Inclusion of lupins in the diets of finisher pigs to reduce the level of cholesterol in pork

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By

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Executive Summary

There has been a misconception amongst consumers that meat consumption is linked with increased health risks (Richardson *et al.*, 1994). The notion of eating foods that enhance consumer health is one that sits well with consumers, therefore nutritionally enhanced pork is likely to have a greater impact on the general health of the population. Feeding commercial pig diets that are high in fat have been reported to benefit pig performance, although impacts on pork cholesterol levels have not been thoroughly investigated. Tallow provides a concentrated energy source, which increases the dietary energy density and allows for the inclusion of alternative ingredients with lower energy densities. This in turn reduces the reliance on the proportion of cereal grains included in pig diets. Lupins are a valuable, high protein legume feed that have been included at up to 35% in pig diets without compromising growth performance. The cholesterol lowering effects of legumes, specifically pea, soybean or soy lecithin have been reported in both animal and human models. Soy protein sources have been effective in lowering plasma triglyceride and cholesterol levels, however few studies have examined their influence on tissue cholesterol levels. Lupins contain high levels of pectin, a soluble fibre source shown to reduce absorption and increase excretion of cholesterol through the gastrointestinal tract. While the cholesterol content of pork varies because of differences in both pig genotype and diet composition, the rationale for this project was to investigate the cholesterol lowering properties of lupins against that of soy lecithin, known for its cholesterol lowering properties, in finisher pigs.

To ascertain a true understanding of the effectiveness of soy lecithin and/or lupins in lowering tissue cholesterol levels and increasing the polyunsaturated fatty acid (PUFA):saturated fatty acid (SFA) ratio of pork, thereby enhancing its nutritional value, an experiment was conducted with individually housed immunocastrated pigs. Pigs were fed a diet with either a low (0.5% tallow) or high (4.5% tallow) cholesterol level and a basal control (wheat), soy lecithin (7.5%) or lupin (30%) diet component during the finishing period from 40-95 kg live weight (LW). The data demonstrated that diets the 30% inclusion of lupins did not negatively impact pig growth performance, however soy lecithin limited daily feed intake. Objective pork quality measurements were not influenced by either the cholesterol level or diet. Soy lecithin significantly lowered both plasma total and low-density lipoprotein (LDL) cholesterol in pigs when compared to pigs fed the control diets. Neither soy lecithin or lupins significantly lowered tissue cholesterol however their inclusion increased the PUFA:SFA ratio in pork, resulting in a healthier final product.

It was concluded that lupins can be used as a nutritional management tool in high fat finisher pig diets, at levels of up to 30%, to effectively manage the fatty acid profile of pork by increasing the PUFA:SFA ratio, thereby maintaining a healthier pork product. Further research of a larger scale is warranted to determine whether the inclusion of lupins can effectively lower the cholesterol content of pork from pigs fed diets high in both cholesterol and fat.

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1. Introduction

There is concern regarding the health status of Australians, in particular the proportion of the population that are classed as overweight, have metabolic diseases or who are at a higher risk of developing cancers and cardiovascular disease. In addition, there is a misconception amongst consumers that meat consumption is linked with health risks, such as an increased incidence of obesity, type 2 diabetes and cardiovascular disease (Richardson *et al.*, 1994). However, there is growing evidence that a diet containing lean meat as a protein source and one which is low in carbohydrate and fat, in conjunction with regular exercise, can effectively reduce the incidence of obesity and improve cardio-metabolic health in humans (Murphy *et al.*, 2012). The concept of eating foods that have beneficial effects on consumer health above that of adequate nutritional value is one that sits well with today's consumers. Nutritionally enhanced pork, as part of our everyday diet potentially has a far greater impact on the general health of the population than the alternative approach of using dietary supplements.

Lupins are a valuable, high protein feed grain. The inclusion of lupins in diets for growing pigs has been limited to 25%, as it was thought that lupins increased the level of dietary non-starch polysaccharides (NSP) and anti-nutritional factors, which in turn compromised both nutrient utilisation efficiency and the growth of pigs. However, recent research indicates that a sweet lupin variety (*L. angustifolius cv.Mandelup*) can be used at higher inclusion levels of 35% in pig diets without compromising growth performance (Kim *et al.*, 2007; 2010). Evidence for the cholesterol lowering effect of legumes in animal and human models has accumulated in recent years (Martins *et al.*, 2004; 2005). However much of this work has been done with peas, soybean or particular components such as soy lecithin. Consumption of soy protein was effective in lowering the levels of serum triglycerides and cholesterol in humans and animals (Anderson *et al.*, 1995; Sirtori *et al.*, 1995). *Lupinus angustifolius* reduced plasma total cholesterol (3.20 mmol/L versus 4.52 mmol/L) and LDL cholesterol (1.67 mmol/L versus 2.69 mmol/L) in pigs fed a lupin diet versus a control diet, respectively (Martins *et al.*, 2005). Further research has found feeding pigs diets containing 5% soy lecithin resulted in reduced levels for both serum total and low density lipoprotein (LDL) cholesterol (Kim *et al.*, 2008). Similarly, D'Souza *et al.* (2005) reported that soy lecithin supplementation tended to reduce plasma cholesterol in pigs and increased the level of polyunsaturated fat content of pork.

