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1	Performance and intestinal responses to dehulling and inclusion level
2	of Australian sweet lupins (<i>Lupinus angustifolius L</i> .) in diets for
3	weaner pigs
4	
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14	
15	Abbreviations: AA, amino acid(s); ADF, acid detergent fibre; ASL, Australian sweet
16	lupins; DM, dry matter; FCR, feed conversion ration; GIT, gastrointestinal tract; NDF,
17	neutral detergent fibre; NSP, non-starch polysaccharides; PUN, plasma urea nitrogen;
18	PWD, post-weaning diarrhoea; TTAD, total-tract apparent digestibility.
19	

20 Abstract

21 A total of 180 entire male weaner pigs weighing 6.4 ± 0.1 kg (mean \pm SEM) 22 and housed in pairs was used in a completely randomised block design with 9 dietary 23 treatments (n=10 pens). Pigs were blocked based on weaning weight. The diets were 24 (i) a wheat-based control diet containing 240 g/kg of milk products (whey and skim 25 milk powder), and (ii) 8 diets containing whole or dehulled lupins (cv. Coromup) that 26 substituted the milk products at 60, 120, 180 and 240 g/kg of diet (replace 25, 50, 75, 27 or 100 % of the milk products in the control diets). The diets were isoenergetic (15 28 MJ/kg), and were formulated to contain the same ileal standardised digestible lysine 29 content (0.85 g/MJ DE) and ideal patterns of other essential amino acids. Pigs 30 receiving 240 g/kg of dehulled lupins grew slower (P<0.05) than pigs fed the other 31 diets mainly due to decreased feed intkae. Pigs fed diets containing more than 180 32 g/kg of dehulled lupins had a higher faecal β -haemolytic *E. coli* score on day 3 after 33 weaning (P<0.05). Moreover, inclusion of 240 g/kg of whole lupin or more than 180 34 g/kg of dehulled lupins increased (P<0.001) plasma urea nitrogen (PUN) levels. Total 35 tract apparent digestibility (TTAD) of dry matter decreased (P<0.001) in all lupin 36 diets compared with the control diet. These data indicate that inclusion of dehulled 37 lupin immediately after weaning should be limited to less than 180 g/kg while whole 38 lupins can be included up to 240 g/kg without deleterious effects on production and 39 intestinal health. 40

Keywords: Australian sweet lupins, performance, intestinal response, weaner pigs,
post-weaning diarrhoea

43

44 **1. Introduction**

Traditionally, high quality piglet feeds have been based on using relatively high percentages of lactose, fat and (or) cooked cereals such as oats as energy sources, and a combination of whey powder, high quality fish meal and dried skim milk as sources of protein. However the cost of many of these ingredients has increased dramatically in recent times and there are certainly indications that their availability will continue to be variable.

51 Australian sweet lupins (*Lupinus angustifolius L*.; ASL) are less expensive 52 than most other sources of protein available for feeding weaner pigs. Recent research in grower pigs demonstrated that ASL can be included at up to 350 g/kg in 53 54 replacement of soybean meal without compromising growth, carcass composition and 55 meat quality (Kim et al., 2011). However, the use of higher levels of lupins in a 56 weaner diet to reduce or replace the more expensive protein sources, such as fishmeal 57 and milk products, has not been examined to date. Lupins contain about 250 g/kg of 58 seed coat (hull), which is mostly insoluble fibre, and its kernel contains about 300 59 g/kg of cell wall materials called polygalacturonans (Kim et al., 2007). Therefore, use 60 of lupins in commercial weaner diets has been limited to 50 to 100 g/kg on the basis 61 that pigs would have limited ability to deal with the high fibre content of whole lupins. 62 In a previous study, however, we showed that yellow lupin seeds could be included at 63 up to 150 g/kg in weaner diets without compromising performance of pigs (Kim et al., 64 2008a). In this regard, it is possible that a similar or greater amount of ASL seeds (Lupinus angustifolius L.) could be used in a weaner diet. Moreover, there is general 65 66 perception that removal of the hull, which is indigestible, from lupins may offer the 67 opportunity for even higher inclusion levels (i.e. > 150 g/kg) as increased amounts of 68 insoluble fibre may physically limit the quantity of lupins that can be incorporated in 69 a diet for weaner pigs.

