

## **Project: Soya bean Lecithin to improve tenderness and health active properties of pork**

<b>ID:</b>	<b>3A-109</b>
<b>Status:</b>	<b>Accepted proposal</b>
<b>Principle Investigator:</b>	<b>Professor Frank DUNSHEA University of Melbourne</b>
<b>Program:</b>	<b>Implement nutritional and genetic strategies</b>
<b>R&amp;D Budget :</b>	<b>\$55552</b>
<b>Probability of success :</b>	<b>49%</b>
<b>Project Net benefit:</b>	<b>n/a</b>

### **Aim:**

The aim of this study is to develop a differentiated pork product that delivers health benefits (lower cholesterol and saturated fatty acids) and improved eating quality (reduced chewiness). It is also possible that fat digestibility of the diet will be improved, with a resultant improvement in the utilisation of dietary fat. This project aims to determine if feeding pigs diets supplemented with soya bean lecithin will improve tenderness and eating quality of pork. The information from this experiment will be used to develop strategies to improve the eating quality of pork.

### **Background:**

Of the many demands put forth by consumers meat tenderness is an important attribute of eating quality. A piece of meat that is tough, dry and generally hard to masticate is not pleasurable to eat and will not be sold. The main contributing factor to the toughness of meat is intramuscular connective tissue, which consists of cross-linked collagen molecules. It is the cross-links that give connective tissue its tensile strength and stability (Kinsman *et al* 1994). As the animal matures, synthesis and turnover of collagen molecules decreases allowing existing fibrillar collagen to progressively cross-link. It is this maturation of collagen that contributes largely to the toughness of meat (Fang *et al* 1999).

A very important aspect of collagen is its high proportion of proline and hydroxyproline. The proportions are positively correlated with the thermal stability of collagen, due to the strong stabilising effect of the added hydroxyl groups on the triple helix (Viidik & Vuust 1980). Without hydroxyproline the polypeptide chains cannot form the triple helical structure, therefore they cannot form collagen fibres and cross-linking cannot occur (Stryer 1988). Based on this knowledge, and the fact that we cannot manipulate age, it can be stated that by decreasing the proportion of hydroxyproline in collagen molecules we will ultimately be reducing the toughness of meat.

The main enzyme responsible for the hydroxylation of proline is prolyl-4-hydroxylase (Bailey & Light 1989). A known inhibitor of this enzyme is polyenylphosphatidylcholine (PPC), an antioxidant phosphatidylcholine (lecithin) mixture which can be extracted from soya beans. PPC also has the ability to attenuate the transformation of lipocytes into collagen-producing transitional cells (Lieber 1997) and to stimulate collagenases, which break down collagen by cleaving peptide bonds (Lieber 2004). All these factors aid in the destabilisation of collagen. It has therefore been proposed that feeding pigs dietary additives of soya bean lecithin that contain PPC may decrease the development of

intramuscular connective tissue and therefore result in a more tender (as assessed by shear force and compression tests) piece of meat (D'Souza et al. 2005) while at the same time increase intramuscular fat without changing back fat. It is also envisaged that the fatty acid profile of intramuscular and adipose tissue fat will be more favourable for health and contain less cholesterol. There may be other benefits of dietary lecithin such as increased digestibility of dietary fat which may be important if we move towards high fat diets.

### **Methodology:**

The experiment will consist of 4 dietary treatments; control and soya bean lecithin supplements administered in 3 dosage treatments. The dosage has been determined by geometric progression as little is known on the effects of lecithin supplementation on connective tissue and intramuscular fat.

1. Control (commercial diet without lecithin)
2. 4 g/kg of feed of soya bean lecithin product during the grower and finisher phase
3. 20 g/kg of feed of soya bean lecithin product during the grower and finisher phase
4. 80 g/kg of feed of soya bean lecithin product during the grower and finisher phase

A total of 36 Large White x Landrace x Duroc crossbred female pigs of similar age will be used in this experiment. The pigs will be selected at 15 weeks of age, individually housed and allowed to acclimatise to the individual pens for a one week period. At 16 weeks of age pigs will be randomly allocated to one of the 4 dietary treatments and fed this diet for a six week test period. The experiment will be conducted in the Boar test facility at Riverlea Australia, Corowa under the Block Funding model. Individual growth performance (average daily gain, feed intake, feed conversion ratio) will be determined as will carcass weight and carcass P2 back fat.