The rising cost of feed is making it increasingly difficult for producers to maintain profit margins. Cholesterol levels in commercial pig diets have not previously been determined. Tallow is the primary source of dietary cholesterol in commercial grower-finisher pig diets and provides a concentrated energy source, increasing dietary energy density, and allowing for the inclusion of alternative ingredients with lower energy densities, thereby reducing the reliance on cereal grains in pig diets. The inclusion of tallow in commercial pig diets is variable and recent Australian research has demonstrated the performance benefits of feeding high fat diets (6% tallow) (Collins *et al.*, 2009).

The cholesterol content of pork (*Longissimus dorsi)* is variable (e.g. 54.17 mg/100 g tissue (Burgos *et al.*, 2010) to 62.3 mg/100 g tissue (Kim *et al.*, 2008)) and this would be primarily due to differences in genotype, diet composition and the amount of intramuscular fat analysed (Table 1.1).

Table 1.1: Cholesterol content (mg/kg) of beef, lamb and pork (Swize *et al.*, 1992).

Species	Loin		Topside	
	Mean	SD	Mean	SD
Beef	63.4	1.7	83.6	3
Lamb	77.4	7.5	77.6	2.7
Pork	76	4.7	78.3	4.2

Many studies investigating cholesterol reduction in pigs measure plasma cholesterol levels but not tissue cholesterol levels (Kingman *et al.*, 1993; Martins *et al.*, 2005). It is hypothesised that the lack of effect on cholesterol content in tissue, compared to plasma cholesterol levels, is due to the modulatory role the liver plays in the cholesterol content of tissue, where excess cholesterol is transported to the liver for excretion in bile salts (Chen *et al.*, 2005; Dongowski, 2007; Visavadiya and Narasimhacharya, 2008). To effectively reduce the cholesterol content of pork we need to manipulate the absorption and excretion of cholesterol. Particular dietary components reduce cholesterol absorption in the small intestine, increase the transport of cholesterol towards the large intestine to ultimately result in a higher cholesterol turnover via increased hepatic bile acid production and faecal excretion of cholesterol and bile salts. Lupins contain high levels of pectin relative to other grains commonly used in pig diets (lupin hulls contain approximately 30% pectin, while the kernel contains about 25% pectin). Pectin, a soluble fibre source, has been shown to inhibit hypercholesterolemia in pigs principally via gel forming properties which increase the viscosity of intestinal digesta, reducing the absorption of cholesterol in the small intestine (Baekey *et al.*, 1988).

Given that lupins are already included in pig diets, it is important that we investigate the hypocholesterolaemic properties of lupins in pigs as this represents a worthwhile and relatively cost-effective strategy to further promote the healthiness of Australian pork. The aim of this experiment was to measure the cholesterol reducing properties of lupins and compare them against the known cholesterol reducing properties of soy lecithin in finisher pigs. The hypothesis was that pigs fed a lupin (30%) based or soy lecithin (7.5%) fortified diet during the finishing period would have lower cholesterol levels in both plasma and tissue compared to pigs fed a control diet. It was also hypothesized that the inclusion of lupins or soy lecithin in the diet would increase the polyunsaturated fatty acid (PUFA):saturated fatty acid (SFA) ratio in pork enhancing its nutritional value without negatively impacting carcass or objective meat quality.

2. Methodology

2.1 Animals and experiment design

Sixty individually housed pigs (Large White x Landrace; immunocastrates approximately 13 weeks of age) weighing 39.6 ± 0.49 kg were allocated by live weight (LW) to a 2 x 3 factorial experiment with respective factors being two dietary cholesterol levels (C; low vs high), and three basal diets (control [wheat], 7.5% soy lecithin and 30% lupins). Pigs within each block were allocated to adjacent individual pens within an insulated naturally-ventilated grower-finisher facility at the Medina Research Station. Diets with low cholesterol levels were equivalent to commercial finisher diets with low tallow content (approximately 0.5%). Those diets that had high levels of cholesterol contained 4.5% tallow and 18.5% full cream milk powder to increase the proportion of cholesterol in the diet. Diets were balanced for available lysine (0.7%), but contained different digestible energy levels due to the addition of both tallow and full cream milk powder (Appendix 1). Pigs were fed the low cholesterol control diet during a one week acclimatisation period, before receiving *ad libitum* feeding of experimental diets until slaughter. Average daily gain (ADG) and average daily feed intake (ADFI) were recorded and from this, average digestible energy intake (ADEI) per day and feed conversion ratio were calculated. At approximately 95 kg pigs were transported to a commercial abattoir for slaughter. Hot standard carcass weight (HSCW), dressing percentage and P2 depth (mm) were measured on the hot carcass by abattoir staff. The first pigs reached slaughter weight by day 42, with the duration for which pigs consumed experimental diets ranging from 42 – 63 days.

2.2 Blood plasma analyses

Prior to the experimental diets being fed to pigs, blood plasma samples were collected via vena puncture to assess the basal levels of total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol (Day 0). Blood samples were also collected from all pigs 24 hours pre-slaughter. Total cholesterol, LDL and HDL cholesterol levels were analysed by an automated clinical chemistry analyser (Olympus AU400) using commercial kits (Olympus catalogue numbers OSR6183, OSR6187 and OSR6116 respectively supplied by Beckman Coulter Australia Pty Ltd).