70 The change in diet from sows' milk to solid feed at weaning disrupts the 71 structure and function of the gastro-intestinal tract (GIT). Post-weaning diarrhoea 72 (PWD) is often a consequence of this malaise. In this regard, the role of dietary fibre 73 in the post-weaning period has been studied, with the source, type, structure and 74 absolute amount of fibre in the diet all known to have effects on the structure and 75 function of the GIT (e.g., Pluske et al., 2002; de Lange et al., 2010). Previous 76 research by Kim et al. (2008b) showed that a small amount of insoluble fibre, as oat 77 hulls, was beneficial for prevention of PWD, while soluble fibres are seemingly 78 associated with proliferation of certain enteric pathogens (e.g., Pluske et al., 1996, 79 Hopwood et al., 2004). As ASL contain two distinctive fibre sources in the hull 80 (insoluble fibre, mostly cellulose, and xylose) and kernel (some pectin-like soluble 81 fibres and largely fermentable fibres, polygalactouronans), increasing amounts of 82 these fibres in diets for weaner pigs may promote or compromise GIT structure and 83 function. No such experiments to measure the impact of lupin inclusion level on 84 digestibility and indices of GIT function have been conducted, to our knowledge. 85 Therefore, the purpose of this study was to examine performance and intestinal 86 responses to whole and dehulled ASL as alternatives to more expensive animal protein 87 sources. The hypotheses tested in this experiment were: (1) performance of weaner 88 pigs will decline as the inclusion level of whole and dehulled lupins increases in the 89 diet; (2) weaner pigs fed dehulled lupins will perform better than pigs fed whole lupins 90 at the same rate; and (3) indices of GIT function such as faecal consistency and β -91 haemolytic *E. coli* score will be compromised when pigs are fed more than 180 g/kg 92 whole or dehulled lupins. 93

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95 **2.** Materials and methods

96 The experimental protocol used in this study was approved by the Department of
97 Agriculture and Food Western Australia Animal Ethics committee (AEC 6-08-41).
98 Animals were handled according to the Australian code of practice for the care and use
99 of animals for scientific purposes (NHMRC, 2004).

100 2.1 Lupins, diets, animals, and experimental design

101 A high protein ASL, cv. Coromup, was selected for the study because it is the 102 variety most likely to be grown in the future in Western Australia (WA). In turn, WA 103 produces approximately 80% of the world's angustifolius lupins (FAOSTAT, 2008). 104 The lupin was collected from the northern agricultural region of WA (Geraldton, WA) 105 which has a reasonably generic soil type (sand over loam). The lupin contained 319 g 106 protein, 346 g insoluble non-starch polysaccharides (NSP) and 24 g soluble NSP (Kim 107 et al., 2009a).

A total of 180 entire male pigs weaned at 21 days of age and weighing $6.4 \pm$ 0.1 kg (mean ± SEM) was acquired from a high health status commercial farm and be transported to the Medina Research Station. Two replicate studies using 90 pigs per replicate were conducted, with an interval of a month between each. Upon arrival, pigs were weighed, ear tagged, housed as pairs (space allowance 0.4 m² per pig) and were allocated to 9 dietary treatments based on weaning weight.

The experiment was a completely randomised block design with 9 dietary treatments, as follows: (i) a wheat-based control diet containing 240 g/kg of whey and skim milk powder, and (ii) 8 diets containing whole or dehulled lupins (cv. Coramup) that substituted the milk products at 60, 120, 180 and 240 g/kg of diet (replace 25, 50, 75, or 100% of the milk products in the control diets). The whole and dehulled lupins were hammermilled to a mean particle size of 700 µm and directly dumped into the

120	mixer. Digestible energy and ileal digestible indispensable AA contents were
121	equalised using soy protein concentrate, canola oil, full fat soya and meat meal. The
122	diets were isoenergetic (15 MJ/kg), and were formulated to contain the same ileal
123	standardised digestible lysine content (Sauvant 2003, 0.85 g/MJ DE) and ideal
124	patterns of other indispensable AA. Composition and chemical contents of diets are
125	presented in Table 1. All diets contained 2 g/kg titanium dioxide as a digestibility
126	marker (Short et al., 1996) to estimate the total tract apparent digestibility (TTAD) of
127	dry matter (DM). Nutrient composition of dehulled lupins was not chemically
128	determined but used tabulated value (Table 2) reported in the previous publication
129	(Kim et al., 2007), as chemical composition of lupin kernels are not variable.
130	
131	2.2 Experimental procedure and measurements

132 Pigs were fed their respective diets as a mash form and on an ad libitum basis 133 for 3 weeks. Fresh water was available throughout the experiment. Pigs were weighed 134 weekly and feed intake was measured on a daily basis. Pigs having diarrhoea (score 4; 135 see below) were treated with Trisoprim-480 (trimethropin 80 mg/ml, sulfadiazine, 400 136 mg/ml, 0.05 ml/kg body weight, Troy Laboratories, Smithfield, NSW, Australia) until 137 considered healthy and the number of antibiotic treatments was recorded. The 138 antibiotic treatment was initiated when the faecal score exceeds 4 and ceased at 3 (Kim 139 et al., 2008b).

