# SOYA BEAN LECITHIN TO IMPROVE TENDERNESS AND HEALTH ACTIVE PROPERTIES OF PORK
# 3A-109

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


**By**

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## Executive Summary

Previous research has shown that dietary lecithin may improve pork eating quality by reducing chewiness and hardness while also improving the fatty acid profile (D'Souza *et al.* 2005a,b). However, little is known about the effects of dietary lecithin on other aspects of pork and carcase quality or the mechanisms of action. The aim of this study was to test the hypothesis that dietary lecithin would improve pork eating quality by reducing chewiness and hardness and in doing so may improve other aspects of carcase and meat quality. In addition, the hypothesis that dietary lecithin would improve meat tenderness by reducing collagen content and collagen cross-linking by measuring the gene expression of collagen precursors and enzymes involved in collagen synthesis and degradation was also tested.

Thirty six Large White x Landrace (PrimeGro™ Genetics) gilts were randomly allocated at 16 weeks of age (62.9±0.56 kg, mean± SE) to finisher diets containing either 0, 4, 20 or 80 g/kg soybean lecithin (ADM Australia Pty Ltd). The pigs were housed individually and had *ad libitum* access to feed and water for 6 weeks (103.2±1.67 kg). The pH of the *Longissimus thoracis* was measured at 45 min and 24h post-slaughter. Muscle samples from the abdomen were collected 25 minutes post-slaughter and frozen in liquid nitrogen for gene expression using real time polymerase chain reaction. Genes measured included Type I (α1) and Type III (α1) procollagens, matrix metalloproteinases-1 and 13 (MMP-1 and MMP-13), tissue inhibitor matrix metalloproteinases-1 and 3 (TIMP-1 and TIMP-3), prolyl-4 hydroxylase and lysyl oxidase. All expression data were reported as the threshold cycle (cT) relative to that of ribosomal 18S (ΔCT). A difference in ΔCT of -1.0 is associated with a doubling and +1.0 a halving of expression. Twenty-four hours after slaughter, the *L. thoracis* was cut and let to bloom for 30 minutes before determination of surface colour (Minolta Chromameter CR-400). Drip loss was also determined at this time. Additional muscle was removed and frozen prior to subsequent hydroxyproline and fatty acid analyses and determination of cooking loss, shear force, compression and hardness. Data were analysed for linear and quadratic dose effects using GENSTAT Release 11.1. Where there were no linear or quadratic effects the contrasts assessed were for control versus pooled lecithin treatment and within pooled lecithin treatment.

Lecithin had no effect on growth performance, P2 back fat or loin muscle depth (data not shown) but increased carcase dressing by 2%. Lecithin had no effect on pork pH, drip loss or cooking loss. Lecithin decreased pork chewiness (1.51 vs. 1.28, P=0.047) and tended to decrease cohesiveness (0.391 vs. 0.371, P=0.069) with these changes associated with decreased collagen hydroxyproline (1.63 vs. 1.18 µg/ml, P=0.043). However, there were no significant effects of lecithin on either shear force or hardness. Dietary lecithin decreased pork surface L (51.2 vs. 49.6, P=0.008) and increased surface a* in a dose-dependent manner. Dietary lecithin decreased Type I (α1) and Type III (α1) procollagen gene expression by 67 and 46%, respectively indicating a decrease in the precursor for collagen synthesis. Lecithin decreased MMP-1 expression by 92% but had no significant effect on MMP-13. Skeletal muscle TIMP-3 expression was low in control pigs (ΔCT=50.5) and was increased 90-fold by lecithin possibly because of decreased collagen. There was no effect of lecithin on TIMP-1 or lysyl-oxidase gene expression whereas prolyl-4-hydroxylase expression was decreased by 50%. These data show that dietary lecithin can decrease procollagen gene expression and alter the expression of genes involved in synthesis and degradation of collagen.

Lecithin increased polyunsaturated fatty acid (PUFA) such as C18:2n-6t, C18:2n-6c, C18:3n-3, C20:2n-6 and C20:3n-3 and the ratio of PUFA to saturated fatty acid (SFA) ratio in pork. However, there was no significant (P>0.05) difference in n-6:n-3 ratio in pork between the dietary treatments. Pigs fed dietary lecithin had lower C10:0, C12:0, C14:0, C16:0, C20:0, C21:0 and consequently lower total SFA composition in pork compared with pigs fed the

control diet. There was no effect of dietary lecithin on pork cholesterol although total intramuscular fat tended (P=0.07) to decrease linearly.

In conclusion, dietary lecithin decreases pork hardness in a dose-dependent manner though decreasing collagen synthesis. It needs to be confirmed whether these chemical and physical improvements in pork quality can be detected as improved pork quality by consumers. While lecithin improves the fatty acid profile of pork, these changes may not be sufficient on their own to justify use of dietary lecithin. An interesting finding is that dietary lecithin may improve carcass weight and dressing percentage, particularly in females. These findings need to be confirmed as does the improvement in feed efficiency observed in an associated study.

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# 1. Introduction

The sustainability of the pork industry depends upon consumer preference and acceptance of its products. In general, consumers prefer more tender pork; therefore, improvement in pork tenderness is particularly important for increasing consumer satisfaction. Previous research has shown that dietary lecithin may improve pork eating quality of pork by reducing chewiness and hardness (D'Souza *et al.* 2005). Chewiness and hardness are strongly influenced by connective tissue (Bouton and Harris, 1972) and intramuscular connective tissue plays an important role in determining meat toughness (Bailey and Light, 1989; Avery et al. 1996; Lepetit, 2008). The influence of connective tissue meat texture is multi-factorial (Lepetit, 2008) and includes the quantitative, chemical and structural aspects (Purslow, 2005). It has been suggested that collagen concentration and extent of cross-linking have an additive effect on the toughening of meat (Lepetit, 2007). Lecithin has anti-fibrogenic property and it has been associated to reduce hepatic collagen content in liver fibrosis (Ma et al. 1996). Therefore, dietary lecithin may improve meat tenderness by reducing collagen content. In addition to possessing anti-fibrogenic properties, lecithin also has been known to have emulsifying and anti-oxidant properties. However, little is known about the effects of dietary lecithin on other aspects of pork eating quality.

Therefore, the aim of this project was to test the hypothesis that dietary lecithin would improve pork eating quality by reducing chewiness and hardness and in doing so may improve other aspects of carcase and meat quality. Some of the mechanisms are hypothesized to be via altering the expression of some of the genes involved in collagen synthesis and degradation and so a series of analyses were undertaken to determine if this was the case. It was also proposed that dietary lecithin may alter the fatty acid and cholesterol profile of pork so these aspects were also investigated.

# 2. Methodology

## *2.1.* ***Animals and experimental procedure***

Thirty six crossbred (Large White x Landrace, PrimeGro™ Genetics) finisher gilts were randomly selected and stratified on live weight at 16 weeks of age (62.9±0.56 kg, mean± SE). The pigs were randomly allocated into individual pens in an experimental finisher shed for acclimatization one week before the beginning of the experiment, during which time they were fed the control diet at 80% of *ad libitum.* Subsequently, all pigs were offered four dietary treatments (i) control (commercial finisher diet) (ADM Australia Pty Ltd); (ii) soybean lecithin at 4 g/kg of commercial finisher diet; (iii) soybean lecithin at 20 g/kg of commercial finisher diet; and (iv) soybean lecithin at 80 g/kg of commercial finisher diet. All pigs had *ad libitum* access to feed and water via nipple drinker for 6 weeks (103.2±1.67 kg). All procedures outlined in this investigation were approved by the Rivalea Animal Care and Use Committee.

At 22 weeks of age, the pigs were transported to a commercial abattoir at Rivalea Foods, Corowa. The pigs were kept overnight in lairage (time off feed- 15h), before the abattoir treatment were imposed just prior to slaughter. The pigs were stunned using a carbon dioxide prior to slaughter. Exsanguination, scalding, dehairing and evisceration were performed according to the standard procedures practiced in commercial abattoirs and the carcasses were split before entering the chiller (air temperature 5°C to -2°C cycle; air speed 4m/sec). Muscle samples

from the abdomen were collected 25 minutes post-slaughter and frozen in liquid nitrogen for gene expression using real time polymerase chain reaction.

### *2.2.* ***Growth performance, carcass and pork quality assessment***

The live weight and feed intake of all pigs were measured weekly and average daily gain (ADG) and feed conversion ratio (FCR) determined every 3 weeks. After slaughter, carcass weight and backfat depth at the P2 site were determined on the hot carcass at 45 min after slaughter. Backfat depth at P2 site was measure using the Hennessy Grading Probe 4 (Hennessy, Auckland, New Zealand). Muscle depth was also measured at the P2 site.