2.3 Tissue analysis

Twenty-four hours post-slaughter, *m.biceps femoris* (ham), belly fat and *m.longissimus dorsi* (loin) tissues were collected. Approximately 1kg of the loin (with fat and skin attached) was removed from the left side of 0carcasses in a posterior direction from the point of the last rib and measurements of objective pork quality performed according to Moore *et al*. (2012). The pH of the loin between the 12th and 13th rib was determined using a portable pH/temperature meter (Jenco Electronic Ltd, Model 6009) fitted with a polypropylene spear-type gel electrode (Ionode IJ42S, Brisbane, QLD) and a temperature probe. Drip loss from the loin muscle was measured using the suspension method described by Honikel (1998). Surface lightness (L^*), redness (a^*) and yellowness (b^*) of the loin muscle were measured with a Minolta Chromameter CR-400, using D65 lighting, a

2° standard observer and a measuring aperture of 8 mm, standardised to a white tile. Shear force of the cooked samples was determined using a Warner Bratzler shear blade fitted to Lloyd texture analyser machine.

Percentage of intramuscular fat in the loin and fatty acid composition of the loin, ham and belly fat tissues were measured by capillary gas chromatography. Cholesterol analyses of loin and ham tissues were performed following using a high-performance liquid chromatography (HPLC) method (Katsanidis and Addis, 1999). Belly fat cholesterol content was determined by a modified HPLC method (Dinh *et al.*, 2008) due to poor recovery of cholesterol associated with triglyceride inhibition when the HPLC method for cholesterol analysis of lean tissue was used.

2.4 Feed chemical analysis

Theoretical analysis, composition and ingredients of the the experimental treatment diets are shown in Appendix 1. Analysis of experimental diets was performed by Agrifood Technology (FeedTest), Werribee, Victoria and shown in Table 2.1. Fatty acid composition of diets were analysed by capillary gas chromatography. The level of SFA reflected the cholesterol level of the diets as expected, whereby the high cholesterol diets had higher levels of SFA (decreasing the PUFA:SFA ratio).

Table 2.1: Analysed composition of experimental treatment diets (g/kg as-fed basis)

Diet	Control		7.5% Soy lecithin		30% Lupins	
Cholesterol level (C)	Low	High	Low	High	Low	High
Ash	61.0	49.0	68.0	61.0	56.0	48.0
Crude fibre	33.0	28.0	34.0	30.0	60.0	59.0
Crude fibre (g/kg of DM)	36.0	31.0	37.0	32.0	66.0	65.0
Protein (g/kg of DM)	197.0	183.0	229.0	242.0	242.0	239.0
Dry Matter	917.0	915.0	924.0	915.0	915.0	919.0
Moisture	803.0	850.0	760.0	820.0	810.0	850.0
Nitrogen free extract	595.0	568.0	500.0	490.0	494.0	488.0
Fatty acid quantities and ratios						
Fat (g/kg of DM)	76.0	97.0	131.0	157.0	103.0	111.0
SFA (g/kg)	16.2	21.8	28.6	36.5	17.2	23.7
PUFA (g/kg)	42.0	30.1	71.8	69.5	45.0	38.7
PUFA:SFA ratio	2.60	1.38	2.51	1.90	2.61	1.63
n-6:n-3 ratio	2.70	7.06	4.48	6.92	3.44	6.78

2.4 Statistical analyses

Data were analysed by two-way ANOVA (Genstat v15). As pigs were slaughtered over four separate dates, both slaughter date and duration on treatment diet (42 – 63 days) were assessed for their impact on cholesterol measurements and neither were found to impact on treatment effects. The individual pig was the

experimental unit for statistical analysis. Results were considered significant when $P<0.05$ and trends were identified when $P<0.10$.

3. Outcomes

3.1 Growth performance and carcass composition

Results of the ANOVA analyses are presented in Table 3.1. For growth performance data from day 0 to 42, neither cholesterol level nor diet had a significant effect on LW or ADG ($P>0.05$), however there was a strong trend for pigs fed the soy lecithin diet to have a lighter LW than pigs fed the control or lupin diets ($P=0.059$). This trend was explained by the significant effect of diet on ADFI ($P=0.001$) as well as the interaction between cholesterol level and diet ($P=0.017$). Pigs fed high cholesterol diets had a higher ADFI than pigs fed the soy lecithin diets and the low cholesterol lupin diet. Pigs fed the high cholesterol soy lecithin diet had the lowest ADFI compared to all other treatments. There was also a trend for pigs fed the low cholesterol diets to have a lower FCR than pigs fed the high cholesterol diets ($P=0.083$).

For overall growth performance, cholesterol level and diet did not affect ADG and FCR. There was a significant effect of diet and the interaction between cholesterol level and diet on ADFI ($P\leq0.001$), where pigs fed the high cholesterol soy lecithin diet had the lowest ADFI, while the pigs fed the high cholesterol control diet had the highest ADFI. As a result of differences in both the ADFI and energy densities of the various treatment diets, there was a significant effect of the cholesterol level, diet and their interaction on ADEI ($P<0.05$).

Neither cholesterol level nor diet effected the number of days pigs took to reach target final LW, the HSCW or P2 fat depth ($P>0.05$). Carcass dressing percentages for pigs fed lupin diets were lower than that of the pigs fed control or soy lecithin diets ($P=0.008$).

