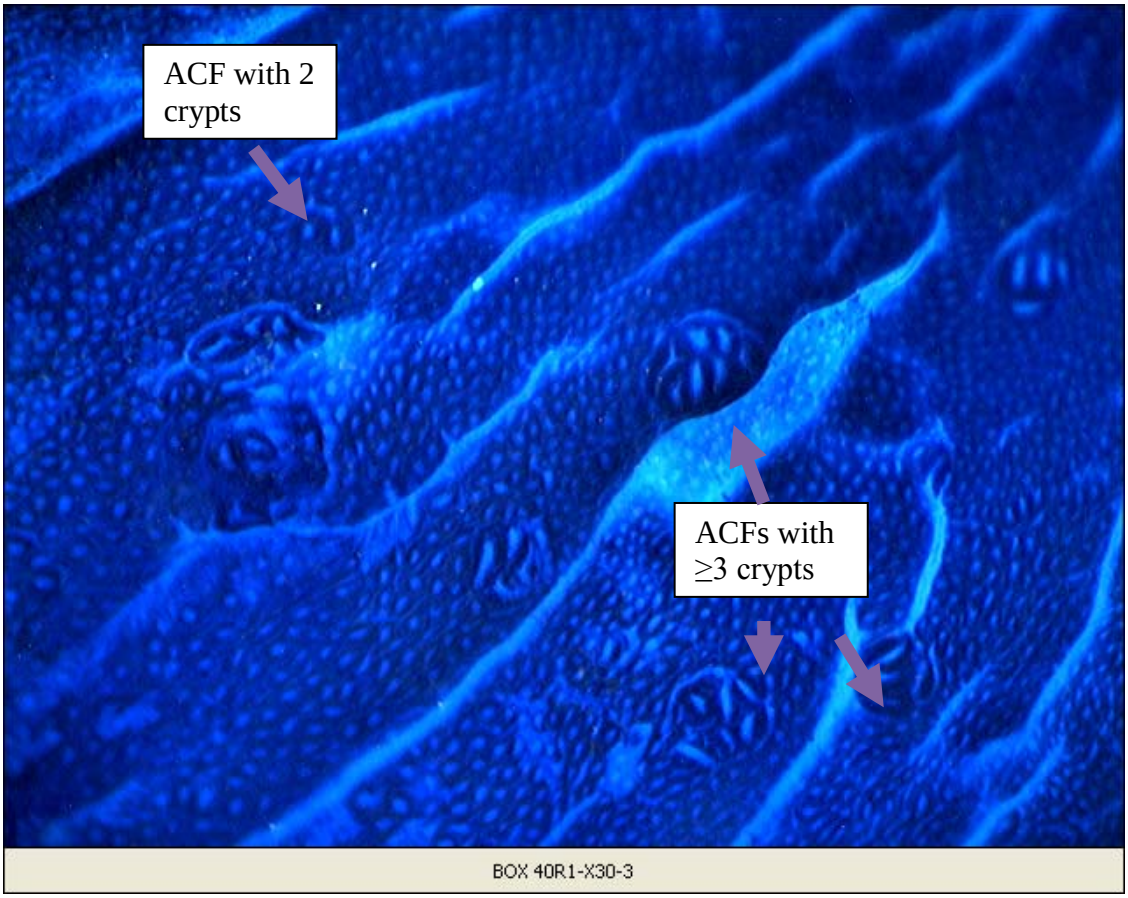

Figure 4 - Azoxymethane induced early colon neoplasia -Aberrant Crypt Foci in Sprague Dawley rats

Effect on Selenoprotein

Gastrointestinal glutathione peroxidise (GPx2) levels in the colon will be measured as an antioxidant function to provide defence against colon cancer. Analyses of gastrointestinal glutathione peroxidise (GPx2) level in the colon tissues are being currently undertaken by Dhammika Jayasooriya and will be reported by September 2009.

4. Application of Research

Opportunities uncovered by the research

1. It is possible to increase Se content, but not iron, in pork through dietary manipulation
2. Selenium enriched pork was found to be effective in preventing pre-neoplastic lesions in rats, an early biomarker of colorectal cancer
3. This observation is significant as the incidence of colorectal cancer is a significant problem in Australia and other Western populations
4. Opportunities for developing a Se-enriched high value pork products
5. Se-enriched pork can be sold as a differentiated product in the market due to its health enhancing benefits
6. Improved image of pork as part of a healthy diet

Commercialization/Adoption Strategies

- Potential benefits to cost of production

  Increasing the Se content of the pig feed will involve a small increase in the cost of feed production. The main benefits from the cost of production go to pig producers such as QAF meats or other pig producers who produce these premium pork product for a niche market that can be sold as nutrient enhanced pork. However the increased cost will be offset by increased value of products.

- Ease of adoption by producers

  The nutritional enrichment process can be adopted into the feeding systems through a premix by feed millers prior to use at piggeries. This process is most likely an integrated chain approach with feed millers and pig producers to maximize adoption likelihood.

- Impact of the research

  The direct benefits of this research will be for consumers who consume the enhanced pork product through improved long term health. Other benefit is for producers through an increased marketing of pork as a differentiated product. Less government health care cost due to better public health.

5. Conclusions

- In the first study conducted in pigs, dietary selenium supplementation significantly increased the selenium content of pork in a linear manner compared with control or non Se-diet treatments
- The organic iron supplementation did not increase the iron content of pork. However, the iron content of pork (loin and ham muscles) was higher in gilts than boars, which needs further investigation
- Neither Se nor iron supplements had an effect on growth performance of pigs, carcass characteristics, or meat quality aspects
- In the second study conducted in rats, animals from all treatments continuously grew from week 1 to week 21 with the largest increase in body weight gain during week 1-week 5
- Azoxymethane injection significantly reduced body weight gain in rats from all treatments
- Bioavailability and efficacy of selenium with Se-pork and Se-yeast diets were significantly higher as indicated by an increased level of blood and tissue (muscle & liver) selenium in rats compared with control and normal pork diets
- Selenium enriched pork diet significantly reduced the colorectal cancer development induced by azoxymethane compared with control or normal pork diets

6. Limitations/Risks

To the application of the research findings

This was the first study on the efficacy of selenium-enriched pork against colorectal cancer (A proof of concept). Further studies are needed to demonstrate:

1. Efficacy against different stages of cancer
2. Determination of intake levels (dose-response)
3. Bioavailability in human

to support the health benefits of selenium-enriched pork.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

1. Further study is warranted to examine the effect of selenium pork on protective effect against different forms and stages of cancer, including in sorts of human clinical trials
2. A detailed study needs to be carried out to understand the enrichment of iron in pork using different sources and forms of dietary supplementation and different pig genotypes

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