The pH of the *Longissimus thoracis* between 12$^{th}$ and 13$^{th}$ rib was measured at 45 min and 24h post-slaughter using a portable pH/temperature meter (Jenco Electronic Ltd, Model 6009, Taipai, Taiwan) fitted with a polypropylene spear-type gel electrode (Ionode IJ42S, Brisbane, QLD) and a temperature probe. The *L. thoracis* was removed from the carcass and meat colour was measured on a 5 cm thick chop at 24 hours post-slaughter following exposure of the cut surface to air for 30 mins. Relative surface lightness (L), redness (a*) and yellowness (b*) at 24 h post-slaughter were measured with a Minolta Chromameter CR-400, using D65 illumination, a 2º observer, and an 8 mm aperture in the measuring head (Minolta Chromameter CR-400). Drip loss was also determined on a sample obtained at this time using the suspension method. Additional muscle was cut and frozen prior to subsequent determination of cooking loss, shear force per the methodology described by (Bouton et al. 1971) and muscle compression as per methodology described by (Channon et al. 2004). Samples for Warner Bratzler shear force assessment were labelled, weighed, individually vacuum packaged and aged for 2 and 7 days post-slaughter. After ageing, samples were then prepared for Warner-Bratzler shear force as described by Bouton et al. (1971). Following cooking, samples were dried with paper towel to remove excess moisture and weighed to determine cooking loss. Following overnight chilling at 4°C, each sample was cut, parallel to the muscle fibres, into five replicates of 1 cm2 cross-sectional area. Tenderness was measured using a Warner-Bratzler shear force blade fitted to an Instron Universal Testing Machine model 4465. The crosshead speed was set at 300 mm/min and a 5 kN load cell was used.

Compression analysis was undertaken on a cooked sample of pork loin aged for 2 days post-slaughter before freezing. Slices of the *L. thoracis* were standardized in size (20 mm in thickness) and weight (65-70g). Following thawing and then cooking, samples were dried with paper towel to remove excess moisture and weighed to determine cooking loss. Following overnight chilling at 4ºC, slices of 1 cm thickness were cut perpendicular to the fibres from each sample. A 0.63 cm diameter plunger was driven 0.80 cm through the 1 cm thick meat sample using the Instron Universal Testing Machine. The peak force required and work done during plunger travel was measured. The plunger was then withdrawn and returned to the same damaged area to measure work done in repeating the first action. A total of six replicates were conducted per sample. These tests determined hardness (the peak force attained during initial penetration), cohesiveness (the proportion of work required for the second penetration to that required for the first penetration) and chewiness (the product of hardness and cohesiveness) (Bouton & Harris, 1972). The crosshead speed was set at 50 mm/min and a 5 kN load cell was used to measure force (Channon et al. 2004).

## 2.3. *Chemical and fatty acid analyses*

Additional muscle samples were removed and frozen prior to subsequent hydroxyproline analyses and fatty acid. Hydroxyproline, as measure of collagen content was determined using colourimetric method developed using methodology of (Kolar, 1990) with few modifications. Samples were weighted to 4g into Erlenmeyer flasks. The samples were hydrolyzed with 7N sulfuric acid in 105±1ºC drying oven for 16 hour (conveniently, overnight). Then, the hydrolysate was diluted to 500ml MilliQ water so that hydroxyproline concentration of dilution was in range 0.5-2.4 µg/ml. After that, the solution was filtered through filter paper to get 2ml of filtrate. Working standard solutions containing 0.6, 1.2, 1.8 and 2.4 µg hydroxyproline/ml, respectively were prepared. Following that, hydroxyproline in working standard and sample solutions were oxidized with chloramines-T to pyrrole. Red-purple colour that developed after addition of 4-dimethylaminobenzaldehyde was measured photometrically at 560nm using Multiscan. Hydroxyproline standard curve was plotted with absorbance values on *y*-axis and hydroxyproline (0.6, 1.2, 1.8 and 2.4 µg) on *x*-axis. Fatty acid analyses were conducted by Victorian Department of Primary Industries using standard gas chromatographic procedures (Ponnampalam et al. 2010).

## 2.4. *Real time PCR analysis of tissue samples*

### 2.4.1. Muscle tissue homogenization and RNA extraction

Frozen muscle tissue samples were pulverised into a fine power in liquid nitrogen using a pre-cooled mortar and pestle. Approximately 200-250 mg of muscle powder was weighed into each of two pre-cooled 2 ml screw cap tubes (Axygen Scientific, California, USA) each containing one sterile 5 mm stainless steel bead (Qiagen, Hilden, Germany) and placed into liquid nitrogen until needed. (Two tubes per animal sample were needed to yield a sufficient quantity of high quality RNA for RT-PCR). To each tube, 1 ml of TRIzol™ reagent (Invitrogen, California, USA) was added and placed into a Mini Bead beater (Biospec Products Inc, USA) for two 20 second bursts, each separated by 1 minute. Following that, all samples were incubated at room temperature for 5 minutes to ensure complete nuclear protein dissociation. Following incubation, 200 µl of chloroform (Sigma, Castle Hill, NSW, Australia), was added to each tube and shaken vigorously by hand for 15 seconds. Samples were further incubated at room temperature for 2 to 3 minutes. Following incubation, samples were centrifuged in a Hettich EBA 12 centrifuge (Hettich, Tuttlingen, Germany) at 12,000 x g for 15 minutes at 4°C to fractionate RNA. Following centrifugation, 600 µl of the upper aqueous phase layer containing the RNA was transferred into a new sterile 1.75 ml Eppendorf tube (Axygen Scientific, California, USA). The transferred aqueous solution was centrifuged a second time at 12,000 x g for 15 minutes at 4°C to separate any remaining muscle tissue debris. Following the second centrifugation, 450 µl of the upper aqueous phase layer was transferred to a new sterile 1.75 ml Eppendorf tube. An equal amount of 70 % ethanol (Fronine Pty Ltd, Riverstone, NSW, Australia) was added to the transferred sample and contents mixed by vortexing (IKA, Staufen, Germany). Using the Invitrogen PureLink™ Micro-to-Midi RNA isolation system kit (Invitrogen, California, USA), two tubes of muscle tissue homogenate from identical samples were combined into one spin column. Purelink spin columns containing combined homogenate were centrifuged at 12,000 x g for 15 seconds at room temperature in an Eppendorf MiniSpin plus bench centrifuge (Eppendorf, Hamburg, Germany). The resulting flow through was discarded and 700 µl of PureLink™ Wash Buffer I was added, and re-centrifuged at 12,000 x g for 15 seconds. The flow through and collection tube were discarded, and the spin column placed into a clean RNA wash tube. To this, 500 µl of Purelink™ Wash

Buffer II was added and centrifuged at 12,000 x g for 15 seconds at room temperature. Flow through was discarded and a further 500 µl of wash buffer II was added and re-centrifuged. Spin cartridges were then centrifuged at 12,000 x g for 60 seconds to dry out the membrane with attached RNA. Collection tubes were discarded at this point and spin cartridges were placed inside Purelink RNA recovery tubes supplied with the kit. To the centre of the spin column, 55 µl of Purelink RNase free water was added and incubated for 1 minute at room temperature. Following incubation, the spin cartridges were centrifuged for 2 minutes at 14,500 x g at room temperature. Following centrifugation, spin cartridges were discarded and 3 µl of the eluent containing RNA was transferred into a sterile 1.75 ml Eppendorf tube and stored in a -80°C freezer for subsequent RNA integrity and yield evaluation with the remainder of the RNA sample.

### 2.4.2. RNA quantification

Yield and integrity of RNA in muscle tissue homogenate was evaluated using the Bio-Rad™ Experion™ automated electrophoresis system (Bio-Rad Laboratories Inc, California, USA) and the Experion StdSens Analysis Kit (Bio-Rad Laboratories Inc, California, USA). All StdSens kit reagents were allowed to equilibrate to room temperature before use with the RNA ladder thawed on ice. The electrodes on the Experion electrophoresis station were cleaned immediately before each run using the recommended procedures, Experion electrode cleaner solution, and DPEC water. The RNA gel from the kit was centrifuged at 1,500 x g for 10 minutes in a spin filter tube provided in the kit prior to use. The Gel stain solution was prepared by combining RNA gel and RNA stain in a 65:1 ratio. A 2 µl samp RNA ladder was transferred to a sterile 1.75 ml Eppendorf tube for each chip run. The tubes containing the 2 µl of RNA ladder and the 3 µl experimental sample tubes were denatured for 2 minutes at 70°C using a dry heat block (Therr Australia). Following heating, samples and ladder were both placed on ice and allowed to cool for 5 minutes. The Experion RNA StdSens chip was primed by addition of 9 µl of filtered gel-stain and subjected to pressure setting B and time setting 1 on the priming station. Following priming, the chip was examined for air bubbles or evidence that would indicate incomplete priming. If adequate priming was not achieved, the chip was discarded and the priming procedure using a new chip initiated again. Following successful priming of the chip, loading of the chip was initiated by addition of a further 9 µl of gel-stain solution, and 9 µl of the filtered gel. To each of the sample wells and the ladder well, 5 µl of loading buffer was added followed by the addition of 1 µl of denatured RNA ladder, and 1 µl of denatured RNA sample into specific wells respectively. The RNA chip was then placed into the Experion vortex station and allowed to mix for 60 seconds. Following vortexing, the chip was placed immediately into the Experion electrophoresis system and analysis was initiated. The electrophoresis system uses a photodiode laser to capture fluorescence detection and display RNA within samples as ribosomal protein bands. The resulting electropherograms were analysed for RNA concentration and RNA integrity. Integrity of RNA was evaluated by the ratio of 28S to 18S ribosomal protein as indicated by the two bands in Figure 1. Samples that exhibited two clean peaks and a ratio close to 1.5 were deemed sufficient quality RNA.