The data demonstrates that the inclusion of lupins had no negative impacts on the growth performance of immunological castrates, however soy lecithin limited ADFI. Inclusion of lupins lowered dressing percentage, possibly due to a higher fibre component in lupin diets increasing gastrointestinal development, leading to an increase in the weight percentage of gastrointestinal organs relative to LW.

Table 3.1: Effect of dietary cholesterol levels and diet on growth performance and carcass characteristics of immunocastrate pigs (n=10).

Diet (D)	Control		7.5% Soy lecithin		30% Lupins			Significance		
Cholesterol (C)	Low	High	Low	High	Low	High	SEM	C	D	C*D
Growth performance (day 0 – 42)										
LW at 42 days (kg)	87.5	86.8	83.8	83.6	87.7	86.6	1.61	0.949	0.059	0.856
Final LW (kg)	95.8	96.5	96.4	94.8	96.3	95.7	2.27	0.432	0.767	0.341
ADG (g)	1138	1113	1076	1067	1108	1130	30.5	0.556	0.201	0.920
ADFI (g)	2426 ^{bc}	2556 ^{c}	2363 ^{b}	2152 ^{a}	2344 ^{b}	2500 ^{bc}	65.6	0.513	0.001	0.017
FCR	2.30	2.57	2.40	2.41	2.34	2.45	0.00	0.083	0.919	0.348
Growth performance (overall)										
LW (day 0; kg)	39.7	40.1	39.1	38.8	40.1	40.2	0.49	0.494	0.383	0.955
ADG (g)	1153	1127	1095	1079	1124	1122	33.9	0.894	0.211	0.782
ADFI (g)	2594 ^{b}	2767 ^{c}	2573 ^{b}	2400 ^{a}	2575 ^{b}	2674 ^{bc}	46.4	0.456	<0.001	0.001
FCR	2.18	2.40	2.22	2.24	2.17	2.27	0.08	0.108	0.664	0.463
ADEI (MJ/day)	39.2 ^{ab}	42.6 ^{c}	40.9 ^{b}	40.6 ^{b}	37.9 ^{a}	40.0 ^{b}	0.72	0.005	0.015	0.034
Days on diet	49	51	54	53	50	40	2.0	0.931	0.177	0.739
Carcass composition										
HSCW (kg)	65.2	65.8	65.9	64.5	64.5	63.7	0.76	0.361	0.178	0.443
Dressing percentage (%)	68.1 ^{b}	68.1 ^{b}	68.3 ^{b}	68.1 ^{ab}	67.0 ^{a}	66.5 ^{a}	0.50	0.533	0.008	0.875
P2 fat (mm)	12.4	13.2	12.7	13.0	11.3	12.8	0.77	0.162	0.531	0.752

SEM, standard error of the mean; ^{abc} Means in a row with different superscripts differ significantly (P<0.05).

3.2 Blood attributes

Results of the ANOVA analyses are presented in Table 3.2, Figure 3.1 and Figure 3.2. Basal total cholesterol, HDL or LDL levels (Day 0) did not vary across allocated treatment groups ($P>0.100$). Neither cholesterol level nor diet had a significant effect on plasma HDL cholesterol levels ($P>0.05$). However, as expected, soy lecithin significantly lowered both plasma total ($P=0.040$) and LDL ($P<0.001$) cholesterol (% change) in both low and high cholesterol diets when compared to the control groups. Furthermore the inclusion of lecithin in finisher diets decreased the plasma LDL:HDL and total cholesterol:HDL ratios significantly (Figures 3.1 and 3.2; $P<0.001$). Lowered LDL:HDL and total cholesterol:HDL ratios are both associated with increased health benefits through lowering the risk of cardiovascular disease.

The lupin diets appeared to lower both plasma LDL and total cholesterol (% change), however this was not significantly different from pigs fed the control diets ($P>0.05$).

Table 3.2: Effect of dietary cholesterol levels and diet on total, HDL and LDL cholesterol contents in plasma of immunocastrate pigs (n=10).

Diet (D)	Control		7.5% Soy lecithin		30% Lupins			Significance		
Cholesterol (C)	Low	High	Low	High	Low	High	SEM	C	D	C*D
Total cholesterol										
Day 0 (mmol/L)	2.67	2.45	2.65	2.53	2.79	2.70	0.10	0.112	0.172	0.761
Pre-slaughter(mmol/L)	2.66 ^{bc}	2.70 ^{bc}	2.53 ^{ab}	2.35 ^{a}	2.77 ^{c}	2.70 ^{bc}	0.09	0.265	0.002	0.465
change	-0.01 ^{ab}	0.25 ^{b}	-0.13 ^{a}	-0.17 ^{a}	-0.01 ^{ab}	-0.01 ^{ab}	0.11	0.543	0.049	0.278
% change	-0.10 ^{ab}	11.33 ^{b}	-4.44 ^{a}	-6.16 ^{a}	-1.45 ^{ab}	-1.94 ^{ab}	4.20	0.339	0.040	0.256
HDL										
Day 0 (mmol/L)	0.95	0.90	0.98	0.91	0.97	0.96	0.04	0.156	0.478	0.614
Pre-slaughter(mmol/L)	1.08	1.07	1.08	1.03	1.10	1.05	0.04	0.263	0.825	0.820
change	0.13	0.17	0.10	0.13	0.13	0.09	0.04	0.338	0.631	0.569
% change	14.36	20.29	10.79	14.77	14.25	11.74	4.87	0.551	0.590	0.666
LDL										
Day 0 (mmol/L)	1.70	1.54	1.65	1.61	1.66	1.75	0.07	0.110	0.467	0.762
Pre-slaughter(mmol/L)	1.61 ^{b}	1.56 ^{b}	1.33 ^{a}	1.28 ^{a}	1.55 ^{b}	1.64 ^{b}	0.06	0.379	<0.001	0.611
change	-0.08 ^{b}	0.02 ^{b}	-0.33 ^{a}	-0.33 ^{a}	-0.11 ^{b}	-0.11 ^{b}	0.07	0.319	<0.001	0.368
% change	-2.16 ^{b}	2.48 ^{b}	-18.80 ^{a}	-19.46 ^{a}	-4.81 ^{b}	-5.62 ^{b}	3.95	0.242	<0.001	0.318

