Variation in digestible energy content of Australian sweet lupins (Lupinus angustifolius L.) and the development of prediction equations for its estimation

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Variation in digestible energy content of Australian sweet lupins (*Lupinus angustifolius* L.) and the development of prediction equations for its estimation[1]

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ABSTRACT: Sixty-three male pigs (Landrace × Large White) weighing 49.5 ± 0.40 kg were used to (1) examine the variation in DE content of *Lupinus angustifolius* L. in relation to variety and geographical growing region and (2) establish prediction equations for DE content from physical and chemical composition. The pigs were randomly allocated to a 4 × 2 factorial treatment design with respective factors being 4 varieties (cv. Belara, Coromup, Mandelup, and Tanjil) and 2 growing locations (northern and southern agricultural areas of Western Australia). In addition, a wheat control diet was fed as a reference for calculation of lupin DE content. The lupins were ground through a hammer mill fitted with a 4-mm screen to a mean particle size of 888 μm. Pigs were fed their respective experimental diets at 3 times maintenance energy level [3 × (0.458 × $BW^{0.75}$)/diet DE] in the study. The DE content of lupins ranged from 13.3 to 15.7 MJ/kg with a mean value of 14.2 MJ/kg. Variety of lupins affected ($P < 0.01$) the DE content, and lupins grown in the northern agricultural region had a greater DE content than the same lupins grown in the southern agricultural area ($P < 0.01$). Although the variation in DE content of lupins was mostly caused by significantly greater DE content of cv. Coromup grown in the northern agricultural region, the results suggest that genetic and environmental conditions during the growth of lupins have a significant impact on the utilization of energy in grower pigs. Simple regression analysis showed that prediction of DE content was possible from the proportion of hulls [$R^2 = 0.88$, residual SD (RSD) = 1.116, $P < 0.001$], 1,000-seed weight ($R^2 = 0.77$, RSD = 1.092, $P < 0.01$), and soluble arabinoxylan content ($R^2 = 0.64$, RSD = 1.072, $P < 0.05$). Multiple regression analysis showed that adding total nonstarch polysaccharide ($R^2 = 0.96$, RSD = 1.187, $P < 0.01$) and soluble nonstarch polysaccharide ($R^2 = 0.95$, RSD = 1.200, $P < 0.01$) to the equation along with the proportion of hull and 1,000-seed weight significantly improved the accuracy of prediction. Results indicate that the DE content of lupins varies by up to 2.4 MJ/kg and that the DE content can be predicted with a good degree of accuracy using physical and chemical characteristics.

Key words: digestible energy, growing location, lupin, pig, variety

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INTRODUCTION

Western Australia (**WA**) produces approximately 70% of the lupin in the world (FAO, 2008). Lupin seeds are an important protein and energy source for the Australian pig industry and in other countries (e.g., in northeastern Europe) where the use of lupins in pig diets is cost effective. Because AA availability of lupins is reported elsewhere (Kim et al., 2009), this paper will focus on the DE content of lupins. Previous studies have demonstrated that the DE content of lupins (*Lupinus angustifolius* L.) ranges from 12.3 to 15.3 MJ/kg with an average value of 14.6 MJ (Petterson et al., 1997; van Barneveld et al., 1997). These values were measured using older varieties of Australian sweet lupins (**ASL**, *Lupinus angustifolius* L.) such as Gungurru (released in 1988), which are no longer recommended for planting and will not be present in pig diets currently fed in WA, or at least not present in any great proportion. The nutritive value for pigs of newer varieties of ASL that have been released after 1998 in WA is unknown. Also, previous studies with lupins have not addressed

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varietal and environmental effects on DE variation, although it is known that particle size can affect DE content (Wigan et al., 1995). Nevertheless, and as has been demonstrated in wheats produced in WA and fed to pigs (Kim et al., 2003, 2004), there is a significant variation in chemical composition and the DE content of grain due to growing region in the state.

In vivo digestibility studies with pigs to estimate the nutritive value of feed ingredients are a time-, cost-, and labor-intensive process; hence, the prediction of DE content from physical characteristics and chemical composition, which can be determined rapidly in vitro, can be a useful tool for addressing ingredient variation and for accurate diet formulation. For example, bushel weight and β-glucan content are correlated to the DE content of barley, whereas xylose and NDF content are correlated to the DE content of wheat (Bhatty et al., 1974; Zijlstra et al., 1999; Kim et al., 2004). Therefore, these characteristics can be used to minimize the impact of DE variation of these ingredients for the design of pig diets. However, such relationships for the accurate prediction of the DE content for lupins are yet to be established.

The aims of the present experiment were to quantify the variation in DE content of *Lupinus angustifolius* L. according to its variety and growing region and to develop in vitro prediction equations for DE content from physical and chemical characteristics of the lupins. The hypothesis tested in the present experiment was that the DE content of lupins will vary in response to the variety and geographical location where it was grown.