140

Faecal consistency and incidence of diarrhoea of individual pigs were visually
assessed daily for the first 2 weeks. Faecal consistency was determined using the
following scoring criteria: 1 = well-formed faeces, firm to cut; 2 = formed faeces, soft
to cut; 3 = faeces falling out of shape upon contact with surfaces, sloppy; 4 = pasty and

145	liquid diarrhoea. Data for faecal consistency were then expressed as the mean faecal
146	consistency value of pigs within a diet having the score 1 (25 %), score 2 (50 %), score
147	3 (75 %), and score 4 (100 %). A faecal consistency score of 4 represented pigs with
148	diarrhoea, and the incidence of PWD was express as the mean proportion of days pigs
149	had faecal score 4 with respect to days (14 d) (after Mateos et al. 2006).
150	
151	Faecal swabs from individual pigs were taken on days 0, 3, 5, 7 and 9 to
152	examine the degree of β -haemolytic <i>E. coli</i> proliferation (Kim et al., 2008b). Swabs
153	were cultured onto sheep blood (50 ml/L) agar plates (Columbia base, Oxoid, London,
154	UK) and then assessed for β -haemolytic colonies displaying the characteristic
155	morphology of <i>E. coli</i> , after overnight incubation at 37°C in air (McDonald et al.
156	2001). The sheep blood agar plates were given a swab score according to the number
157	of streaked sections that contained viable haemolytic <i>E. coli</i> , where: $0 = no$ growth, $1 =$
158	haemolytic <i>E. coli</i> in 1^{st} section, $2 =$ haemolytic <i>E. coli</i> in 2^{nd} section, $3 =$ haemolytic <i>E</i> .
159	<i>coli</i> in 3^{rd} section, $4 =$ haemolytic <i>E. coli</i> in 4^{th} section, $5 =$ haemolytic <i>E. coli</i> present
160	right out to the 5 th section of the plate.
161	
162	Faecal samples were collected from the pen floor as voided by the pigs for 3
163	consecutive days at the end of weeks 1 and 3 to determine faecal moisture content and
164	the TTAD of DM. Samples were pooled per pen at the end of each collection and
165	subsamples were stored at -20°C until analyzed.
166	
167	Blood samples were collected from individual pigs on days 7 and 21. Samples
168	were collected from anterior vena cava into lithium heparin coated vacutainer. The

169 blood samples were immediately placed on ice and then centrifuged at $2,000 \times \text{g}$ for

170 10 minutes at 5°C. Plasma was stored at -20°C until analyzed for plasma urea nitrogen
171 (PUN).

172

173 2.3 Chemical analyses

174	The PUN level was determined using an enzymatic (urease) kinetic method
175	(Randox, Crumlin, Co. Antrim, UK). Dry matter was measured using the AOAC
176	official method 930.15 (AOAC 1997). Titanium dioxide (TiO ₂) was measured
177	spectrophotometrically at 410 nm after acid hydrolysis (7·4 M-H ₂ SO ₄ ; Short et al.,
178	1996). The nitrogen (N) content was determined using combustion method 990.03
179	(AOAC 1997). Crude protein content was calculated as N content \times 6.25. Crude fat
180	content was determined using AOAC official method 2003.06 (AOAC, 1997). The
181	neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined
182	using the AOAC official method 925.10 (AOAC, 1997). Gross energy content was
183	determined using a ballistic bomb calorimeter (SANYO Gallenkamp, Loughborough,
184	UK). Total phosphorus (P) was determined using inductively-coupled atomic emission
185	spectroscopy as described by McQuaker et al. (1979). Phytate-P content was
186	determined spectrophotometrically using the principle that phytate forms stable
187	complexes with ferric ions in dilute acid solution (Xu et al., 1992). The insoluble and
188	soluble NSP content of the lupin samples was determined as alditol acetates by gas-
189	liquid chromatography (GLC) using the method of Theander and Westerlund (1993).
190	Total NSP content was calculated by adding the insoluble and soluble NSP contents.
191	The sum of insoluble and soluble NSP was calculated using the following
192	polymerization factors:

194 Sum of total, insoluble and soluble NSP = $(Rha + Fuc + Rib) \times 0.89 + (Ara + Xyl) \times$ 195 $0.88 + (Man + Gal + Glu) \times 0.90$,

196

197 where Rha = rhamnose; Fuc = fucose; Rib = ribose; Ara = arabinose; Xyl = Xylose;

198 Man = mannose; Gal = galactose; Glu = glucose.

199

200 Polymerization factors were used to correct for differences in total molecular weights

201 due to dehydration during the polymerization process. For example, each glucosidic

202 linkage for a glucose (hexose) molecule loses one molecule of water during

203 polymerization. Therefore, a factor of 0.9 was used in the calculation to account for

204 differences in molecular weights between glucose (180) and water (18) [i.e., (180 –

18) / 180 = 0.9]. The same calculation was applied for deoxysugars (molecular weight

206 164 and hence a factor of 0.89) and pentoses (molecular weight 150 and hence a factor

207 of 0.88).

208

209 2.4 Statistics

210 There were no replication effects and data were pooled for subsequent 211 statistical analyses. The individual pig was considered as the experimental unit for all 212 measurements (n=20) except performance indices, faecal DM content and faecal DM 213 digestibility, in which a pen was the experimental unit (n=10). The treatment effects 214 were assessed by one-way analysis of variance (ANOVA) and faecal score was analysed using repeated measure ANOVA as it was recorded daily for 14 days. When 215 216 significant diet effect was found in the ANOVA test, then the variables were tested for 217 Fisher's-protected least significant difference analysis to separate means where 218 significant main effect occurred under the ANOVA analysis. Pigs were blocked based

219	on weaning weight and the block was used as a random factor in the model for all
220	measured experimental variables. Initial BW was used as a covariate for growth data
221	analyses. Where significant treatment effect was evident, then the data were subjected
222	to a simple linear regression analysis to establish linear relationship between dietary
223	concentration of whole or dehulled lupins and TTAD DM. Statistically significant
224	difference between treatments was accepted at P<0.05. The statistical analyses were
225	conducted using the statistical package Genstat 10.0 for Windows (VSN International
226	Ltd., Hemel Hempstead, UK).

- 227
- 228
- **3. Results**
- 230 *3.1 Post-weaning performance*

Pigs fed diets containing whole lupins up to 240 g/kg and dehulled lupins up to 180 g/kg ate comparable amounts of feed and had similar feed conversion ratio (FCR) and daily gains compared to pigs fed the milk-powder-based control diet. Although FCR was comparable, pigs receiving 240 g/kg of dehulled lupins grew slower (P<0.05) than pigs fed the other diets, predominantly due to decreased feed intake (Table 3).

236

237 3.2 Indices of GIT function

Faecal consistency, the number of antibiotic treatments, and the incidence of PWD were generally low and unaffected by up to 240 g/kg inclusion of whole or dehulled lupins in the diet (Table 4). However, pigs fed diets containing 180 g/kg and 240 g/kg of dehulled lupins had greater faecal β -haemolytic *E. coli* scores on day 3 after weaning (P<0.05, Figure 1). Faecal β -haemolytic *E. coli* scores were not different in the other days. Inclusion of 240 g/kg of whole lupin or more than 180 g/kg of dehulled lupins increased (P<0.001) the PUN level. Increased dispensable amino acid
levels in the diets with greater lupin concentrations showed a positive relationship to
the PUN level (Figure 2).

247

248 *3.3 Total tract apparent digestibility of dry matter*

The TTAD of DM decreased (P<0.001) as inclusion of lupins was increased, and the extent of the reduction was greater in the whole lupin diet than in the dehulled lupin diet (Figure 3). The TTAD of DM was negatively correlated to the NDF and ADF concentration of the diets (P<0.001, Figure 3).