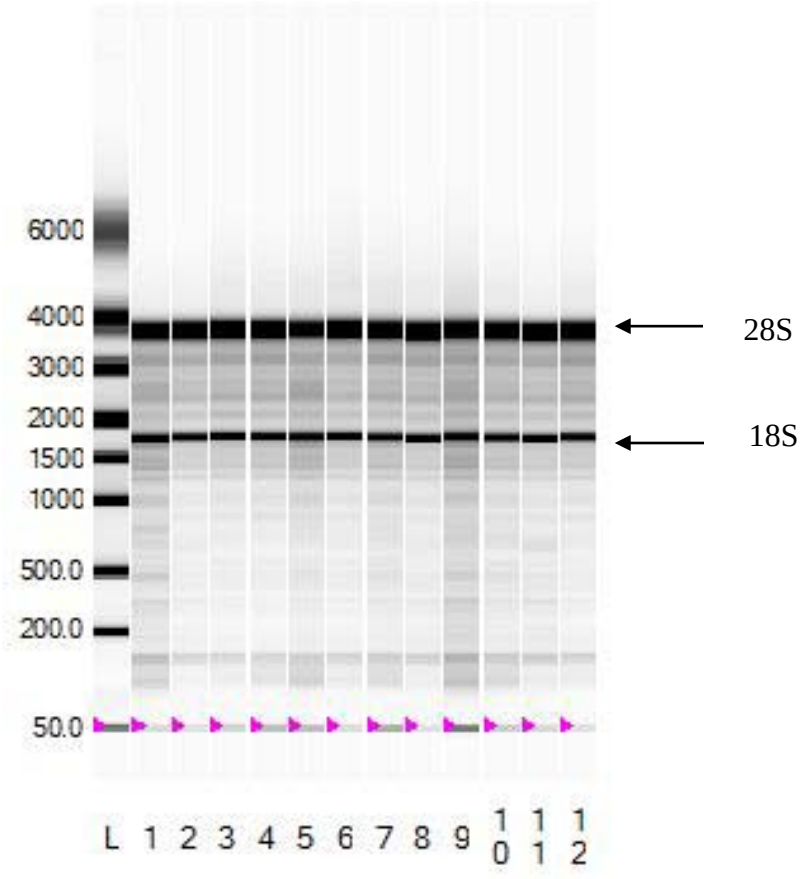


**Figure 1 - RNA electrograph showing the 28S and 18S ribosomal protein bands, RNA ladder (L) made up of multiple nucleotide base lengths ranging in size from 200 to 6000 bases in length. Lanes 1 thru 12 are ovine adipose tissue sample RNA. The Marker indicating 50 bases in length is a reference lane marker common to all samples as to accurately align samples with ladder to an identical starting point.**

### 2.4.3. cDNA synthesis

Following acceptable RNA being extracted, complimentary DNA (cDNA) was synthesised from transcribed RNA using the Superscript III First Strand Synthesis System for RT-PCR (Invitrogen, California, USA). In brief, this method required 8 µl of RNA from each sample aliquoted in triplicate into a 96 well PCR plate (Simport, Quebec, Canada) and combined with 1 µl of 50 ng/ µl random hexamer and 1 µl of 10 mM dNTP mix. The plate was incubated at 65°C for 5 minutes inside a Corbett Palm Cycler PCR (Corbett Research, Sydney, Australia). A cDNA synthesis mix was prepared using the following protocol. In brief, 2 µl of 10x RT buffer, 4 µl of 25mM $MgCl_2$, 2 µl of 0.1M Dithiothreitol (DTT), 1 µl of 40 U/µl RNase OUT, and 1 µl of 200 U/µl SuperScript III RT. Following the 5 minute incubation, 10 µl of the cDNA mix was added to each sample well, briefly centrifuged (Eppendorf, Hamburg, Germany) and then placed in the Corbett Palm Cycler for further incubation. This second incubation period consisted of 25°C for 10 minutes, 50°C for 50 minutes and, 85°C for 5 minutes. Following incubation, the plate was removed and cooled on ice for approximately 2 minutes. The final step of cDNA synthesis involved the addition of 1 µl of RNase H to each sample well and incubation at 37°C for 20 minutes. Following cDNA synthesis, 2 µl of each sample was removed from the plate and pooled in a sterile Eppendorf micro tube, and 20 µl portions were aliquoted from this cDNA pool. Following sample pooling, all cDNA plates and

pooled aliquots were frozen at -80°C until required for RT-PCR. The 20 µl aliquots were used as positive controls during RT-PCR optimisation and gene expression procedures.

### 2.4.4. Reverse transcriptase polymerase chain reaction (RT-PCR)

All gene expression evaluation was undertaken using a Bio-Rad MyIQ single colour Real Time PCR Detection System (Bio-Rad Laboratories Inc, California, USA). All PCR reactions were evaluated using 0.2 ml iCycler 96 well PCR plates (Bio-Rad Laboratories Inc, California, USA) and sealed with iCycler IQ optical tape ( Bio-Rad Laboratories Inc, California, USA). Each sample PCR reaction consisted of an in-well reaction Supermix containing 12.5 µl of SYBR Green Supermix (Bio-Rad Laboratories Inc, California, USA), 2 µl of cDNA synthesised from sample tissue, 1-3 µl of forward primer (5' – 3'), and 1-3 µl of reverse primer ( 3' – 5'). Primer volume would fluctuate depending on optimised primer concentration, and RNase free water was added to the Supermix to make up a total volume of 25 µl in each well. Control wells were also implemented on each plate in duplicate, with positive controls comprising 25 µl of the above stated Supermix with cDNA, negative controls comprising 23 µl of Supermix and 2 µl of RNase free water, and plate blanks comprising 25 µl of RNase free water.

### 2.4.5. Primer design

Because the complete genome of the pig (*Sus scrofa*) is currently only partially sequenced, and the complete genome of the mouse (*Mus musculus*) and human (*Homo sapiens*) have been mapped, nucleotide primers that were used in RT-PCR reactions were designed using these three species messenger RNA (mRNA) sequences. These sequences are published in the internet nucleotide database of the National Centre for Biotechnology Information (NCBI) http://www.ncbi.nlm.nih.gov/sites/entrez. The published genome sequences in NCBI were copied into the Invitrogen OligoPerfect™ Designer software http://www.invitrogen.com/content.cfm?pageid=9716. The OligoPerfect™ Designer software locates primer sequences within the given mRNA sequence that meets individual specifications of size, annealing temperature, Glycine and Cysteine (GC) content, region of analysis, product size, salt concentration and primer concentration. The potential forward and reverse primers were then imported into Premier Biosoft International's Netprimer primer design software http://www.premierbiosoft.com/netprimer/netprlaunch/netprlaunch.html to evaluate the structural and biochemical properties of the oligonucleotide sequence. A primer was considered appropriate if its rating out of a possible 100 was above 98, and adverse secondary structures including runs, repeats, palindromes, dimers, and hairpins were minimal. Only the best forward and reverse primer pairs which displayed the least number of adverse characteristics were selected (Table 1). These custom primers were ordered through GeneWorks Pty Ltd (Hindmarsh, South Australia, Australia). The forward and reverse primers were made up to a stock concentration of 100 µM through the addition of an appropriate amount of 1 x Tris-EDTA (TE) buffer (10 mM Tris-HCL, 1 mM EDTA, pH 8.0) depending on the concentration (nmol/tube) of the primer ordered. From this 100 µM stock, 5 µM, 3.2 µM and 2500 nM stocks were obtained by diluting an aliquot of the 100 µM stock solution with an appropriate amount of 1 x TE buffer. The 5 µM and 2500 nM stocks were used to optimise the concentration of primer needed to produce the most efficient standard curve for each primer set, while the 3.2 µM stocks were used for sequencing.

### 2.4.6. RT-PCR optimization

Following primer sequence design, conditions of both temperature, and primer concentration were optimised to obtain the most efficient annealing temperature, and a dilution series for creating an efficient standard curve for accurately evaluating gene expression.

All RT-PCR reactions used the following protocol:

Cycle 1: Step 1, 95.0°C for 03:00 minutes.

Cycle 2: Step 1, 95.0°C for 00:10 minutes, Step 2, 55.4°C for 00:45 minutes. Cycle 2 was repeated 40 times.

Cycle 3: Step 1, 95.0°C for 01:00 minute.

Cycle 4: Step 1, 95.0°C for 01:00 minute.

Cycle 5: Step1, 55.0°C for 01:00 minute.

Cycle 6: Step 1, 55.0°C for 00:10 minute. Cycle 6 was repeated 80 times.