SEM, standard error of the mean; ^{abc} Means in a row with different superscripts differ significantly (P<0.05).

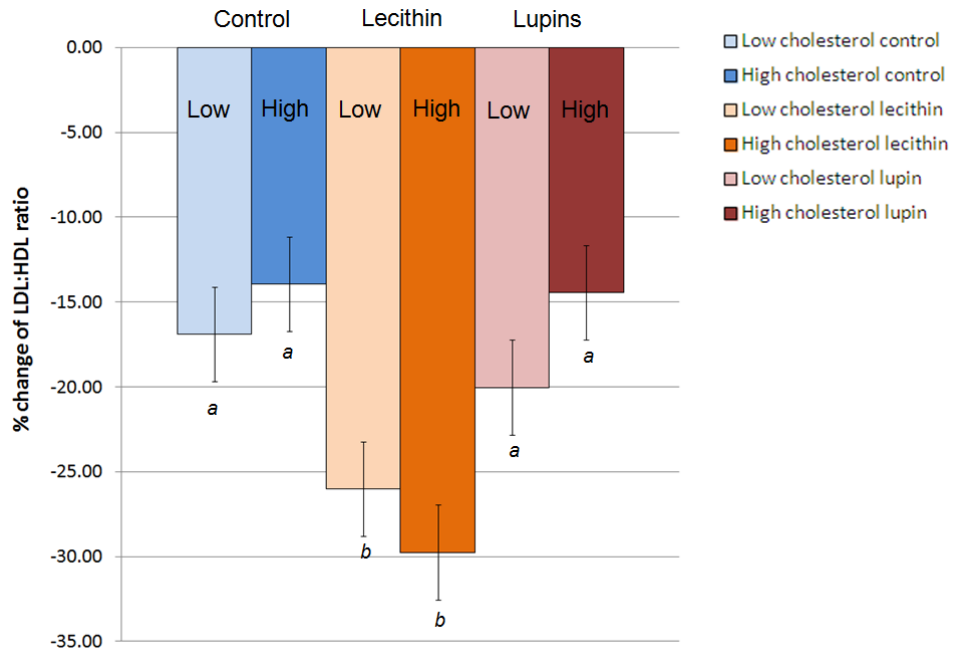

Figure 3.1 The percentage change in the plasma LDL:HDL ratio of immunocastrate pigs fed control, soy lecithin (7.5%) or lupin (30%) diets with either a low or high cholesterol level.

[ab] Means with different superscripts differ significantly (P<0.05).

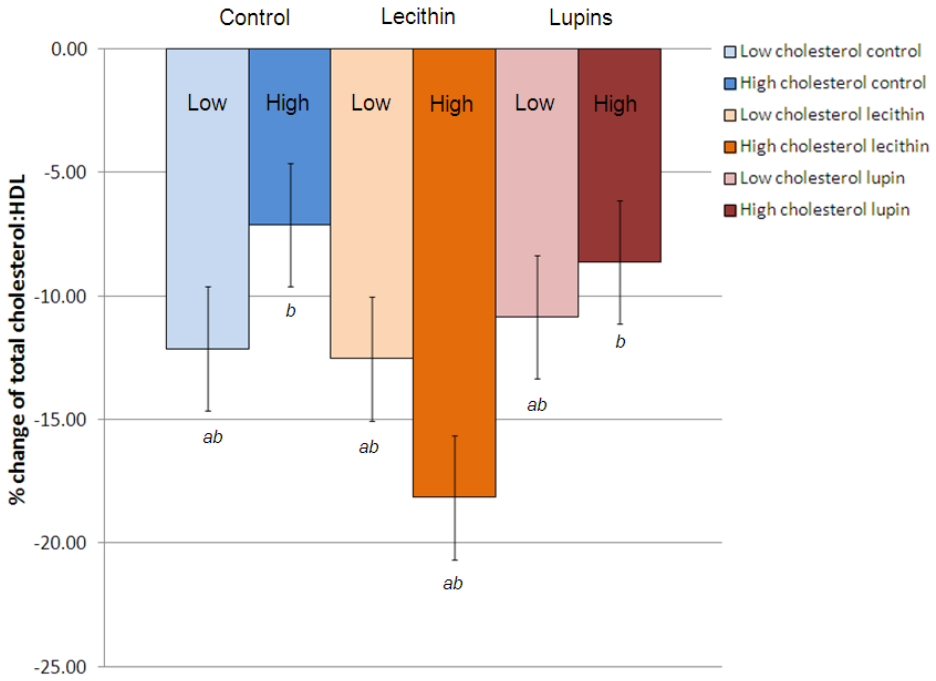

Figure 3.2 The percentage change in plasma total cholesterol:HDL ratio of immunocastrate pigs fed control, soy lecithin (7.5%) or lupin (30%) diets with either a low or high cholesterol level.