253

4. Discussion

255 It was hypothesised that feed intake, daily gain and FCR of weaner pigs would 256 be significantly reduced when both whole and dehulled lupins above the current 257 recommended level of 150 g/kg were included. This expectation was based on the 258 previous findings that (1) lupins contain greater levels of NSP in hulls and kernels, 259 which may alter the physiological properties of digesta and influence digestibility 260 (Gdala et al., 1997; Kim et al., 2007); (2) ileal digestibility of energy decreased 261 linearly with increasing lupin kernel concentration in 45 kg grower pigs (van 262 Barneveld et al., 1995); (3) feeding lupins increased endogenous protein loss due 263 mainly to higher NSP contents (Salgado et al., 2002; Rubio et al., 2005); and (4) 264 inclusion of slowly-digestible insoluble NSP in a weaner pig diet limits the physical 265 capacity of the GIT and hence limits voluntary feed intake (Kyriazakis and Emmans, 1995). 266

In the present study, weaner pigs responded differently to insoluble NSP (hulls)
and partly soluble NSP (kernels) of the lupin variety fed. Lupin hulls contain 880 g/kg

269 NSP with 500 g/kg being cellulose, whereas lupin kernels contain 300 g/kg NSP with 270 more than 200g/kg being galacturonic acids (Evans et al., 1993). Pigs fed 240 g/kg 271 dehulled lupins ate less feed and hence grew slower than other pigs, although their 272 short-term growth retardation disappeared in the growing-finishing phases of growth, 273 whereas pigs fed 240 g/kg whole lupins showed comparable intake and daily gain to 274 the pigs fed the milk powder-based control diet. As FCR was not affected by any of 275 the experimental diets, reduced daily again in pigs fed 240g/kg dehulled lupins was 276 caused by decreased feed intake. Inclusion of lupin kernels, containing approximately 277 300 g fermentable NSP, can decrease the net energy value of the diet (Taverner et al., 278 1983; Kim et al., 2007). However, decreased feed intake but not FCR indicates that 279 dietary energy was not a limiting factor in the 240 g/kg dehulled lupin diet (Ferguson 280 et al., 2003). Rather, the results suggest that ASL in either form (i.e., dehulled or 281 whole) does not influence nutrient utilisation efficiency but excess inclusion of 282 fermentable lupin kernel fibre limits the physical capacity of the GIT and hence 283 reduces feed intake. This is most likely due to the greater amount of structural cell wall 284 component of lupin kernel endosperms (Vincken et al., 2003). These structural 285 polysaccharides may have altered the physico-chemical properties of the digesta (i.e., 286 increased viscosity and fermentation by-products) in the GIT and limited feed intake, 287 possibly by increasing gastric distension and increasing digesta transition time once 288 the inclusion levels of the dehulled lupin exceeded 180 g/kg (Kyriazakis and Emmans, 289 1995; Choct et al., 1996; Choct and Kocher, 2000; Dunshea et al., 2001). On the other 290 hand, these findings also indicate that supplementation of lupin hulls, which is mostly 291 insoluble NSP, can remove the deleterious effect of lupin kernel endosperm 292 polysaccharides, although the mechanism behind this was not explored herein. 293 Nevertheless and based on these findings, it is clear that inclusion of dehulled lupins in the diet for young pigs should be limited to current recommended inclusion level of

295 150 g/kg, while inclusion of whole lupins can be increased up to 240 g/kg.

296 Although NDF content of the diets were not analysed and used calculated 297 values from previous publications, it is worth to discuss possible impact of dietary 298 NDF due to increasing lupin concentration on the TTAD of DM. A linear reduction in 299 TTAD of DM was expected with the increased amounts of both whole and dehulled 300 lupins in the diet, as dietary NDF increases with increasing inclusion of dehulled and 301 whole lupins. However and as Figure 3 shows, only whole lupin concentration linearly 302 decreased the TTAD of DM whereas increasing dehulled lupin concentration 303 decreased TTAD DM in a non-linear manner. This finding indicates that a significant 304 proportion of lupin kernel fibre was fermented in the large intestine while fermentation 305 of lupin hull fibre was minimal, as previously reported (Taverner et al., 1983; Kim et 306 al., 2009b). Therefore, the significantly decreased TTAD of DM in ASL-supplemented 307 diets can be attributed to the dilution effect of insoluble fibre but not to the fermentable 308 lupin kernel endosperm structural fibre, as found in the relationship between fibre 309 content and the TTAD of DM (Figure 3). A similar relationship between NDF and the 310 TTAD of energy was reported previously (Le Goff et al., 2002), where TTAD of 311 energy was decreased by 0.1% per 1 g increase in dietary NDF. Our study showed a 312 0.19 and 0.23% reduction in TTAD DM per gram increase in dietary NDF and ADF. 313 respectively.