MATERIALS AND METHODS

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

Lupins, Animals, and Experimental Design

Three major ASL (*Lupinus angustifolius* L.) varieties based on the quantity produced by WA lupin growers and hence representing the greatest proportions of ASL produced (cv. Belara, Mandelup, and Tanjil) were selected. Also, a recently released increased protein ASL cv. Coromup was selected because it is a candidate variety that is likely to be extensively grown in WA in the near future. The 4 varieties of lupins comprised more than 80% of ASL produced in WA in the 2006 to 2007 growing season. Other minor varieties such as Kalya, Wonga, Myallie, Quilinock, and Jenabillup comprise less than 5% each and less than 20% of the total volume produced in WA (B. Buirchell, senior lupin breeder, Lupin Taskforce Team, Western Australian Department of Agriculture and Food, personal communication). The 4 varieties of ASL samples used in the present study were aggregate samples collected from 3 locations in the northern (Mingenew, Marchagee, Yorkrakine) and southern (Katanning, Wongan Hills, Williams) agricultural regions of WA. Each of these areas has a reasonably generic soil type (sand over loam for the north and loam over clay for the south), and the agronomic practices (e.g., row spacing, seed density, fertilizer application, pesticide application, harvesting techniques) used to grow lupins are largely identical in each region.

Sixty-three individually housed intact male pigs (Large White × Landrace) weighing 49.5 ± 0.40 kg were used. The experiment was designed as a 4 × 2 factorial arrangement of treatments plus a wheat control group (n = 7) with the respective factors being the 4 lupin varieties (Belara, Coromup, Mandelup, and Tanjil) and 2 growing locations (northern and southern agricultural region of WA). A wheat control diet was included to calculate the DE value of lupins by difference (Kim et al., 2004).

Housing, Diets, Feeding, and Sample Collection

Pigs were weighed on arrival and randomly allocated to 1 of the 9 diets based on BW and pen position in the building. Diets were manufactured using the 8 test lupin samples ground through a hammer mill fitted with a 4-mm screen. The mean particle size of the ground lupins was 888 ± 12 μm. Wheat was separately ground using a 6-mm screen, and the control diet was formulated to contain wheat plus other supplements to determine the DE content of the dietary components other than test lupins. The 8 test lupin diets were formulated by replacing 350 g of wheat/kg with test lupins from the control diet. Titanium dioxide (TiO_2, 1 g/kg) was added as an indigestible marker to calculate digestibility of DM, GE, and CP (Table 1). To minimize the effect of feed intake on energy digestibility, all diets were offered at 3 times maintenance energy level based on the individual metabolic BW [3 × (0.458 MJ × $BW^{0.75}$)/diet DE; about 90% of ad libitum intake]. Fresh water was freely available at all times through a nipple drinker. Pigs were fed each diet for a total of 10 d, comprising a 7-d adaptation period followed by a 3-d grab sample collection of feces. Feces were collected at 0800, 1000, 1200, 1400, and 1600 h daily for the 3-d collection period. Feces were then bulked per pig and subsampled for analysis. The DE content of the lupins was determined by comparing the DE content of the control diet and the lupin-based diets using the following formula:

$$\text{Lupin DE (MJ/kg)} = \{\text{diet DE} - [(\text{wheat DE} \times 0.5783) - (\text{canola oil DE} \times 0.03)]\}/0.35,$$

where the numbers represent the proportions of each ingredient in the test diets, wheat DE was determined

Table 1. The composition of the experimental diet offered to grower pigs (g/kg, air-dry basis)

Item	Wheat control diet	Lupin test diet
Ingredient		
Wheat	928.3	578.3
Test lupins[1]	—	350
Canola oil	30	30
Dicalcium phosphate	20	20
Limestone	15	15
Salt	5	5
Vitamin and mineral mix[2]	0.7	0.7
Titanium dioxide[3]	1	1
Calculated composition, g/kg		
DE, MJ/kg[4]	14.2	13.8
SID lysine[5]	2.66	6.01
CP	111	181
Total starch	572	366
Crude fat	45.5	59.7
NDF	118	154
Phosphorus	6.6	6.5
Available phosphorus[6]	4.6	4.6
Calcium	1.2	1.2

[1]Four varieties (Belara, Coromup, Mandelup, and Tanjil) of lupins grown in the northern or southern agricultural regions of Western Australia (WA).

[2]BJ Grower 1, BioJohn Pty Ltd., WA, Australia. Provided the following nutrients (per kg of air-dry diet): vitamins: A 4,900 IU, D_3 980 IU, E 14 mg, K 0.7 mg, thiamine 0.7 mg, riboflavin 2.1 mg, pyridoxine 1.05 mg, B_{12} 10.5 μg, calcium pantothenate 7.5 mg, folic acid 0.13 mg, niacin 8.4 mg, biotin 21 μg; minerals: Co 0.14 mg (as cobalt sulfate), Cu 7 mg (as copper sulfate), iodine 0.35 mg (as potassium iodine), iron 42 mg (as ferrous sulfate), Mn 28 mg (as manganese oxide), Se 0.21 mg (as Na selenite), Zn 70 mg (as zinc oxide), antioxidant as Endox (Kemin Industries Inc., Des Moines, IA) 14 mg.