**Table 1 - Primer name and nucleotide database accession number, Nucleotide sequences of forward and reverse RT PCR primers, and location and length of nucleotide sequence relative to the Porcine genome.**

| NAME | ACCESSION NUMBER | FORWARD 5'-3' | REVERSE 5'-3' | LENGTH AND LOCATION OF PRIMER SEQUENCE |
|---|---|---|---|---|
| TYPE I α-1 ProCollagen(a) | BC050014 | GTCTGGTTTGGAGAGAGCAT | CTTCTTGAGGTTGCCAGTCT | 189 BP's (4036-4224) |
| Type III α-1 Procollagen | NM_009930 | TGATGTCAAGTCTGGAGTGG | TCCTGACTCTCCATCCTTTC | 223 BP's (687-909) |
| MMP-1 | EU722905 | GTTCCACAAATGAGTGCTGA | ATAATAACGACGGCTCATCC | 212 BP's (1778-1989) |
| MMP-13 (a) | BC125320 | GTGACTGGCAAACTTGATGA | TCACATCAGACCAGACCTTG | 211 BP's (279-489) |
| TIMP-1 (a) | BC034260 | CCCAGAAATCAACGAGA | TGGGACTTGTGGGCATA | 154 BP's (219-372) |
| TIMP-3 | BC014713 | ACACGGAAGCCTCTGAAA | TGGAGGTCACAAAACAAGG | 231 BP's (512-742) |
| Lysyl oxidase | M65142 | CTGCTTGATGCCAACACA | TGCCGCATAGGTGTCATA | 156 BP's (1160-1315) |
| Prolyl 4-hydroxylase | BC009654 | CCCAGTCAGGTCTGCTATTC | GGAACAGTCTCTGGACAACC | 204 BP's (2202-2405) |

Based on the Net-primer melting temperature ($T_m$) data, a range of temperatures were evaluated to determine optimal annealing temperature. An annealing temperature gradient ranging between 51.3°C and 63°C was performed and all genes of interest. The specific temperature that gave the lowest threshold cycle ($C_T$) value was selected as the optimal annealing temperature (refer to Figure 2). Annealing temperatures for all genes are shown in Table 2.

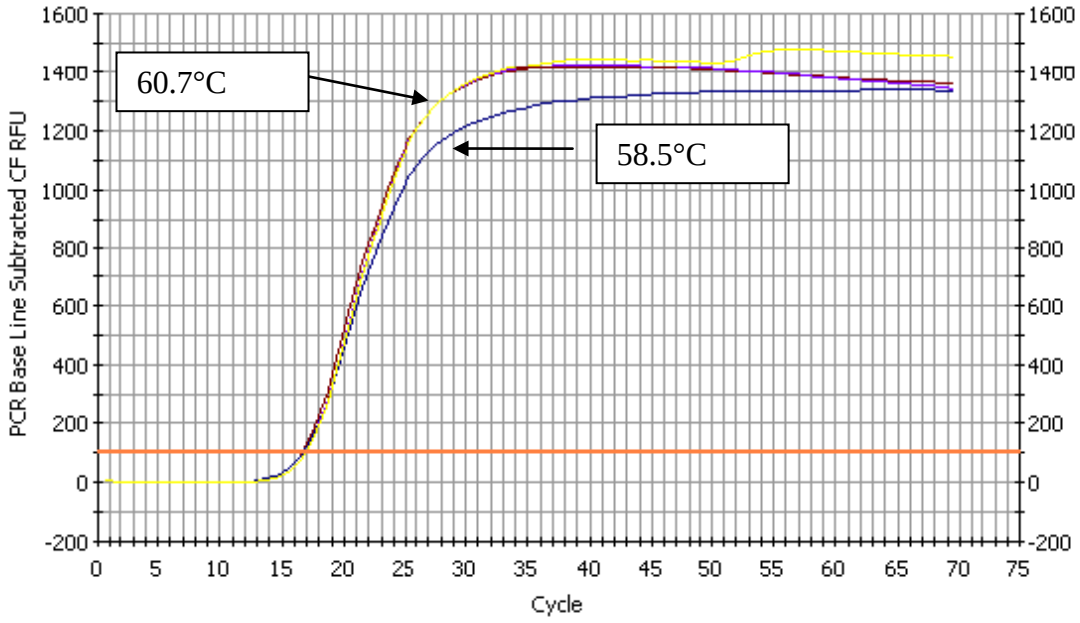


**Figure 2 - PCR melt curve indicating ultimate annealing temperature for Hormone Sensitive Lipase (HSL) occurred at 60.7 °C.**

Following optimal annealing temperature evaluation, optimal primer concentration was elucidated by generating a standard curve using a ten-fold dilution series from 1:1 thru 1:1,000,000 of positive control template cDNA in triplicate. The dilution series produced evenly spaced amplification curves each in close proximity to the ideal 3.32 cycles apart. The data was plotted to determine linearity and efficiency, with a resulting Pearson's correlation coefficient (r) > 0.990, and PCR efficiency > 90% through a minimum of four dilution series points deemed sufficient to warrant linearity. The $C_T$ number that corresponded with the conditions indicating linearity was the selected $C_T$ used to evaluate expression of genes of interest. Following the determination of $C_T$ number, linearity and efficiency, gene of interest primer sets were deemed efficient and optimised, and the entire PCR plate frozen at -80°C for gene sequencing. The optimised conditions of each gene evaluated are shown in Table 2.

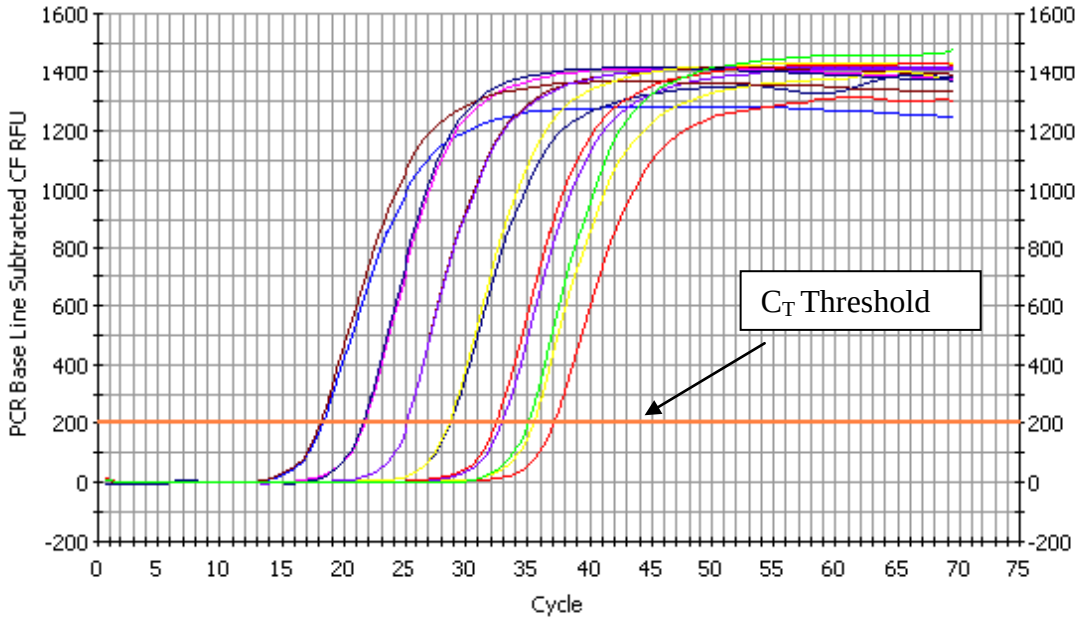


**Figure 3 - PCR Amplification curve showing a dilution series of 350 nM Hormone Sensitive Lipase (HSL) and the Threshold cycle ($C_T$) (201.5) which corresponds to the standard curve in Figure 4 located below.**

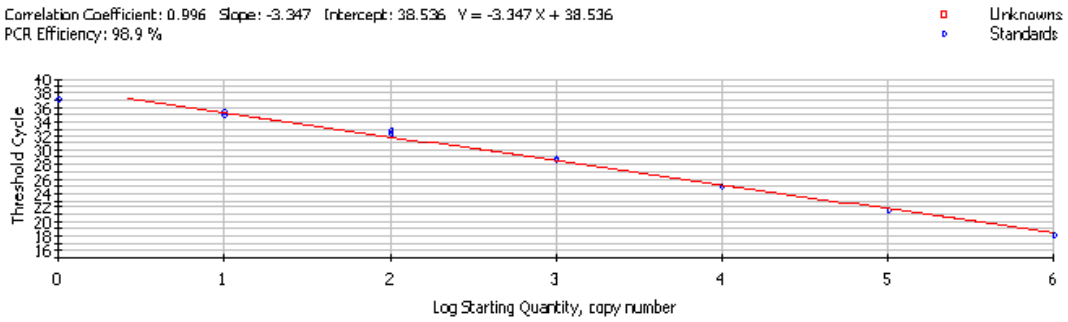


**Figure 4 - PCR standard curve for 350 nM Hormone Sensitive Lipase (HSL) indicating optimal conditions as indicated by a Pearson's correlation coefficient of 0.996, and PCR efficiency of 98.9%.**