A purported benefit of feeding greater amounts of highly-digestible milk powder in diets after weaning is amelioration of the post-weaning growth check (e.g., increased daily gain in the first week after weaning; Kim et al., 2010) and improved 'health' of the GIT. Inclusion of lactose through dietary milk products has been shown to improve indices of intestinal health, for example by reducing digesta pH through 319 production of lactic acid (Pierce et al., 2006), increasing Lactobacilli populations 320 (Dillon et al., 2010; Kim et al., 2010) and decreasing the coliform population (Kim et 321 al., 2010), although supplementation of lactose did not prevent villous atrophy (Vente-322 Spreeuwenberg et al., 2003). With this in mind, the current experiment attempted to 323 replace milk products with a mixture of insoluble (whole lupins) and partly soluble 324 (dehulled lupins) fibres or partly soluble fibre solely, and examined indices of GIT 325 function and whole-of-life performance. Only a very small number of pigs developed 326 PWD, and therefore a definitive conclusion as to whether these different types of fibre 327 affected intestinal health cannot be made. However, the significant increase in the 328 faecal β -haemolytic *E. coli* score in pigs fed more than 180 g dehulled lupins/kg diets 329 (Figure 1) suggests that fermentable lupin fibre may have negative effects on GIT 330 function, however this was not observed in more PWD. It is likely that 331 polygalacturonans (major polysaccharides in lupin kernel) increased water holding 332 capacity and increased digesta retention time in the GIT, which is evident in the 333 decreased feed intake in 240 kg/kg dehulled lupin-fed pigs. In turn, increased retention 334 time and availability of fermentable fibre contributed to higher proliferation of β -335 haemolytic E. coli.

336

5. Conclusions

Use of whole Australian sweet lupins up to 240 g/kg in diets for weaner pigs did not affect performance and indices of intestinal health most likely because of a insoluble NSP in the hull, that in turn possibly altered physico-chemical property of the digesta. However, feeding a diet containing greater than 180 g/kg dehulled lupins significantly compromised feed intake and hence growth of the pigs, and stimulated the proliferation of β-haemolytic strains of *E. coli* in the gastrointestinal tract.

344	
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473	

475 Table 1

Ingradiant	Control ^a	Whole lupin, g/kg				Dehulled lupin, g/kg			
ingredient	Control	60	120	180	240	60	120	180	240
Wheat	417	407	407	411	397	430	453	448	446
Cooked oats	150	150	150	150	150	150	150	150	150
Whole lupin	0	60	120	180	240	-	-	-	-
Dehulled lupin	-	-	-	-	-	60	120	180	240
Full fat soya	25	50	43	24	19	30	3	11	9
Soycomil ^b	53	26	10	10	10	24	10	10	7
Meat meal	4	11	23	22	38	15	26	10	19
Fishmeal	100	100	100	100	100	100	100	100	100
Skim milk	120	90	60	30	-	90	60	30	-
Whey	120	90	60	30	-	90	60	30	-
Canola oil	-	5	15	27	34	-	5	6	5
_L -Lysine	0.4	1.1	1.9	2.6	2.6	1.4	2.4	2.6	2.6
_{DL} -Methionine	0.7	0.9	1.2	1.4	1.4	1.0	1.3	1.4	1.4
Threonine	-	0.08	0.47	0.79	0.80	0.22	0.72	0.87	0.89
Tryptophane Vitamin/Mineral	-	-	0.04	0.17	0.24	-	0.09	0.11	0.15
Premix ^c	1	1	1	1	1	1	1	1	1
Limestone	3.7	3.3	1.6	3.8	0.4	2.3	0.9	8.8	7.6
Dicalcium									
Phosphate	-	-	-	-	-	-	-	3.9	4.6
Salt	1	1	1	1	1	1	1	1	1
Znic oxide	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Choline Chloride	-	-	-	-	-	-	0.22	0.47	0.65
TiO ₂ ^d	2	2	2	2	2	2	2	2	2
Calculated compos	sition [g/kg	as-fed]							
DE [MJ/kg]	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
NE [MJ/kg]	10.2	10.3	10.5	10.7	10.8	10.2	10.4	10.4	10.5
Crude protein	230	230	230	230	240	230	230	234	245
Crude fat	34	47	60	71	81	40	45	49	52
Calcium	9.0	9.0	9.0	9.2	9.0	9.0	9.0	10.8	11.0
FD P ^e	5.2	5.0	5.0	4.5	4.7	5.1	5.0	4.5	4.5
NDF	70	85	99	112	124	77	83	87	92
ADF	15	28	39	49	60	1.6	16	16	16
Lignin	4.6	5.7	6.6	7.4	8.2	4.8	4.7	4.8	4.7

