

FATTY ACID COMPOSITION, CHOLESTEROL- AND INTRAMUSCULAR FAT- CONTENT OF LOIN MUSCLES FROM FINISHER PIGS FED SOY LECITHIN SUPPLEMENT

3A-112

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Co-operative Research Centre for an Internationally
Competitive Pork Industry

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Executive Summary

This project was undertaken by the Victorian Department of Primary Industries to provide quantitative analysis of samples collected from an experiment undertaken by Pork CRC researchers in pigs fed a dietary supplement involving with soy lecithin.

The analysis presented covers the fatty acid composition and cholesterol content of these samples.

The analysis will be used in combination with other findings of the research study to better understand the dietary factors affecting pig production and pork meat quality.

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Introduction

An experiment was undertaken with pigs fed a diet supplemented with soy lecithin by Prof. Frank Dunshea at the University of Melbourne Dookie campus animal facilities. Pork muscle samples (*longissimus dorsi*) were collected from pigs at 24 h post-slaughter during this study. These muscle samples were transferred to the Department of Primary Industries, Werribee Centre, in November 2010 for the determination of fatty acid composition and cholesterol content. Samples were supplied with ID, but no information on treatment codes.

DPI was to be responsible for the completion of the analytical work and provide results in Excel form with a description of the methodologies used for the analysis.

Methodology

Individual fatty acid content and the total composition were quantified by capillary Gas Chromatography (see Appendix 1 for details). Fatty acid peaks were identified using a reference standard (Supelco C4-C24 mix, Sigma Aldrich), which was run in each batch. Fatty acid and total fat results are expressed as mg/100 g of meat.

The cholesterol content of the pork was determined using Gas Chromatography - Flame Ionization Detector (see Appendix for 2 for details). The concentration of cholesterol in samples was calculated from the quadratic regression of concentration against the ratio of cholesterol response to an internal standard response.

Outcomes

Results were tabulated in excel sheet format, with the results attributed to the sample ID as supplied. The electronic documents have been uploaded to Pork CRC iMap management system.

The details of individual fatty acid content and total fatty acid composition in pork are contained the Excel document - **Fatty acid RESULTS-lecithin study.xls**. The results are also presented in Table 1. Values for total fat do not include results of C4:0 as the uniformly high valued for this component are unusual. In general C4:0, C6:0 and C8:0 fatty acids are very small compared with other medium and long chain saturated fatty acids and monounsaturated fatty acids in meat (refer articles Enser et al. (1996); Haak et al. (2008) for details.). The observed level for C4:0 in the present study might have been due to highly volatile contaminants introduced during the preparation of the samples for analysis.

The details of cholesterol content in pork are contained in the excel document - **Lecithin study- cholesterol results.xls**. The results are also presented in Table 2.

Table 1 - Fatty acid composition and total fat content (mg/100 g meat)