**Table 2 - Optimised RT-PCR conditions for genes of interest**

| Gene | Optimised annealing temperature (°C) | Optimised primer concentration (nM) | Optimised threshold Cycle ($C_T$) | Pearson's Correlation coefficient (r) | PCR efficiency (%) |
|---|---|---|---|---|---|
| **Type I α-1 Procollagen** | 60.9 | 50 | 302.89/420.14 | 0.965/0.950 | 90.1/93.9 |
| **Type III α-1 Procollagen** | 53.4 | 200 | 121.85/128.8 | 0.977/0.976 | 99.2/100 |
| **MMP-1** | 60.9 | 100 | 50.53/335.42 | 0.978/0.963 | 92.4/99.8 |

| Gene | Optimised annealing temperature (°C) | Optimised primer concentration (nM) | Optimised threshold Cycle ($C_T$) | Pearson's Correlation coefficient (r) | PCR efficiency (%) |
|---|---|---|---|---|---|
| **MMP-13** | 58.7 | 700 | 201.9/310.65 | 0.946/0.929 | 90/95.2 |
| **TIMP-1** | 58.7 | 1100 | 105.75/189.7 | 0.931/0.924 | 96.3/99.9 |
| **TIMP-3** | 60.9 | 300 | 62.78/125.02 | 0.996/0.994 | 99.9/99.9 |
| **Lysyl oxidase** | 58.7 | 200 | 185.36/195.72 | 0.954/0.952 | 99.2/100 |
| **Prolyl 4-hydroxylase** | 51.0 | 400 | 103.42/280.84 | 0.932/0.923 | 97.6/98.0 |

### 2.4.7. PCR product gene sequencing

Gene sequencing of PCR amplicon was necessary to evaluate primer design, and to validate the homology of the sequence with the specific species genome in order to guarantee that the sequence which was amplified during RT-PCR was in fact the sequence specific to the gene of interest. Following RT-PCR optimisation, samples were prepared for gene sequencing using the following procedure. Firstly, an agarose gel (1.5 %) was prepared by dissolving 1.5 grams agarose powder (Scientifix, Australia) in 100 ml of 0.5 x TAE buffer containing Ethidium Bromide (( 50 µl per litre), Sigma, Castle Hill, Australia) followed by heating mixture in a microwave oven until boiling. The agarose solution was allowed to cool to approximately 60 °C and poured into an agarose gel mould (Bio-Rad Laboratories Inc, Riverstone, NSW, Australia), and a 20 well comb inserted into the gel and allowed to set. Once the agarose gel had set, the 20 well comb, and gel seals were removed from the mould and the gel placed into an electrophoresis gel tank (BioRad Laboratories Inc, California USA). The gel tank was then filled with 0.5 x TAE buffer until gel was submerged and sample wells had filled. Next, PCR products were prepared by using 15 µl of the 1:1 dilution sample well product and adding 2.5 µl of loading buffer (Yeastern Biotech Corporation, Taiwan) to each sample in a 0.2 ml sterile micro-tube (BioRad Laboratories Inc, California USA). All samples were briefly mixed by vortexing and 5 µl of 100 base pair ladder (Yeastern Biotech Co. ltd) was loaded into lane 1, and 15 µl of each sample mixture was loaded into the remaining wells in duplicate. Following the loading of samples and ladder, an electrical charge was applied to the gel tank at 90 volts for 50 to 60 minutes using a BioRad Powerpac 300 (Bio-Rad, California, USA). Following gel electrophoresis for 60 minutes, the gel was removed from the tank and placed onto an ultraviolet (UV) trans-illuminator (Wealtec Corporation, USA). The Ethidium bromide fluoresced under ultraviolet light and enabled the DNA fragment from each sample to be excised by using a clean scalpel and forceps to a sterile 2.0 ml micro-tube (Axygen Scientific, California USA). The excised fragment from each sample was weighed and recorded. Using the Mini Elute Gel extraction kit (Qiagen, Germany), DNA fragments were prepared for gene sequencing using the following procedure. To each sample, 3 volumes of buffer QG were added to 1 volume of gel (v/w) which equated to 300 µl of buffer QG to 100 mg of excised gel fragment. Samples were then incubated at 50°C for 10 minutes on a dry heat block (Thermoline, Australia) to dissolve agarose gel. During this incubation step, samples were vortexed briefly every 2 to 3 minutes to assist in dissolving the gel. Following incubation, 1 gel volume of isopropanol (Sigma-Aldrich, St Louis, USA) was added to each sample and mixed by vortex. The samples were then

transferred to a mini elute spin column provided with the kit and centrifuged at room temperature for 1 minute at 10,000 x g. The flow through was discarded and spin column placed back into the same collection tube. To each spin column, 500 µl of buffer QG was added and re-centrifuged at room temperature for 1 minute at 10,000 x g. Flow through was discarded and spin column re-inserted into the collection tube. To each spin column, 750 µl of buffer PE was added and allowed to incubate at room temperature for 2-5 minutes and then centrifuged at room temperature for 1 minute at 10,000 x g. Flow through was discarded, the spin column re-inserted into the collection tube and re-centrifuged for a further 1 minute at room temperature at > 10,000 x g. Following centrifugation, the spin column was placed into a sterile 1.5 ml microtube. To elute the DNA fragment, 10 µl of buffer EB was added to the centre of the spin column of each sample and incubated at room temperature for 1 minute. Following incubation, all samples were centrifuged at room temperature for 1 minute at 10,000 x g. Approximately 9 µl of eluent was recovered and 30 µl of 3.2 µM forward primer for each gene being sequenced was also prepared. Both eluent and primer were kept at -80°C prior to being sent for sequencing the following day. A complete genome sequencing service was carried out by AgGenomics Pty Ltd (Bundoora, VIC, Australia). The complete genome sequencing service produced a chromatograph displaying the corresponding gene sequence and this sequence was then imported into the 'Blast' website of the National Centre for Biotechnology Information (NCBI) and cross matched for nucleotide similarities. The species genome was specified as highly homologous with *Ovis aries* for all genes except for UCP2 which was designed using and subsequently found homologous with the *Bos taurus* genome. All PCR amplicon's sequenced matched genes of interest using this reference database.

## *2.5. **Calculations and statistics***

### 2.5.1. General

Data were analyzed for linear and quadratic dose effects using GENSTAT Release 11.1. Where there were no linear or quadratic effects the contrasts assessed were for control versus pooled lecithin treatment and within pooled lecithin treatment.

### 2.5.2. RT-PCR gene expression evaluation and data analysis

Following confirmation that the designed primers were amplifying the specific gene of interest, gene expression was evaluated on all muscle tissue samples following the procedure outlined earlier. Threshold cycle ($C_T$) values were calculated as the cycle when fluorescence of the sample exceeded a set threshold level corresponding to the baseline fluorescence determined during the optimisation process. A sample that contains a higher concentration of the gene of interest will cross the threshold at an earlier cycle than a similar sample with a lower concentration of the gene of interest. All samples had a $C_T$ value for both the gene of interest and the reference/housekeeper gene (β-Actin), with the difference between the two $C_T$ values evaluated as the ΔCt.

When using RT-PCR to evaluate gene expression in samples obtained from multi-factorial experiments, the ΔCt method is required to statistically analyse the data arising from such experiments (French *et al*. 2006; Yuan *et al*. 2006). The magnitude of the ΔCt of a gene indicates the level of gene expression, with the lower the ΔCt indicating a higher gene expression, and the higher the ΔCt indicating less gene expression. Following ΔCt evaluation of all samples, statistical analysis was carried out by REML using Genstat computational software. All expression data were reported as the threshold cycle (cT) relative to that of ribosomal 18S (ΔCT). A difference in ΔCT of -1.0 is associated with a doubling and

-1.0 a halving of expression. Since there were no significant linear or quadratic dose effects the contrasts assessed by ANOVA were for control versus pooled lecithin treatment and within pooled lecithin treatment using GENSTAT Release 11.1.

# 3. Outcomes

## *3.1.* ***Growth performance and carcass quality***

Growth performance, carcass quality and meat quality are presented in Table 3. There was no effect of dietary lecithin on daily gain, food intake, feed conversion rate (FCR), muscle depth or P2 back fat. However, carcase dressing percentage was increased by 2% (Table 3). Lecithin had no effect on pork pH, drip loss or cooking loss (data not shown). Lecithin decreased pork chewiness (1.51 vs. 1.28, P=0.047) and tended to decrease cohesiveness (0.391 vs. 0.371, P=0.069) with these changes associated with decreased collagen hydroxyproline (1.63 vs. 1.18 µg/ml, P=0.043) (Table 3). However, there were no significant effects of lecithin on either shear force or hardness (Table 3). Dietary lecithin decreased pork surface L (51.2 vs. 49.6, P=0.008) and increased surface a* in a dose-dependent manner (Table 3). Lecithin had no effect on pork pH, drip loss or cooking loss.