476 Composition and calculated analysis of the experimental diets (g/kg, as-fed basis)

Calculated SID^f AA content [g/kg as-fed] Indispensable AA

Lysine	14.4	14.4	14.5	14.5	14.6	14.4	14.3	14.2	14.3
Methionine	5.4	5.3	5.3	5.3	5.2	5.3	5.3	5.2	5.1
Threonine	9.5	9.3	9.3	9.3	9.4	9.3	9.3	9.3	9.4
Tryptophan	3.1	3.0	2.9	2.9	2.9	2.9	2.8	2.8	2.9
Isoleucine	10.6	10.2	9.8	9.4	9.5	10.0	9.4	9.4	9.5
Leucine	17.8	17.3	16.6	16.1	16.4	17.1	16.3	16.2	16.5
Valine	11.9	11.5	11.2	10.9	11.1	11.4	10.9	10.7	10.8
Arginine	12.3	13.7	15.0	16.3	18.4	13.6	14.8	16.6	18.8
Histidine	5.8	5.8	5.7	5.7	5.9	5.7	5.6	5.7	6.0
Phenylalanine	10.3	10.1	9.8	9.6	9.9	9.9	9.5	9.7	10.0
Dispensable AA									
Alanine	8.2	9.2	9.8	9.8	10.6	9.1	9.6	9.5	10.2
Aspartic	13.8	16.2	17.3	17.5	18.8	15.8	16.3	17.4	18.8
Glutamine	33.9	38.4	41.3	42.7	45.5	38.6	41.4	44.1	47.8
Glycine	8.5	9.9	11.1	11.4	12.9	10.0	11.1	10.9	12.2
Proline	12.8	13.6	14.0	13.7	14.2	13.6	13.8	13.3	13.6
Serine	8.5	9.5	9.9	9.9	10.5	9.4	9.7	10.1	10.7
Tyrosine	6.6	7.1	7.2	7.1	7.2	7.1	7.2	7.4	7.7
Cystine	3.3	3.4	3.4	3.5	3.7	3.4	3.4	3.6	3.8

477 ^aBasal diet contained none of either whole or dehulled lupins

^bSoycomil, soy protein concentrate (ADM Specialty Ingredients, Netherlands)

479 ^cProvided the following nutrients (per kg of air-dry diet):

480 Vitamins: A 7000 IU, D₃ 1400 IU, E 20 mg, K 1 mg, B₁ 1 mg, B₂ 3 mg, B₆ 1.5 mg, B₁₂

481 15 μg, Calcium pantothenate 10.7 mg, Folic acid 0.2 mg, Niacin 12 mg, Biotin 30 μg.

482 Minerals: Co 0.2mg (as cobalt sulphate), Cu 10 mg(as copper sulphate), Iodine 0.5 mg

483 (as potassium iodine), Iron 60 mg (as Ferrous sulphate), Mn 40 mg (as manganous

484 oxide), Se 0.3 mg (as Sodium Selenite). Zn 100 mg (as zinc oxide). (BJ Grower 1,

- 485 BioJohn Pty Ltd., WA, Australia)
- ^dTitanium dioxide (TiO₂; Sigma Chemical Company, St. Louis, MO, USA)
- 487 ^eFD P: faecal digestible phosphorus
- 488 ^fSID: standardised ileal digestible
- 489

493	Table 2. Analysed chemical	composition ((g/kg,	as-fed basis)	of Australian	sweet lupin
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Item	Whole lupin, Coromup	Dehulled lupin ^b
Dry matter	916	900
Gross energy, MJ/kg	18.4	18.9
Crude protein	319	380
Crude fat	62	71
Neutral detergent fibre	244	58
Acid detergent fibre	222	32
Lignin	19	0.8
Total NSP ^a	370	270
Rhamnose	2.8	7.2
Fucose	1.1	-
Ribose	0.9	-
Arabinose	54	35
Xylose	41	6
Mannose	8	1.8
Galactose	159	181
Glucose	146	13.5
Insoluble NSP	346	231
Rhamnose	2.5	5.4
Fucose	1.1	-
Ribose	0.6	-
Arabinose	49	31.4
Xylose	37	5.1
Mannose	4.7	-
Galactose	146	154
Glucose	146	12.6
Soluble NSP	24	39
Rhamnose	0.3	1.8
Fucose	-	-
Ribose	0.3	-
Arabinose	5.1	3.6
Xylose	4.1	0.9
Mannose	3.4	1.8
Galactose	13	27
Glucose	0.5	0.9
Total phosphorus	3.5	1.0
Phytate phosphorus	2.2	

495 ^aNSP: non-starch polysaccharides

^bTabulated value (Evans et al., 1993; King et al., 2000; Kim et al., 2007)

491

497 Table 3

498 Effect of dehulling and concentration of lupins in the diet for weaner pigs on performance of weaner pigs measured for 21 days after weaning.