Sample No	C4:0	C6:0	C8:0	C10:0	C11:0	C12:0	C13:0	C14:0	C14:1	C15:0	C15:1	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1n6c+	C18:2n6t	C18:2n6c	C18:3n6	C18:3n3	#CLA-1	#CLA-2	#C18:4n3	C20:0	C20:1n9	C20:2n6	C20:1	C20:3n6	C20:4n6	C20:3n3	C22:0	C20:5n3	C22:1n9	C22:2n6	C23:0	C22:3n6	C22:4n6	C24:0	C22:5n3	C24:1n9	C22:6n3	total muscle fat		
1	153.73	0.00	0.31	2.17	0.00	1.59	0.00	27.77	0.76	1.69	0.00	567.82	77.73	8.08	0.12	284.77	990.54	1.27	228.66	1.93	12.95	0.65	0.04	0.00	4.38	22.33	7.29	2.51	7.56	47.38	2.01	0.35	2.46	0.54	0.00	0.00	0.00	8.73	0.00	7.74	0.00	8.43	0.24	4.91	2325.56
2	149.58	0.00	0.22	1.54	0.00	1.20	0.00	21.67	0.66	1.44	0.00	415.87	62.96	6.37	0.11	204.46	696.39	1.22	217.09	2.07	11.37	0.63	0.05	0.00	2.62	15.22	6.28	2.18	7.51	49.53	1.81	0.24	2.98	0.37	0.00	0.00	0.00	7.74	0.00	8.43	0.24	4.91	1755.40		
3	173.89	0.00	0.33	2.71	0.00	2.11	0.00	36.47	0.84	1.80	0.00	706.04	83.04	7.90	0.05	378.07	1125.73	0.60	524.60	1.83	41.14	0.65	0.06	0.00	6.01	23.84	19.16	2.26	8.38	48.39	6.31	0.43	2.79	0.48	0.00	0.00	0.12	8.10	0.00	9.69	0.25	4.62	3054.81		
4	158.38	0.00	0.09	0.66	0.00	0.47	0.00	8.47	0.24	0.69	0.00	201.49	25.41	2.86	0.09	111.38	290.68	0.96	146.07	2.35	5.14	0.18	0.05	0.00	1.35	6.18	2.93	2.18	6.69	45.03	0.62	0.23	2.69	0.21	0.00	0.00	0.00	6.35	0.00	6.61	0.10	3.90	881.93		
5	150.32	0.00	0.23	1.65	0.00	1.10	0.00	18.48	0.36	0.95	0.00	369.70	46.08	3.80	0.11	199.77	635.32	0.65	195.97	1.97	9.21	0.45	0.06	0.00	2.58	13.74	5.02	2.06	7.55	42.78	1.44	0.27	3.01	0.36	0.00	0.00	0.00	6.73	0.00	7.47	0.23	4.32	1583.43		
6	138.45	0.00	0.15	1.13	0.00	0.81	0.00	14.29	0.27	0.80	0.00	290.97	39.02	3.40	0.10	150.97	492.97	0.65	177.72	1.45	8.12	0.40	0.06	0.00	2.08	10.17	4.43	1.68	6.87	45.68	1.21	0.26	2.43	0.29	0.00	0.00	0.00	6.11	0.00	7.04	0.16	4.08	1275.79		
7	139.86	0.00	0.12	0.96	0.00	0.75	0.00	13.39	0.28	0.98	0.00	293.49	33.98	4.41	0.09	170.27	464.63	0.81	170.97	1.46	8.27	0.36	0.06	0.00	1.73	11.28	4.91	2.69	6.34	40.89	1.28	0.26	2.48	0.31	0.00	0.00	0.00	6.99	0.00	7.03	0.18	2.45	1254.09		
8	147.71	0.00	0.14	1.22	0.00	0.88	0.00	15.81	0.32	0.90	0.00	317.76	41.43	3.87	0.07	174.74	521.17	0.83	176.60	1.79	7.97	0.37	0.07	0.00	2.09	9.48	4.15	2.20	7.30	41.25	1.11	0.20	2.41	0.26	0.00	0.00	0.00	6.18	0.00	7.36	0.15	4.14	1354.23		
9	168.69	0.00	0.24	2.57	0.00	2.19	0.00	37.50	1.09	1.43	0.00	633.57	91.41	6.49	0.06	335.29	924.83	1.06	210.71	2.00	11.23	0.54	0.08	0.00	4.20	18.69	5.65	2.87	7.81	45.59	1.59	0.33	2.83	0.43	0.00	0.00	0.00	7.89	0.00	7.47	0.19	2.94	2370.78		
10	164.96	0.00	0.12	0.82	0.00	0.63	0.00	12.17	0.28	1.25	0.00	276.20	31.77	5.36	0.12	151.13	466.44	0.97	231.18	2.20	12.66	0.44	0.07	0.00	1.71	10.31	5.98	1.98	8.47	48.74	1.57	0.24	3.04	0.27	0.00	0.00	0.00	7.04	0.00	8.80	0.17	4.69	1296.80		
11	164.06	0.00	0.26	2.29	0.00	1.93	0.00	33.19	0.76	1.42	0.00	597.90	74.75	6.24	0.11	308.64	935.30	0.57	330.27	1.89	23.55	0.57	0.06	0.00	4.16	21.35	11.98	1.70	8.23	44.71	4.00	0.35	2.40	0.48	0.00	0.00	0.29	7.85	0.00	7.23	0.17	2.77	2437.37		
12	144.54	0.00	0.11	0.88	0.00	0.66	0.00	11.49	0.20	0.80	0.00	246.63	27.87	2.69	0.13	137.57	317.37	0.24	214.97	1.62	10.65	0.23	0.06	0.00	1.41	6.83	5.24	1.60	7.06	43.45	1.52	0.21	2.70	0.19	0.00	0.00	0.00	5.96	0.00	6.83	0.12	3.29	1060.57		
13	157.96	0.00	0.20	1.38	0.00	1.13	0.00	20.56	0.43	1.30	0.00	445.04	46.16	6.68	0.14	262.34	713.41	1.07	234.41	1.69	12.73	0.53	0.06	0.00	2.59	14.79	7.08	2.23	7.91	47.95	2.19	0.26	3.31	0.32	0.00	0.00	0.00	6.04	0.00	8.26	0.14	6.33	1858.64		
14	160.33	0.00	0.16	1.29	0.00	1.03	0.00	17.30	0.44	1.35	0.00	332.44	47.42	5.16	0.14	160.02	456.51	0.43	289.86	1.65	17.03	0.39	0.05	0.00	1.66	9.04	6.88	1.54	7.28	45.33	2.24	0.25	3.01	0.21	0.00	0.00	0.00	6.28	0.00	8.13	0.13	5.00	1429.64		