[ab] Means with different superscripts differ significantly (P<0.05).

3.3 Objective meat quality

Results of the ANOVA analyses are presented in Table 3.2, Figure 3.1 and Figure 3.2. Results of the ANOVA analyses are presented in Table 3.3. Objective pork quality was not influenced by the main treatment effects of cholesterol level or diet ($P>0.05$), nor was there an effect of their interaction ($P>0.100$) (data not presented). There was a strong trend for pork from pigs fed high cholesterol diets to have a lower percent cook loss ($P= 0.054$) and a trend for pork from pigs fed low cholesterol diets to have a higher L^* value ($P=0.074$) indicating that pork colour was lighter. These data indicate that the inclusion of lupins or soy lecithin in diets with a low or high level of cholesterol had no influence on objective pork quality measures.

Table 3.3: Effect of dietary cholesterol levels and diet on objective meat quality measures from immunocastrate pigs (n=10).

Diet (D)	Control		7.5% Soy lecithin		30% Lupins			Significance	
Cholesterol (C)	Low	High	Low	High	Low	High	SEM	C	D
Intramuscular fat (%)	1.3	1.7	1.3	1.3	0.8	1.1	0.20	0.474	0.153
pH_{24}	5.47	5.47	5.49	5.50	5.46	5.47	0.032	0.834	0.520
Drip loss (%)	4.75	4.94	4.99	3.83	5.26	4.49	0.508	0.162	0.584
*L**	51.7	50.3	51.2	49.9	51.8	50.1	1.01	0.074	0.874
*a**	6.31	6.77	6.98	6.33	6.49	6.74	0.332	0.929	0.943
*b**	3.31	3.38	3.51	2.85	3.49	3.25	0.347	0.338	0.822
Cook loss (%)	26.6	26.2	27.0	24.6	26.8	25.9	0.73	0.054	0.631
Shear force (N)	34.0	38.6	33.8	35.8	32.2	36.5	3.22	0.373	0.819

3.4 Tissue cholesterol

Results of the ANOVA analyses are presented in Table 3.4. As expected, dietary cholesterol level was found to significantly influence loin and ham tissue cholesterol levels ($P<0.05$), but this was not observed for belly fat. Although pigs fed soy lecithin or lupin diets had lowered tissue cholesterol levels when compared to the control group, the effect of diet was not significant for the loin and belly fat tissues, though a trend was observed for ham tissue ($P=0.062$).

These results suggest that the level of cholesterol in pork is regulated by dietary cholesterol levels and likely also influenced by feed composition.

Table 3.4: Effect of dietary cholesterol levels and diet on cholesterol level of loin, ham and belly fat tissues of immunocastrate pigs (n=10).

Diet (D)	Control		7.5% Soy lecithin		30% Lupins			Significance		
Cholesterol (C)	Low	High	Low	High	Low	High	SEM	C	D	C*D
Cholesterol (mg/100g)										
Loin	43.6 [ab]	45.8 [a]	41.7 [a]	43.5 [ab]	42.1 [a]	43.7 [ab]	1.15	0.049	0.153	0.977
Ham	48.9 [ab]	50.6 [b]	43.3 [a]	47.4 [ab]	43.9 [a]	48.1 [ab]	2.00	0.042	0.062	0.791
Belly fat	70.4	75.2	66.1	66.3	68.7	70.1	3.41	0.474	0.153	0.755

SEM, standard error of the mean; [ab] Means in a row with different superscripts differ significantly ($P<0.05$).

3.5 Tissue fatty acid composition

Results of the ANOVA analyses are presented in Tables 3.5.1, 3.5.2 and 3.5.3. Pigs fed a diet with a low cholesterol had significantly different PUFA:SFA ratios ($P<0.05$) in their tissues (loin, ham and belly fat) when compared to pigs fed a high cholesterol diet ($P<0.001$). Diet also significantly affected the fatty acid profile of the loin, ham and belly fat ($P<0.05$). . The PUFA:SFA ratios from pigs fed the low cholesterol control diets were intermediate. For pigs fed low cholesterol diets, lupins were more effective in maintaining the n-6:n-3 ratios in loin, ham and belly fat tissues when compared to soy lecithin fed pigs ($P<0.001$).

The data show that the inclusion of lecithin in finisher diets increases the ratio of PUFA:SFA ratio in the lean and fat tissues of pork, resulting in a healthier product. Similarly, the inclusion of lupins in high cholesterol (high fat) finisher diets improves the fatty acid profile of pork so that the PUFA:SFA ratio is similar to that for pork from pigs fed a low cholesterol (low fat) diet. Hence, lupins possess further advantages other than being a nutrient source for finisher pigs, shown to be a potential nutritional management tool when included in finisher diets to effectively enhance the nutritional value of pork.
Lupins have advantages other than nutrient supply for finisher pigs –something like this?

Table 3.5.1: Effect of dietary cholesterol level and diet on fatty acid composition of the loin tissue from immunocastrate pigs (n=10).