499

Item	Control ^a	Whole lupin, g/kg			Dehulled, g/kg				SEM ^b	P- value ^c	
		60	120	180	240	60	120	180	240		1 1000
Average daily gain	324	351	342	364	344	362	359	326	305	6.4	0.040
Average daily feed intake	509	523	524	519	498	516	502	507	424	10.6	0.202
Feed conversion ratio	1.56	1.46	1.55	1.43	1.43	1.44	1.36	1.52	1.49	0.029	0.734

^aBasal diet contained none of either whole or dehulled lupins.

501 ^bPooled standard error of mean.

502 ^cSignificance level: NS: Not significant.

503

505 Table 4

- 506 Effect of dehulling and concentration of lupins in the diet for weaner pigs on faecal consistency, faecal dry matter, incidence of PWD and
- 507 number of antibiotic treatment.

Item	Control ^a -	Whole lupin, g/kg					Dehulled	CEN (b	D 1 C		
		60	120	180	240	60	120	180	240	- SEM	P-value
n=	20	20	20	20	20	20	20	20	20		
Faecal consistency ^d , %											
d 1-7	22.1	22.6	22.0	21.6	24.4	24.6	22.6	24.1	22.3	0.410	NS
d 8-14	22.4	23.7	21.6	20.6	21.9	22.9	22.6	22.9	22.6	0.355	NS
Faecal DM,	% ^e										
d 7	31.8	37.1	34.0	30.6	35.3	31.3	34.4	32.9	35.2	0.69	NS
d 21	29.5	28.4	28.2	29.2	30.9	28.4	27.5	29.3	30.1	0.26	NS
Incidence of PWD ^f . %											
d 1-7	2.1	0.7	0.7	0.7	2.9	2.9	2.9	2.1	0.7	0.42	NS
d 8-14	0.0	0.7	0.0	0.0	0.7	0.7	0.0	0.0	1.4	0.21	NS
Number of a	antibiotic treat	ments ^g									
d 1-7	0.1	0.1	0.1	0.0	0.3	0.2	0.2	0.2	0.1	0.03	NS
d 8-14	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.01	NS

^aBasal diet contained none of either whole or dehulled lupins.

^bPooled standard error of mean.

- 510 ^cSignificance level: NS: Not significant.
- ^dFaecal consistency was expressed as % cumulative score per day of pigs having more liquid faeces; higher values are associated with more
- 512 liquid faeces.
- ⁶The pen was experimental unit for faecal DM analysis (n=10).
- ⁵14 ^fA faecal consistency score of either 4 or 5 represented pigs with PWD, observed during the first14-days after weaning, is expressed as the mean
- 515 percentage of days with diarrhoea relative to the total 14 days after weaning. Data are mean values per treatment combination assessed between

516 days 1-14.

^gMean for total number of pigs injected with antibiotics with respect to the number of pigs in each feeding group.

518



520

521 Figure 1 Effect of dehulled (\Box) or whole (\blacksquare) lupin concentration on faecal β -haemolytic *E. coli* score (log₁₀) at day 3 after weaning.

522 Significance: * P < 0.05

523



526 Figure 2

(a) Effect of dehulled (\bigcirc) or whole (\bullet) lupin concentration on plasma urea nitrogen (PUN) concentration (P < 0.001); and (b) relationship between dietary dispensable amino acids (AA) and PUN concentration (P<0.001). The PUN concentration was measured on day 6 and 12 after weaning from 20 pigs per treatment (n=40).

530



Figure 3. (a) Effect of dehulled (\bigcirc) or whole (\bullet) lupin concentration on total tract apparent digestibility (TTAD) of dry matter (DM) (P < 0.001); and (b) linear relationship between TTAD of DM and dietary fiber concentration [Acid detergent fibre (ADF): y = -0.14x + 82.6, R² = 0.63; Neutral detergent fibre (NDF): y = -0.16x + 92.9, R² = 0.77] (P<0.001). Total tract apparent digestibility of DM was repeatedly measured on day 7 and 21 after weaning from 10 pens per treatment (n = 20).