15	161.79	0.00	0.16	1.22	0.00	0.93	0.00	16.82	0.37	1.10	0.00	332.46	41.24	4.83	0.10	174.13	612.36	1.01	196.11	1.74	10.58	0.57	0.03	0.00	1.93	13.65	5.87	1.87	7.37	46.93	1.64	0.22	2.81	0.31	0.00	0.00	0.00	6.44	0.00	7.89	0.19	5.06	1497.96		
16	148.77	0.00	0.17	1.36	0.00	1.18	0.00	20.80	0.47	0.94	0.00	363.19	45.17	3.81	0.12	197.77	512.69	0.60	192.19	1.79	8.17	0.36	0.05	0.00	2.29	10.55	4.94	1.90	6.32	43.16	1.26	0.26	3.01	0.28	0.00	0.00	0.00	6.05	0.00	7.54	0.15	2.78	1440.69		
17	151.34	0.00	0.10	0.80	0.00	0.60	0.00	10.89	0.24	0.82	0.00	237.00	27.29	3.69	0.07	144.13	371.53	0.64	169.76	1.74	7.16	0.28	0.02	0.00	1.33	7.09	3.36	1.88	6.93	46.06	0.84	0.17	2.92	0.18	0.00	0.00	0.00	5.88	0.00	7.66	0.14	4.80	1066.03		
18	164.70	0.00	0.16	1.38	0.00	1.13	0.00	19.75	0.40	1.14	0.00	382.26	48.48	4.74	0.10	199.79	545.98	0.37	289.26	1.32	17.20	0.33	0.03	0.00	2.40	10.34	8.80	1.83	7.20	42.01	2.73	0.31	2.51	0.28	0.00	0.00	0.00	6.46	0.00	7.25	0.13	4.19	1610.23		
19	143.85	0.00	0.24	1.94	0.00	1.45	0.00	24.69	0.51	1.04	0.00	446.98	55.45	4.87	0.06	243.38	749.49	0.82	201.77	1.82	10.04	0.53	0.04	0.00	3.02	15.17	5.07	2.51	8.21	46.33	1.34	0.29	2.87	0.39	0.00	0.00	0.00	7.65	0.00	7.43	0.23	3.09	1848.71		
20	173.81	0.00	0.27	2.13	0.00	1.56	0.00	27.05	0.61	1.30	0.00	515.04	57.57	6.63	0.07	293.42	905.77	1.35	229.66	1.94	13.22	0.61	0.04	0.00	4.77	21.31	8.10	2.20	8.62	49.49	2.42	0.34	2.23	0.48	0.00	0.00	0.16	8.65	0.00	7.41	0.19	4.50	2179.06		
21	213.22	0.00	0.40	4.61	0.00	3.93	0.00	67.10	1.86	2.01	0.00	1074.55	147.73	9.74	0.11	581.84	1637.00	1.45	296.62	1.74	19.70	0.92	0.07	0.00	7.42	31.20	9.92	4.80	8.81	35.39	3.16	0.45	2.63	0.75	0.00	0.00	0.07	8.15	0.00	7.82	0.23	3.31	3975.49		
22	172.29	0.00	0.23	2.02	0.00	1.59	0.00	26.73	0.54	1.31	0.00	468.41	60.52	5.95	0.12	246.11	736.25	0.40	269.72	2.25	16.52	0.39	0.06	0.00	3.05	13.74	7.48	1.29	7.52	50.40	2.26	0.31	2.77	0.30	0.00	0.00	0.00	7.02	0.00	7.28	0.16	2.65	1945.36		
23	159.86	0.00	0.18	1.32	0.00	1.10	0.00	19.77	0.62	1.32	0.00	428.48	66.01	5.62	0.05	208.50	666.23	0.85	233.92	1.83	12.26	0.53	0.04	0.00	2.89	15.47	6.31	2.39	8.12	52.11	1.86	0.29	3.23	0.38	0.00	0.00	0.00	7.81	0.00	9.02	0.20	5.20	1763.90		
24	186.69	0.00	0.34	2.84	0.00	2.13	0.00	36.76	0.82	1.14	0.00	651.56	83.73	5.98	0.06	346.45	1122.72	1.26	242.01	1.75	13.10	0.69	0.03	0.00	5.36	21.69	7.60	1.94	8.37	43.34	2.38	0.40	2.94	0.58	0.00	0.00	0.00	7.12	0.00	7.76	0.21	4.35	2627.40		
25	157.00	0.00	0.10	0.67	0.00	0.44	0.00	7.89	0.19	0.69	0.00	180.34	26.26	2.42	0.25	99.42	281.16	0.44	147.61	1.51	5.69	0.19	0.04	0.00	1.21	6.23	3.27	1.39	5.85	39.31	0.90	0.18	2.42	0.20	0.00	0.00	0.00	5.98	0.00	6.57	0.09	2.81	831.72		
26	166.97	0.00	0.21	1.72	0.00	1.31	0.00	23.02	0.69	1.00	0.00	444.24	69.18	4.17	0.06	209.70	683.78	0.86	183.35	2.01	8.74	0.44	0.03	0.00	2.99	13.31	4.23	1.83	6.33	42.09	1.16	0.25	2.47	0.34	0.00	0.00	0.00	7.38	0.00	6.12	0.15	3.23	1726.40		
27	158.43	0.00	0.13	0.97	0.00	0.69	0.00	11.48	0.24	0.66	0.00	260.72	32.24	2.96	0.06	142.14	405.53	0.29	216.04	1.08	10.42	0.19	0.02	0.00	1.46	8.57	5.94	1.56	5.89	43.77	1.73	0.19	2.38	0.19	0.00	0.00	0.00	5.50	0.00	6.82	0.12	3.34	1173.21		
28	165.42	0.00	0.17	1.33	0.00	1.02	0.00	17.39	0.44	1.06	0.00	355.66	46.85	4.90	0.08	192.48	622.99	0.89	197.42	2.01	9.62	0.48	0.07	0.00	2.42	13.01	5.43	1.66	7.45	49.35	1.47	0.28	2.96	0.33	0.00	0.00	0.00	7.00	0.00	7.46	0.20	6.10	1560.20		
29	174.05	0.00	0.27	2.33	0.00	1.78	0.00	30.82	0.75	1.00	0.00	577.41	69.00																																