Diet (D)	Control		7.5% Soy lecithin		30% Lupins			Significance		
Cholesterol (C)	Low	High	Low	High	Low	High	SEM	C	D	C*D
Total (mg/100g)										
SFA	951	1226	813	883	753	926	117.4	0.075	0.064	0.684
MUFA	1206 ^{ab}	1394 ^{b}	920.3 ^{a}	927 ^{a}	978 ^{ab}	1019 ^{ab}	158.1	0.543	0.048	0.830
PUFA	517 ^{a}	574 ^{ab}	651 ^{bc}	709 ^{c}	584 ^{ab}	628 ^{abc}	43.4	0.657	0.013	0.362
PUFA:SFA ratio	0.66 ^{ab}	0.49 ^{a}	0.84 ^{bc}	0.83 ^{bc}	0.91 ^{c}	0.70 ^{b}	0.072	0.031	<0.001	0.351
n-6 (%)	17.50 ^{ab}	15.81 ^{a}	23.19 ^{c}	25.00 ^{c}	22.94 ^{c}	21.27 ^{bc}	1.677	0.692	<0.001	0.494
n-3 (%)	4.63 ^{c}	2.31 ^{a}	4.65 ^{c}	3.27 ^{b}	4.72 ^{c}	3.03 ^{b}	0.125	0.078	<0.001	0.095
n-6:n-3 ratio	3.75 ^{a}	6.84 ^{c}	5.01 ^{c}	7.66 ^{d}	4.79 ^{b}	7.09 ^{cd}	0.284	<0.001	0.002	0.388

SEM, standard error of the mean; ^{abc} Means in a row with different superscripts differ significantly (P<0.05).
MUFA, monounsaturated fatty acids.

Table 3.5.2: Effect of dietary cholesterol level and diet on fatty acid composition of the ham tissue from immunocastrate pigs (n=10).

Diet (D)	Control		7.5% Soy lecithin		30% Lupins			Significance		
Cholesterol (C)	Low	High	Low	High	Low	High	SEM	C	D	C*D
Total (mg/100g)										
SFA	1061	1497	1048	1146	983	1060	169.0	0.149	0.316	0.497
MUFA	1465	1823	1333	1337	1415	1368	251.6	0.617	0.438	0.684
PUFA	686 [a]	715 [a]	955 [ab]	1050 [b]	722 [ab]	775 [a]	110.1	0.972	0.016	0.775
PUFA:SFA ratio	0.70 [b]	0.50 [a]	0.97 [d]	0.93 [cd]	0.86 [c]	0.72 [b]	0.040	<0.001	<0.001	0.146
n-6 (%)	17.84 [a]	15.94 [a]	25.77 [c]	27.10 [c]	21.67 [b]	21.41 [b]	1.123	0.765	<0.001	0.363
n-3 (%)	4.90 [d]	2.27 [a]	4.91 [d]	3.36 [b]	4.69 [d]	2.92 [c]	0.142	<0.001	0.002	0.002
n-6:n-3 ratio	3.63 [a]	6.98 [d]	5.23 [c]	8.09 [e]	4.61 [b]	7.33 [d]	0.187	<0.001	<0.001	0.221

SEM, standard error of the mean; [abcde] Means in a row with different superscripts differ significantly (P<0.05).

Table 3.5.3: Effect of dietary cholesterol level and diet on fatty acid composition of the belly fat tissue from immunocastrate pigs (n=10).

Diet (D)	Control		7.5% Soy lecithin		30% Lupins			Significance		
Cholesterol (C)	Low	High	Low	High	Low	High	SEM	C	D	C*D
Total (mg/100g)										
SFA	27529 ^{b}	33488 ^{e}	27816 ^{bc}	29544 ^{cd}	25014 ^{a}	29810 ^{d}	608.3	<0.001	<0.001	0.004
MUFA	37110 ^{cd}	34732 ^{b}	32448 ^{a}	30982 ^{a}	37762 ^{d}	35818 ^{bc}	671.0	<0.001	<0.001	0.794
PUFA	15515 ^{b}	12303 ^{a}	22280 ^{d}	21642 ^{d}	18171 ^{c}	16255 ^{bc}	624.0	<0.001	<0.001	0.132
PUFA:SFA ratio	0.57 ^{b}	0.37 ^{a}	0.81 ^{c}	0.74 ^{c}	0.73 ^{c}	0.55 ^{b}	0.032	<0.001	<0.001	0.129
n-6 (%)	14.03 ^{a}	12.73 ^{a}	21.12 ^{b}	22.09 ^{b}	16.89 ^{c}	16.51 ^{c}	0.537	0.585	<0.001	0.116
n-3 (%)	4.94 ^{d}	2.22 ^{a}	5.28 ^{d}	3.66 ^{b}	5.11 ^{d}	2.89 ^{c}	0.115	<0.001	<0.001	<0.001
n-6:n-3 ratio	2.85 ^{a}	5.72 ^{d}	4.02 ^{c}	6.03 ^{e}	3.30 ^{b}	5.73 ^{d}	0.084	<0.001	<0.001	<0.001

SEM, standard error of the mean; ^{abcde} Means in a row with different superscripts differ significantly (P<0.05).