**Table 3 - Effect of dietary lecithin on growth performance, carcase characteristics and pork eating quality.**

| | Dietary lecithin (g/kg) | | | | | P-value | |
|---|---|---|---|---|---|---|---|
| | 0 | 4 | 20 | 80 | SED[1] | Lecithin | Within Lecithin |
| Daily gain (kg/d) | 0.967 | 0.983 | 0.979 | 0.981 | 0.0481 | 0.93 | 0.80 |
| Feed intake (kg/d) | 2.45 | 2.42 | 2.49 | 2.47 | 0.117 | 0.96 | 0.81 |
| Feed:gain | 2.55 | 2.56 | 2.57 | 2.52 | 0.102 | 0.97 | 0.91 |
| Carcase weight (kg) | 76.5 | 77.9 | 78.9 | 78.8 | 1.82 | 0.19 | 0.83 |
| Carcase dressing (%) | 73.7 | 75.5 | 75.6 | 75.5 | 0.81 | 0.009 | 0.98 |
| P2 back fat (mm) | 9.7 | 9.3 | 10.1 | 9.9 | 1.13 | 0.91 | 0.78 |
| Loin depth (mm) | 50.9 | 49.7 | 51.9 | 52.0 | 2.28 | 0.88 | 0.52 |
| Chewiness | 1.51 | 1.39 | 1.21 | 1.24 | 0.137 | 0.047 | 0.13 |
| Hardness (kg) | 3.84 | 3.71 | 3.26 | 3.37 | 0.308 | 0.13 | 0.20 |
| Cohesiveness | 0.391 | 0.375 | 0.370 | 0.367 | 0.014 | 0.069 | 0.32 |
| Shear force (kg) | 3.21 | 3.91 | 3.55 | 3.44 | 0.382 | 0.18 | 0.45 |
| pH @ 45 min | 6.07 | 6.08 | 5.97 | 6.09 | 0.0795 | 0.68 | 0.25 |
| pH @ 24 h | 5.47 | 5.49 | 5.47 | 5.46 | 0.0233 | 0.76 | 0.56 |
| Colour L | 51.2 | 49.9 | 49.6 | 49.3 | 0.60 | 0.008 | 0.77 |
| Colour a*[2] | 5.20 | 5.42 | 5.97 | 6.22 | 0.254 | 0.010 | 0.037 |
| Colour b* | 2.63 | 2.74 | 2.69 | 2.94 | 0.247 | 0.53 | 0.68 |
| Drip loss (%) | 5.0 | 4.2 | 5.2 | 5.7 | 0.72 | 0.91 | 0.14 |
| Cooking loss (%) | 26.4 | 27.0 | 25.8 | 27.3 | 0.013 | 0.80 | 0.54 |
| Hydroxyproline (µg/ml) | 1.63 | 1.22 | 1.17 | 1.15 | 0.186 | 0.043 | 0.93 |

[1]Standard error of the difference for effect of lecithin; for within lecithin multiply by 1.22.
[2]Significant linear (P<0.001) and quadratic (P=0.042) effects of lecithin.

### *3.2.**Fatty acid and cholesterol concentrations***

Lecithin increased polyunsaturated fatty acids (PUFA) such as C18:2n-6t, C18:2n-6c, C18:3n-3, C20:2n-6 and C20:3n-3 and the ratio of PUFA to saturated fatty acid (SFA) ratio in pork (Table 4). However, there was no significant (P>0.05) difference in n-6:n-3 ratio in pork between the dietary treatments. Pigs fed dietary lecithin had lower C10:0, C12:0, C14:0, C16:0, C20:0, C21:0 and consequently lower total SFA composition in pork compared with pigs fed the control diet. There was no effect of dietary lecithin on pork cholesterol or total intramuscular fat (Table 4).

**Table 4 - Effect of dietary lecithin on skeletal muscle fatty acids, saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), total fat and cholesterol concentrations (mg/100g fresh muscle).**

| | Dietary lecithin (g/kg) | | | | | P-value | |
|---|---|---|---|---|---|---|---|
| | 0 | 4 | 20 | 80 | SED | Linear | Quadratic |
| C14:0 | 28.0 | 18.0 | 18.0 | 21.0 | 5.10 | 0.037 | 0.78 |
| C16:0 | 515 | 355 | 370 | 415 | 79.6 | 0.045 | 0.74 |
| C18:0 | 270 | 191 | 201 | 218 | 43.0 | 0.068 | 0.82 |
| C18:1n-9c+t | 837 | 587 | 596 | 635 | 127.1 | 0.033 | 0.93 |
| C18:2n-6t | 1.05 | 0.91 | 0.82 | 0.39 | 0.110 | <.001 | <.001 |
| C18:2n-6c | 208 | 189 | 209 | 289 | 26.9 | 0.35 | 0.002 |
| C18:3n-6 | 1.73 | 1.93 | 1.82 | 1.70 | 0.138 | 0.44 | 0.26 |
| C18:3n-3 | 11.1 | 9.2 | 10.7 | 18.0 | 2.62 | 0.47 | 0.005 |
| C20:2n-6 | 6.16 | 4.98 | 5.54 | 8.76 | 1.233 | 0.79 | 0.009 |
| C20:4n-6 | 43.0 | 46.5 | 44.8 | 44.9 | 1.79 | 0.10 | 0.57 |
| C20:3n-3 | 1.76 | 1.36 | 1.63 | 2.74 | 0.430 | 0.68 | 0.007 |
| C20:5n-3 | 2.62 | 2.74 | 2.86 | 2.61 | 0.136 | 0.31 | 0.21 |
| C22:4n-6 | 7.34 | 7.05 | 6.54 | 6.69 | 0.372 | 0.065 | 0.38 |
| PUFA | 221 | 199 | 220 | 308 | 29.6 | 0.37 | 0.002 |
| SFA | 545 | 374 | 389 | 438 | 84.9 | 0.045 | 0.74 |
| PUFA:SFA | 0.456 | 0.556 | 0.601 | 0.729 | 0.0597 | 0.001 | 0.019 |
| n6:n3 | 13.79 | 14.02 | 13.76 | 13.4 | 0.62 | 0.89 | 0.61 |
| Total muscle fat | 2060 | 1509 | 1560 | 1763 | 294.2 | 0.070 | 0.66 |
| Cholesterol | 468 | 472 | 456 | 427 | 38.2 | 0.59 | 0.49 |

### *3.3.**Gene expression***

Dietary lecithin decreased Type I (α1) and Type III (α1) procollagen gene expression by 67 and 46%, respectively (Table 5) indicating a decrease in the precursor for collagen synthesis. Lecithin decreased MMP-1 expression by 92% but had no significant effect on MMP-13. Skeletal muscle TIMP-3 expression was low in control pigs (ΔCT=50.5) and was increased 90-fold by lecithin possibly because of decreased collagen. There was no effect of lecithin on TIMP-1 or lysyl-oxidase gene expression whereas prolyl-4-hydroxylase expression was decreased by 50% (Table 5).

**Table 5 - Effect of dietary lecithin on skeletal muscle gene expression**

| | Dietary lecithin (g/kg) | | | | | P-value | |
|---|---|---|---|---|---|---|---|
| | 0 | 4 | 20 | 80 | SED[1] | Lecithin | Within Lecithin |
| Type I α1 procollagen[2] | 19.83 | 21.18 | 22.23 | 20.94 | 0.708 | 0.029 | 0.30 |
| Type III α1 procollagen[2] | 18.09 | 19.66 | 19.08 | 19.09 | 0.523 | 0.031 | 0.59 |
| MMP-1[2] | 26.31 | 31.24 | 28.26 | 30.60 | 1.693 | 0.035 | 0.33 |
| MMP-13[2] | 32.76 | 35.88 | 34.76 | 30.95 | 1.741 | 0.53 | 0.067 |
| TIMP-1[2] | 36.08 | 37.09 | 34.04 | 36.78 | 1.578 | 0.95 | 0.24 |
| TIMP-3[2] | 50.50 | 42.70 | 46.10 | 43.30 | 3.450 | 0.071 | 0.70 |
| Lysyl-oxidase[2] | 37.65 | 37.88 | 39.73 | 36.77 | 2.042 | 0.82 | 0.50 |
| Prolyl-4-hydroxylase[2] | 26.57 | 27.23 | 28.01 | 27.43 | 0.500 | 0.056 | 0.43 |

[1]Standard error of the difference for effect of lecithin; for within lecithin multiply by 1.22
[2]Data are presented as ΔCt relative to the housekeeper gene (ribosomal 18S).

## 4. Application of Research

The observation from the present research that there was an effect of dietary lecithin on dressing percentage has resulted in further interest within the CRC community. Therefore, two other studies have been conducted (one at Rivalea (Collins unpublished) and one at APFG (Edwards unpublished)) to confirm it there was an effect of low doses of dietary lecithin on dressing percentage. These studies have been reported in separate Pork CRC final reports but in brief there were no significant effects of dietary lecithin on dressing percentage although there was an improvement in feed conversion. However, further dissemination of the data suggested that there may be an interaction with sex such that dietary lecithin increased dressing percentage in females but not in males. To examine this further the data from a number of published and unpublished studies with dietary lecithin were analyzed to a meta-analyses. This analyses show that dietary lecithin increased ($P<0.001$) dressing percentage in a dose dependent manner in females but not in males (Figure 5).