Table 2 - Cholesterol content of meat samples (mg/100 g meat)

muscle sample No	cholesterol	
	mg/kg	mg/100g
1	505	51
2	581	58
3	518	52
4	511	51
5	470	47
6	477	48
7	482	48
8	559	56
9	538	54
10	448	45
11	428	43
12	408	41
13	420	42
14	442	44
15	458	46
16	344	34
17	310	31
18	434	43
19	391	39
20	261	26
21	291	29
22	319	32
23	465	46
24	444	44
25	480	48
26	452	45
27	408	41
28	593	59
29	530	53
30	488	49
31	393	39
32	529	53
33	472	47
34	533	53
35	513	51
36	513	51

Application of Research

The results presented in this report will be used along with other measurements in scientific publications and industry articles related to the effect of soy lecithin on pig production and meat quality.

Conclusion

Analysis of meat samples has been conducted to determine the amounts of fatty acids, total fat and cholesterol.

Limitations/Risks

These results are to be used in combination with the appropriate codes to allow interpretation with respect to the treatments used in the study.

Recommendations

None

References

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M. Enser, K. Hallett, B. Hewitt, G.A.J. Fursey and J.D. Wood. (1996). Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. *Meat Sci.*, 42, 443-456.

Ponnampalam, E.N., Warner, R.D., Kitessa, S., McDonagh, M.B., Pethick, D.W., Allen, D., Hopkins, D.L., 2010. Influence of finishing systems and sampling site on fatty acid composition and retail shelf-life of lamb. *Anim. Prod. Sci.* 50, 775-781.

Appendix 1: Method for fatty acid extraction and quantification

Muscle samples were freeze-dried and a homogeneous sample of 0.6 g of ground material was used for the determination of fatty acid composition using a rapid modified procedure developed from the method reported by O'Fallon *et al.* 2007.

One hundred μL of nonadecanoic acid methyl ester (C19:0, Sigma Aldrich, Castle Hill, NSW, Australia) was added to muscle samples as an internal standard dissolved in chloroform (10 mg C19:0/mL CHCl_3). The contents were hydrolysed using 0.7 mL of 10 N KOH in water and 5.3 mL of methanol to form free fatty acids. After mixing well with vortex, the contents were incubated at 55°C for 1.5 h with vigorous mixing at 20-min intervals and then cooled to room temperature using tap water. Upon cooling, the contents were mixed with 0.6 mL of 24 N sulfuric acid in water and mixing, incubation and cooling process proceeded as above. After cooling the tubes to room temperature, the fatty acid methyl ester (FAME) was separated with 1 mL of hexane solvent by mixing for 5 min and centrifuging at 2000g for 10 min. Two-hundred μL of hexane containing FAME was collected into a GC vial and fatty acid fractions were quantified by capillary GC (HP INNOWAX 60 m \times 0.25 mm, 0.5 micron, Agilent J & W Scientific, Santa Clara, CA, USA).