4. Application of Research

These results have demonstrated that the dietary inclusion of lupins at 30% effectively manages the ratio of PUFA:SFA in the tissue of pigs fed high fat diets without impacting upon performance. Lupins possess further advantages other than being a nutrient source for finisher pigs and as they are a commonly utilised feed ingredient, this management strategy can be easily adopted to allow producers to use tallow as a concentrated energy source when alternative feed ingredients that possess low energy densities are utilised.

5. Conclusion

Soy lecithin effectively lowered plasma total and LDL cholesterol levels, however the inclusion of soy lecithin or lupins in finisher pig diets did not significantly lower tissue cholesterol levels. Pork from pigs fed soy lecithin or lupins had a higher PUFA:SFA ratio indicating that lupin fed pigs can produce healthier pork with enhanced nutritional value and fatty acid profile. The inclusion of soy lecithin or lupins in finisher pig diets had no negative impacts upon pig growth performance, with the exception of soy lecithin fed pigs, which had lighter LW at Day 42 and lower ADFI (Day 42 and overall). There were also no observed effects of diet type on objective pork quality measurements.

6. Limitations/Risks

For producers including high levels of tallow in their finisher diets to provide a concentrated energy source and limit the impact of rising feed costs attributed to cereal grain prices, lupins would not be an effective dietary strategy in lowering cholesterol content of pork tissues. However, lupins could maintain cholesterol levels and enhance the fatty acid profile (PUFA:SFA) of pork resulting in a healthier pork product. Although soy lecithin lowered plasma cholesterol levels as expected, it is not an affordable nutritional strategy. Investigation into the inclusion of lupins to lower the cholesterol level and enhance the fatty acid composition of pork from pigs in a larger commercial environment (group housing environment) would be warranted to assess the effectiveness of this nutritional strategy.

Future research should be directed to clarify:

- Effects of including lupins to lower blood plasma and tissue cholesterol levels of group housed finisher pigs in a larger scale study, fed either a diet with a high or low cholesterol level.
- Different inclusion rates of lupins to assess their influence on the fatty acid composition, processing variability and shelf-life of fresh pork.

7. Recommendations

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

- Lupins can be incorporated into high fat finisher pig diets at levels of up to 30% to supply further advantages other than being a nutrient source for finisher pigs, whereby they can effectively manage the fatty acid profile (PUFA:SFA ratio) of pork to maintain healthier pork product from pigs fed a standard low fat diet.
- Further research, with a larger number of pigs (group housed in a commercial environment) is required to assess whether the inclusion of lupins can effectively lower the cholesterol content of pork when pigs are fed high fat diets.

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Appendix 1 – Composition and theoretical analysis of base feeds

Composition of experimental treatment diets (g/kg as-fed basis)

Diet	Control		7.5% Soy lecithin		30% Lupins	
Cholesterol and SFA (CF) level	Low	High	Low	High	Low	High
Ingredients						
Wheat	400.0	200.0	339.2	171.8	200.0	100.0
Barley	100.0	200.0	200.0	200.0	100.0	200.0
Lupins	50.0	0.0	50.0	50.0	300.0	292.1
Soybean meal	50.0	10.2	50.0	50.0	35.9	0.0
Blood meal	10.0	0.0	10.0	10.0	10.0	3.39
Meat meal	63.1	50.0	82.9	70.6	63.8	30.0
Fish meal	35.5	0.0	33.2	0.0	0.0	0.0
Full cream milk powder	0.0	214.2	0.0	187.2	0.0	165.0
Tallow	0.0	42.9	0.0	65.0	0.0	65.0
Soy lecithin	0.0	0.0	75.0	75.0	0.0	0.0
Canola oil	62.0	0.0	50.0	0.0	70.2	0.0
L-Lysine	2.13	2.85	2.72	2.31	1.48	0.99
DL-Methionine	0.40	0.67	0.61	1.10	0.98	0.63
L-Threonine	0.53	1.19	0.89	1.09	0.0	0.09
L-Tryptophan	0.04	0.23	0.17	0.21	0.0	0.0
Vit/Min[1]	2.50	2.50	2.50	2.50	2.50	2.50
Limestone	0.0	0.93	0.0	1.54	0.46	9.59
Dicalcium phosphate	0.0	10.0	0.0	0.0	3.24	5.21
Salt	2.00	2.50	2.50	2.50	2.00	2.50
Choline chloride	0.40	0.45	0.40	0.40	0.40	0.40
Total	1000	1000	1000	1000	1000	1000
Calculated composition (g/kg)						
DE	14.5	15.0	15.5	17.0	14.5	15.2
Protein	190.8	167.7	195.9	195.9	215.5	208.2
Fat	89.6	120.6	154.8	212.4	105.1	140.0
Crude fibre	41.2	37.3	43.2	38.9	72.5	7.11
Available lysine	9.4	9.7	10.0	11.1	9.4	9.9
NDF	151.3	157.7	161.0	140.5	180.0	184.5
Cholesterol	0.00	0.24	0.00	0.24	0.00	0.22

[1]Provided per kg of air-dry diet: vitamins: A 4900 IU, D3 980 IU, E 14 mg, K 0.7 mg, B1 0.7 mg, B2 2.1 mg, B6 1.05 mg, B12 10.5 mg, calcium pantothenate 7.5 mg, folic acid 0.13 mg, niacin 8.4 mg, biotin 21 mg; minerals: Co 0.14 mg, Cu 7 mg, iodine 0.35 mg, iron 42 mg, Mn 28 mg, Se 0.21 mg, Zn 70 mg.