A small muscle sample will be collected immediately post slaughter for subsequent gene expression analyses, while urine will be collected from the bladders after slaughter for analyses of the products of collagen breakdown.

The loin will be collected from one side of the carcass on each animal (36 animals) and aspects of pork quality (i.m. fat, muscle pH, colour, drip loss, shear force, compression tests) determined. Also, muscle hydroxyproline, proline, fatty acid and cholesterol concentrations will be determined. The expression of genes involved in collagen synthesis will also be determined. Samples of loin will be obtained and frozen to be used in consumer panel studies.

If it is demonstrated that the nutrient content (fatty acid profile, cholesterol content) of the pork can be altered then further studies will then be conducted to test the effects of pork from pigs supplemented with lecithin on aspects of cardiovascular health and diabetes in a rodent model. Pork from pigs fed lecithin will be included in a high fat diet and fed to mature rats. The study will involve rats (housed in pens of three) that will be fed a high fat diet containing pork from pigs fed the 0 and 80 g/kg lecithin diets compared to a casein diet (normal rat diet) for 8 weeks. Casein is a normal component of a rat chow but is also hypercholesterolemic compared to pork protein (Brandsch et al. 2006) so will serve as a negative control. Feed intake and growth rate will be determined on a pen basis. At the end of the study plasma will be collected for measurement of metabolite, triglyceride, hormone and cholesterol status and tissue samples will be obtained for measurement of cholesterol and expression of key genes involved in cardiovascular health.

### **Outcomes/Deliverables:**

Cost effective nutrient manipulation of pork products that promote health and wellbeing of humans. Improved digestibility of dietary fat in finisher pigs.

**Benefit To Industry:**

Increased value of pork products through reduced chewiness and improved health benefits.

A secondary (but if successful not insignificant) benefit may be improved digestibility of dietary fat through the emulsifying effects of lecithin, particularly in pigs fed high fat diets.

**Risks:**

There is very little risk that lecithin won't improve chewiness. There is some minor risk that the dose of lecithin required to improve the cholesterol and saturated fatty acid profile of pork is cost-prohibitive relative to return on carcass value. The secondary benefit of improved fat digestibility may not be as apparent in finisher pigs.

**Commercialization/Adoption:**

The Pork CRC will work with ADM (manufacturer of soy products) and a commercial, vertically integrated pork producer(s) interested in producing branded products for the retail and restaurant sector. A concurrent Honours project is assessing the willingness of consumers to pay for improved health benefits (specifically reduced cholesterol and saturated fatty acids) to inform the commercialisation process.

**Budget Justification:****Budget Justification**Total cash requested Rivalea:

\$1,500 - cost associated with analysing muscle pH, colour and drip loss from the loin (36 samples).

\$2440 - cost associated with collecting the loin from one side of the carcass (approx 3 kg/pig, 36 pigs)

\$1800 36 loin samples for Warner Bratzler testing (analyses performed in duplicate).

**Total cash requested Rivalea - \$5740**In kind 2009/10:

Salaries: Research manager 0.01 FTE @ \$120,000 = 1,200

Experimental overhead: Individual grower/finisher 36 x 49 days x \$1.35/pig = 2,381.40

Non-experimental facility overhead: \$0

Base funding agreement 2009/10

44 pig equivalents - **Base funding allocation \$98/pig = 4,312**

Costs in second year are associated with the conduct of the rat studies, chemical analyses and gene expression work. There is also a top-up of \$3500 pa for a student stipend included as the student is on a lowly paid international stipend. All laboratory work and the rat studies will be conducted by the student, so there is no request for technical support.

Funding per year	2009-10	2010-11	2011-12
<b>CRC cash requested</b>	<b>\$23,240.00</b>	<b>\$28,000.00</b>	<b>\$0.00</b>
Rivalea	\$5,740.00	\$0.00	\$0.00
University of Melbourne	\$17,500.00	\$28,000.00	\$0.00

<b>Research Organisation Contribution Cash</b>	\$0.00	\$0.00	\$0.00
<b>Capital</b>	\$0.00	\$0.00	\$0.00
<b>Base Funding</b>	<b>\$4,312.00</b>	<b>\$0.00</b>	<b>\$0.00</b>
Rivalea	\$4,312.00	\$0.00	\$0.00
Organisation 2	\$0.00	\$0.00	\$0.00
<b>Total of All Funding Sources:</b>	<b>\$27,552.00</b>	<b>\$28,000.00</b>	<b>\$0.00</b>