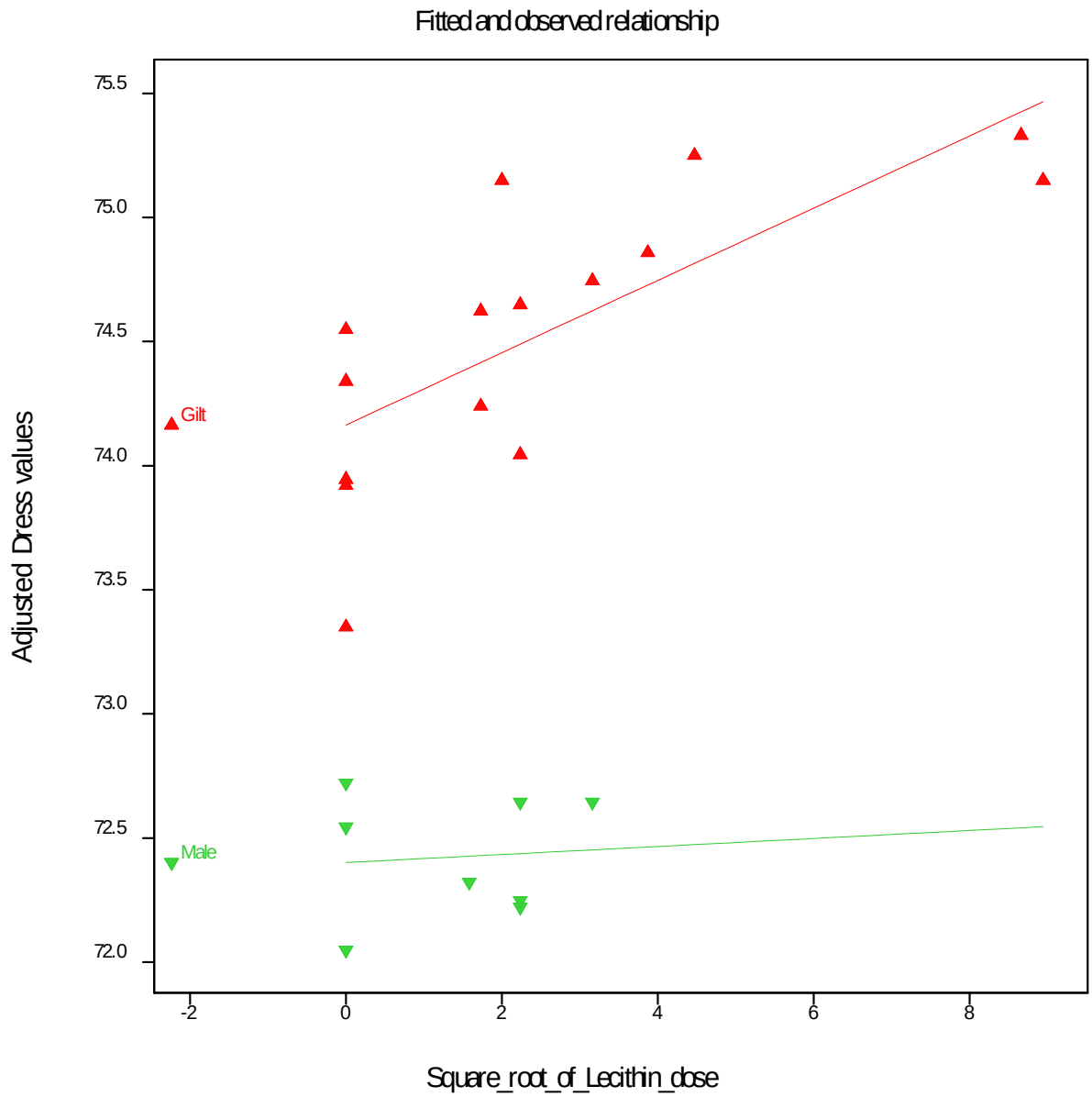


**Figure 5 - Effect of dietary lecithin on dressing percentage (adjusted for study) in females and males.** Data are from D'Souza et al. (2005), Edmunds et al. (2005), Kim et al. (2008), Edwards (unpublished), Collins (unpublished) and the present study.

Samples of pork were obtained from the study of Collins (unpublished) and analysed for hydroxyproline and for gene expression of major genes involved in collagen metabolism as described for the previous study. There was no effect of dietary lecithin on the expression of any genes although dietary lecithin tended ($P<0.10$) to decrease the expression of lysyl-oxidase, particularly in the immunocastrates as indicated by the interaction ($P=0.056$) (Table 6). There was also no effect of lecithin on muscle hydroxyproline concentrations although it should be noted that muscle hydroxyproline concentrations in this study were much lower than in the previous study. Also, although there was no significance interaction between sex and dietary lecithin the hydroxyproline concentrations in pork from gilts treated with lecithin was 13% than the controls. Compression analyses are yet to be conducted on these samples.

A Fulbright Fellow from Kansas State University (Hyatt Frobose) is conducting a study investigating the effect of sex (boar, gilt and immunocastrate)

and dietary lecithin (8 g/kg) on dressing percentage and pork quality at the University of Melbourne, Dookie campus. Samples from this study will be used to supply pork for some for the eating quality studies being conducted by Heather Channon. It is important to be able to link the improvements in compression with eating quality data.

Given the relatively minor effect of lecithin on muscle cholesterol in the present study it was decided not to pursue the rat studies looking at any health benefits of pork from pigs consuming lecithin, and instead focus on gene expression and eating quality research in future. However, one other piece of associated research was the study by Amy Bellhouse (Honours student at the University of Melbourne) who conducted a study to investigate the willingness to pay for pork with health benefits, specifically lower cholesterol. This research predicted a significant financial premium for the reduced cholesterol product at the retail level, with increased willingness to pay for and consume reduced cholesterol pork by the average pork consuming family. Based simply on what these surveyed consumers said they would do, the possible increase in demand for pork that was low cholesterol by the average pork consuming family was up to 32 per cent and they could spend up to 43 per cent more than they currently do with regular pork. Willingness to pay was found to be significantly higher for females and those aged 65 and above. However, as these results are the product of a stated choice analysis and not a revealed preference study, and therefore simply reasonable expectations, it is likely that the reported increase in demand in both quantity and price by potential consumers is overstated to some extent. This work was recently published in Australasian Agribusiness review (Bellhouse et al. 2010).

**Table 6 - Effect of dietary lecithin and sex on skeletal muscle hydroxyproline and gene expression from the experiment by Collins et al (unpublished).**

| Sex | Gilt | | Immonocastrate | | | P-value | |
|---|---|---|---|---|---|---|---|
| Lecithin (g/kg) | 0 | 5 | 0 | 5 | SED[1] | Lecithin | Sex |
| Type I α1 procollagen[2] | 19.68 | 19.84 | 19.41 | 19.22 | 0.352 | 0.95 | 0.076 |
| Type III α1 procollagen[2] | 19.60 | 19.29 | 18.87 | 19.16 | 0.369 | 0.96 | 0.11 |
| MMP-1[2] | 32.10 | 31.55 | 31.12 | 31.69 | 0.495 | 0.99 | 0.25 |
| MMP-13[2] | 35.32 | 35.78 | 34.98 | 35.20 | 0.530 | 0.37 | 0.22 |
| TIMP-1[2] | 33.53 | 33.64 | 33.38 | 33.48 | 0.479 | 0.76 | 0.66 |
| Lysyl-oxidase[2, 3] | 31.43 | 31.31 | 30.63 | 32.20 | 0.607 | 0.10 | 0.92 |
| Prolyl-4-hydroxylase[2] | 29.43 | 29.47 | 28.90 | 29.64 | 0.434 | 0.22 | 0.56 |
| Hydroxyproline (µg/ml) | 0.76 | 0.66 | 0.72 | 0.74 | 0.068 | 0.38 | 0.65 |

[1]Standard error of the difference for effect of lecithin; for within lecithin multiply by 1.22
[2]Data are presented as ΔCt relative to the housekeeper gene (ribosomal 18S). [3] P-value for the interaction between lecithin and sex was 0.056.

## 5. Conclusion

Lecithin had no effect on growth performance, P2 back fat or loin muscle depth but increased carcase dressing by 2%. Lecithin has the potential to serve as an exogenous emulsifier to enhance the utilization of dietary fat. It was shown to enhance utilization of dietary fat by young pigs (Frobish, 1969). However, research investigating the response of growing-finishing pigs to lecithin is inconsistent and limited. Lecithin as an emulsifier did not improve the utilization of soy oil by the growing-finishing pig and it was not an effective dietary fat source when added to a corn and soybean meal diet for growing-finishing pigs (Overland et al. 1993). Bunger et al. (1941;1942) found no positive effect of the addition of crude lecithin to diets on growth performance of growing-finishing pigs. Hellberg (1965) however, found an increase in overall weight gain by 7.8% with the addition of lecithin to barley and oat-based diets for growing and finishing pigs, respectively. Other factors that affect dressing percentage are gut fill and visceral weight and it is possible that dietary lecithin may on one or other of these through mechanisms unknown.

Lecithin decreased pork chewiness and cohesiveness suggesting that lecithin decreases muscle collagen content or cross-linking. Intramuscular collagen plays an important role in determining meat toughness and chewiness and cohesiveness of meat is strongly influenced by connective tissue; the higher the connective tissue content in muscle, the higher the compression value {Bouton and Harris, 1972). Previous studies have shown that dietary lecithin improved the eating quality of pork by reducing chewiness and hardness (D'Souza et al. 2005; Edmunds et al. 2005). D'Souza et al. (2005) suggested that collagen level and extent of cross-linking may be responsible in improving compression characteristics of pork from pigs fed dietary lecithin. The changes in pork chewiness and cohesiveness in the present study were associated with decreased collagen hydroxyproline indicating that the effects are on collagen content. However, there were no significant effects of lecithin on either shear force or hardness. The previous study by (D'Souza et al. 2005) has also shown no significant result on shear force. Tenderness depends on the strength of the myofibrillar component and the stability of collagen (Jeremiah et al. 2003). Shear force values are a direct measure of the resistance of muscle fibres to a blade passing through them. The evidence here was that lecithin has destabilized the collagen and left the myofibrillar component unchanged. These values were not changed by lecithin, suggesting that it was the collagen component that was most affected.