Fatty acid peaks were identified using a reference standard (Supelco C4-C24 mix, Sigma Aldrich), which was run in each batch. Fatty acid levels in the muscles are reported in mg/100 g meat as this relates to nutrition information for the labelling of foods according to Food Standards Australia & New Zealand (FSANZ).

Reference: Ponnampalam, E.N., Warner, R.D., Kitessa, S., McDonagh, M.B., Pethick, D.W., Allen, D., Hopkins, D.L., 2010. Influence of finishing systems and sampling site on fatty acid composition and retail shelf-life of lamb. *Anim. Prod. Sci.* 50, 775-781.

Appendix 2: Method summary for determination of cholesterol in pork

Extraction:

1. Weigh 2g of sample into a 150 mL QF (Quickfit™) Erlenmeyer flask and record the mass to the nearest 0.01 g. Include an homogenised luncheon meat control sample and a spiked recovery of cholesterol in each batch.
2. Add 40 mL special methylated spirits to the flask and add 10 mL of 100% KOH
3. Connect the flask to a condenser and reflux on a stirrer/hotplate for 30 minutes with magnetic stirring.
4. Remove the flask from the condenser and cool rapidly in an ice bath.
5. Remove the stirrer from the flask and rinse any residue on the stirrer into the flask with a small amount of water followed by a small amount of special methylated spirits.
6. Transfer the contents of the flask quantitatively to a 250 mL separating funnel using 50 mL of water followed by 10 mL of special methylated spirits then 50mL of hexane. Shake the flask vigorously for 2 minutes.
7. Allow phases to separate. If emulsions have formed, they may be broken by adding approximately 5 mL of special methylated spirits and swirling the flask gently, or alternatively by centrifuging the solution.
8. Transfer the lower aqueous fraction to another separating funnel and re-extract with 20 ml of hexane. Remove hexane layer and combine with original hexane extract.
9. Re-extract aqueous fraction with a further 20 ml of hexane, and combine with previous hexane extracts.
10. Wash the combined hexane extracts with 3 x 100 mL of water.
11. The washed hexane is then transferred to a dry 100 mL volumetric flask containing sufficient anhydrous sodium sulphate to dry the extract and made to the mark with hexane and mixed.
12. Transfer a 10 mL, aliquot to a graduated 15 mL tube and add 100 µL of internal standard solution (500 mg/L 5 α -cholestane).
13. Reduce sample to 2 mL under a flow of nitrogen and transfer 1mL to a GC vial.
14. Make up four GC calibration standards of cholesterol 10, 20, 50 & 100 mg/L containing internal standard solution at a matching level to the sample solutions.

GC-FID method:

Instrument - Agilent 6890 GC-FID with 7673 autosampler
OVEN

Initial temp: 220 °C (On) Maximum temp: 325 °C
Initial time: 4.00 min Equilibration time: 0.50 min

Ramps:

No. :Rate: Final temp :Final time

1 :20.00°C/min:280 °C:5.00 min

2 0.0(Off)

Post temp: 0 °C

Post time: 0.00 min

Run time: 12.00 min

INLET (SPLIT/SPLITLESS)

Mode: Split

Initial temp: 250 °C (On)

Pressure: 19.1 psi (On)

Split ratio: 20:1

Split flow: 50.0 mL/min

Total flow: 54.1 mL/min

Gas saver: On Saver flow: 15.0 mL/min

Saver time: 2.00 min

Gas type: Helium

COLUMN

Capillary Column

Model Number: JW 1231732

Methyl siloxane (50% Phenyl) DB-17

Max temperature: 300 °C

Nominal length: 15.0 m

Nominal diameter: 320.00 µm

Nominal film thickness: 25.00 µm

Mode: constant flow

Initial flow: 2.5 mL/min

Nominal init pressure: 19.1 psi

Average velocity: 69 cm/sec

DETECTOR (FID)

Temperature: 300 °C (On)

Hydrogen flow: 40.0 mL/min (On)

Air flow: 420.0 mL/min (On)

Mode: Constant makeup flow

Makeup flow: 45.0 mL/min (On)

Makeup Gas Type: Nitrogen

The concentration of cholesterol in samples was calculated from the quadratic regression of concentration against the ratio of cholesterol response (peak area) to internal standard response (peak area).