<b>In-kind contributions Per Year</b>	<b>2009-2010</b>	<b>2010-2011</b>	<b>2011-12</b>
<b>Rivalea</b>			
<b>Category of in-kind staff (FTE)</b>			
Cherie Collins	0.01		
\$120,000 FTE	\$1,200.00	\$0.00	\$0.00
Prof Frank R Dunshea	0.05	0.05	
	\$8,500.00	\$8,500.00	\$0.00
<b>Sub-total 1 - in-kind contributions (\$)</b>	<b>\$9,700.00</b>	<b>\$8,500.00</b>	<b>\$0.00</b>
<b>Other non-staff in-kind contributions</b>			
Experimental overhead	\$2,381.40	\$0.00	\$0.00
Non experimental facility overhead	\$0.00		
<b>Sub-total 1 - other non-staff in-kind contributions (\$)</b>	<b>\$2,381.40</b>	<b>\$0.00</b>	<b>\$0.00</b>
<b>ThirdParty in-kind</b>			
<b>Category of in-kind staff (FTE)</b>			
Research Higher Degree Student Research and Administrative Services Support Costs			
	\$40,820.00	\$40,820.00	\$0.00
Name - Category			
	\$0.00	\$0.00	\$0.00
<b>Sub total 2 - in-kind contributions (\$)</b>	<b>\$40,820.00</b>	<b>\$40,820.00</b>	<b>\$0.00</b>
<b>Other non-staff in-kind contributions</b>			
Type 1	\$0.00	\$0.00	\$0.00
Type 2			\$0.00
<b>Sub-total 2 - other non-staff in-kind contributions (\$)</b>	<b>\$0.00</b>	<b>\$0.00</b>	<b>\$0.00</b>
<b>Total in-kind staff contributions (\$)</b>	<b>\$50,520.00</b>	<b>\$49,320.00</b>	<b>\$0.00</b>
<b>Total other non-staff in-kind contributions (\$)</b>	<b>\$2,381.40</b>	<b>\$0.00</b>	<b>\$0.00</b>

**Animal Ethics Approval:**

Rivalea Animal Research Authority approved 12/11/09 and document uploaded in iMAP 1/12/09

**Budget:**

<b>Year</b>	<b>Direct (\$)</b>	<b>In Kind (\$)</b>
2009	27552	52901
2010	28000	49320
<b>Total</b>	<b>55552</b>	<b>102221</b>

**Tasks:**

<b>Task</b>	<b>Summary</b>	<b>Start date</b>	<b>End date</b>
	Group housing - dose/response - F Dunshea to re-budget under base funding proposal Financial: N Completed	24/02/2009	31/03/2009
	Report on the effect of lecithin on bioactive components Financial: N 0 % complete	6/07/2009	30/11/2009
	Experiment 1 to be completed and reported on. The experiment will consist of 4 dietary treatments; control and soya bean lecithin supplements administered in 3 dosage treatments.	31/08/2009	31/12/2009
	R&D Committee determined that this was the Go/No Go decision step Financial: N 0 % complete		
	Report on the advantages of lecithin for improving health aspects and eating quality of pork Financial: N 0 % complete	1/09/2009	28/02/2010
	Demonstrated effect on cholesterol content of pork Financial: N 0 % complete	30/11/2009	28/02/2010
	Report on the health effects effect of lecithin Financial: N 0 % complete	11/01/2010	30/07/2010
	After demonstrating health benefits need to look for genes of key interest in cardiovascular disease to show if up or down regulated as a measure of bioefficacy. This approach has been used in colon cancer work with selenium Financial: N 0 % complete	30/07/2010	30/10/2010
	Report due via Status Reports tab Financial: N 0 % complete	13/10/2009	11/01/2010
	Report due via Status Reports tab Financial: N 0 % complete	12/01/2010	12/04/2010
	Report due via Status Reports tab Financial: N 0 % complete	13/04/2010	12/07/2010
	Report due via Status Reports tab Financial: N 0 % complete	13/07/2010	11/10/2010
	Financial: N 0 % complete	1/11/2010	31/01/2011
	Financial: N 0 % complete	1/02/2011	31/03/2011