Dietary lecithin decreased Type I (α1) and Type III (α1) procollagen gene expression by 67 and 46%, respectively indicating a decrease in the precursors for collagen synthesis. Lecithin decreased MMP-1 expression by 92% but had no significant effect on MMP-13. Skeletal muscle TIMP-3 expression was low in control pigs (ΔCT=50.5) and was increased 90-fold by lecithin possibly because of decreased collagen. There was no effect of lecithin on TIMP-1 or lysyl-oxidase gene expression whereas prolyl-4-hydroxylase expression was decreased by 50%. These data show that dietary lecithin can decrease procollagen gene expression and alter the expression of genes involved in synthesis and degradation of collagen.

Dietary lecithin decreased pork surface L and increased surface a* in a dose-dependent manner. These indicate that the pork appeared redder with increased a* values and lower lightness with decreased L values. Hunter a* values have been suggested to be related to meat color stability in pork (Monahan et al. 1994), with higher a* values relating to improved colour stability of meat.

Polyenylphosphatidylcholine (PPC), present in lecithin extracted from soy beans has been shown to reduce lipid oxidation (Lieber, 1997; Poniachick et al. 1999; Aleynik et al. 2001). Meat discolouration is affected by the rate of myoglobin oxidation (Monahan et al. 1994). Lipid oxidation and pigment oxidation in fresh meat are closely related and therefore delaying lipid oxidation should result in a similar delay of meat discoloration (Asghar et al. 1991). In this experiment, we suggest that dietary lecithin reduced lipid oxidation and improved pork colour. However, in a recent study by Kim et al. (2008) pork colour was not affected by dietary lecithin. Another attribute that could contribute to the change of pork colour in this experiment is iron content of pork. Meat with higher pigment content has lower lightness (L*) and higher redness (a*) (Gil et al. 2001). It has been suggested the increased a* values of postmortem muscle may be a result of accumulation of myoglobin (Hagler et al. 1981) in response to dietary Fe level. Iron supplementation in pigs has been shown to produce redder pork (higher a* value) than pigs supplemented with lower dose of iron (Apple et al. 2007). In an associated study dietary lecithin had no effect on pork Fe content (Collins et al. unpublished).

These data indicate significant increase in linoleic acid and α-linolenic acid in pork from pigs fed dietary lecithin that would benefit human health. Similarly, dietary lecithin has also been reported in previous studies to increase linoleic acid (D'Souza et al. 2005) and α-linolenic acid (Kim et al. 2008) in pork as compared with the control diet. These fatty acids are precursors for the longer chain, PUFA of the n-6 and n-3 families. In this experiment, there was evidence that dietary lecithin supplementation increase the longer chain PUFA such as C20:2n-6 that derived from linoleic acid and C20:3n-3 from α-linolenic acid. It has been proposed that meat fatty acid composition can be changed via the diet easily in single-stomached pigs where the linoleic, α-linolenic and the other long-chain PUFA content respond quickly to raise dietary concentrations (Wood, 1997). This is supported by a study using grains and oilseeds that are high in linoleic acid in pigs where the proportion of this fatty acid in tissues increases linearly as the dietary intake increases (Wood et al. 2008). In addition, a more recent study by Kim et al. (2008) using dietary lecithin supplementation in pigs suggested the increase in α-linolenic acid of pork in their study may be attributed to the greater amount of this fatty acid in lecithin. Therefore, the higher value of linoleic acid and α-linolenic acid in pork from pigs supplemented with dietary lecithin compared to the control diet in this experiment could be due to the greater amount of these fatty acids in lecithin, particularly at high inclusion rates. Another beneficial effect of dietary lecithin supplementation was indicated by the increase in PUFA:SFA ratio of pork from pigs supplemented with lecithin that gave more than 0.4 ratio which are considered suitable for human consumption (Teye et al. , 2006). However, another study in pigs using dietary lecithin as supplement was unable to find any significant difference in PUFA:SFA ratio in pork as compared with the control diet (D'Souza et al. 2005). A study in human on the other hand, showed similar results to the present study where dietary lecithin supplementation increased the ratio of PUFA:SFA in both serum and erythrocytes (Spilburg et al. 2003). High levels of linoleic acid in pork leads to high values of PUFA:SFA ratios in pork (Jeong et al. 2010). So, the increase PUFA:SFA ratio in this study may be due to the increase in linoleic acid in pork from pigs supplemented with dietary lecithin. Therefore, these data in the present study suggest that dietary lecithin could be used to increase linoleic acid and α-linolenic acid in pork thus, manipulate the fatty acid content of muscle to enhance pork nutritional value. In the case of n-6:n-3 ratio, the results from the present experiment reported no significant difference between the dietary lecithin treatment and the

control diet. Both the total of n-6 as well n-3 fatty acids were higher in pork from pigs fed lecithin supplement compared with the control diet. These are likely due to the increase in linoleic and α-linolenic in pork from pigs fed dietary lecithin supplement.

SFA in dietary fat has been implicated to elevate blood cholesterol leading to coronary heart disease and PUFA on the other hand, lowered blood cholesterol in human (McNiven et al. 2004). The results from this experiment suggest that dietary lecithin has the potential to improve fatty acid profile of pork to be more favorable for health as evidence by higher PUFA:SFA ratio in pork from pigs fed dietary lecithin. Not all SFA are equally hypercholesterolemic. For example, the hypercholesterolemic SFA appear to be more effective in increasing the cholesterol level in blood are those with a medium-chain length (i.e. C12:0, C14:0, C16:0), and C14:0 has four times the hypercholesterolemic effect of the others (Ulbricht and Southgate, 1991). Dietary lecithin in the present study indicated lower level of those fatty acids in pork from pigs fed dietary lecithin than the control diet. Furthermore, the dietary lecithin supplementation in this experiment increased the concentration of the hypocholesterolemic fatty acids like linoleic and α-linolenic fatty acids. These data are consistent with other works using dietary lecithin as supplement in pigs that showed decrease C14:0 (D'Souza et al. 2005) and increased linoleic acid (D'Souza et al. 2005) and α-linolenic (Kim et al. 2008) in pork compared with the control diet without dietary lecithin. Therefore, the results from the present study further support the suggestion that dietary lecithin supplementation in pigs could produce pork with a superior fatty acid composition relative to human health.

These data show that dietary lecithin increases dressing percentage and results in less chewy and darker pork. These data also confirmed that dietary lecithin decreased chewiness and that tehse effects appear to be mediated by a reduction in collagen synthesis. Since most responses appeared to be maximised at the lowest dose (4 g/kg) investigated, futerue research should focus on the impact of lower doses of dietary lecithin supplementation on growth performance and carcass characteristics of group housed finisher pigs. Further investigation, should also focus on the impact of lecithin supplementation during the finisher period on aspects of meat quality including the iron content of pork (see associated activity).

## 6. Recommendations

Dietary lecithin decreases pork hardness in a dose-dependent manner though decreasing collagen synthesis. It needs to be confirmed whether these chemical and physical improvements in pork quality can be detected as improved pork quality by consumers. While lecithin improves the fatty acid profile of pork, these changes may not be sufficient on their own to justify use of dietary lecithin. An interesting finding is that dietary lecithin may improve carcase weight and dressing percentage, particularly in females. These findings need to be confirmed as does the improvement in feed efficiency observed in the study of Collins et al. (unpublished).

## 7. Publications Arising from the Project

Akit, H., Collins, C.L., Fahri, F.T., Hung, T-Y., Leury, B.J. and Dunshea, F.R. (2010). Dietary lecithin may improve meat quality and the health attributes of

pork. Invited paper presented at the 34th annual scientific meeting of The Nutrition Society of Australia. Proceedings of the Nutrition Society 34:65.

Akit, H., Collins, C.L., Fahri, F.T., Hung, T.Y., D'Souza, D.N., Leury, B.J. and Dunshea, F.R. (2011). Dietary lecithin improves eating quality, dressing percentage and meat colour in finisher gilts. In "Manipulating Pig Production XIII", ed R.J. van Barneveld. (Australasian Pig Science Association: Werribee), (in press).

Akit, H., Fahri, F.T., Hung, T.Y., D'Souza, D.N., Leury, B.J., Ponnampalam, E.N. and Dunshea, F.R. (2011). Dietary lecithin improves the ratio of polyunsaturated to saturated fatty acids in pork. In "Manipulating Pig Production XIII", ed R.J. van Barneveld. (Australasian Pig Science Association: Werribee), (in press).

Akit, H., Collins, C.L., Fahri, F.T., Hung, T.Y., D'Souza, D.N., Leury, B.J. and Dunshea, F.R. (2011). Dietary lecithin alters the expression of genes involved in skeletal muscle collagen synthesis and degradation. In "Manipulating Pig Production XIII", ed R.J. van Barneveld. (Australasian Pig Science Association: Werribee), (in press).

Bellhouse, A., Malcolm, L.W., Griffith, G. and Dunshea, F.R. 2010. Australian consumers' willingness to pay and willingness to purchase a hypothetical lower cholesterol pork product. *Australasian Agribusiness Review* 18: 161-192.

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