[3]Titanium dioxide (TiO_2; Sigma Chemical Company, St. Louis, MO), used as a digestibility marker.

[4]DE value used for formulation: wheat: 13.9 MJ/kg (Sauvant et al., 2004); canola oil: 36.7 MJ/kg (NRC, 1998).

[5]Standardized ileal digestible lysine (Sauvant et al., 2004).

[6]Total tract digestible phosphorus (Sauvant et al., 2004).

with the wheat control diet (13.23 MJ/kg as-fed), and a book value was used for canola oil DE, 36.7 MJ/kg (NRC, 1998).

Chemical Analyses

Dry matter content was determined using AOAC official method (930.15, AOAC, 1997). The N content was determined using combustion method (990.03, AOAC 1997). Crude fat content was determined using AOAC official method 2003.06 (AOAC, 1997). The NDF, ADF and lignin were determined using the AOAC official methods 925.10 (AOAC, 1997). Gross energy content was determined using a Ballistic Bomb Calorimeter (SANYO Gallenkamp, Loughborough, UK). Total P was determined using inductively coupled atomic-emission spectroscopy as described by McQuaker et al. (1979). Phytate-P content was determined spectrophotometrically using the principle that phytate forms stable complexes with ferric ions in dilute acid solution (Xu et al., 1992). Crude protein content was calculated as N content × 6.25. The TiO_2 contents of diet and fecal samples were determined using the method described by Short et al. (1996).

The 1,000-seed weight (g/1,000 seeds) was measured in each sample of test lupins by first cleaning it of all foreign materials and then counting 1,000 seeds. The proportion of hulls and kernels in each sample of test lupins was determined by soaking 50 g of seeds in distilled water for 24 h at 4°C. Hulls from individual seeds were removed from the kernel and dried for 72 h at 75°C. The samples were then cooled in a desiccator for 45 min and weighed. Water and ethanol extractable fractions were determined following methods described by Martinez-Villaluenga et al. (2006). Briefly, and for determination of the water extractable fraction, 50 g of seeds from each test lupin were soaked in 200 mL of distilled water for 24 h at 4°C and drained. This process was repeated 3 times, and the samples were dried for 72 h at 75°C before weighing. The weight difference before and after the extraction process was expressed as the water extractable fraction (g/kg). For the ethanol extractable fraction, a 50-g seed sample from each test lupin was soaked in 50% ethanol (vol/vol) and incubated for 24 h at 40°C, and drained of ethanol. This process was repeated 3 times, and the samples were washed with distilled water before drying for 72 h at 75°C. The weight difference was expressed as the ethanol extractable fraction (g/kg). The insoluble and soluble nonstarch polysaccharide (**NSP**) content of the lupin samples was determined as alditol acetates by GLC using the method of Theander and Westerlund (1993). Total NSP content was calculated by adding insoluble and soluble NSP contents. The sum of insoluble and soluble NSP was calculated using the following polymerization factors:

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

where Rha = rhamnose; Fuc = fucose; Rib = ribose; Ara = arabinose; Xyl = Xylose; Man = mannose; Gal = galactose; Glu = glucose.

Polymerization factors were used to correct for differences in total molecular weights due to dehydration during the polymerization process. For example, each glucosidic linkage for a glucose (hexose) molecule loses one molecule of water during polymerization. Therefore, a factor of 0.9 was used in the calculation to account for 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 164 and hence a factor of 0.89) and pentoses (molecular weight 150 and hence a factor of 0.88).

Statistical Analysis

The pig was considered as the experimental unit for data analyses except the regression analyses. The

treatment effects were assessed by 2-way ANOVA for a factorial design with the main effects being lupin variety and growing location. Fisher's protected LSD analysis was conducted to separate means where significant main and interactive effects occurred under the ANOVA analysis. The effects were considered as fixed effects in the model and the statistical analyses were conducted using the statistical package Genstat (VSN International Ltd., Hemel Hempstead, UK). Furthermore, individual DE content and the physical and chemical measures of lupins were subjected to regression analyses. Pearson's correlation analysis, step-wise regression analysis, and simple and multiple regression analyses were conducted to establish prediction equations (Minitab Inc., State College, PA).

RESULTS

Physical Properties, Chemical Composition, and DE Content

The physical properties and chemical composition of the 8 test lupin samples are presented in Table 2. Thousand seed weight and the proportion of hull ranged from 124 to 204 g and 21 to 26%, respectively. The CV for most of the fiber components such as NDF, lignin, and NSP was greater than 10%, whereas CP and crude fat content were less variable.

The DE content of the 8 test lupin samples and apparent total tract digestibility (**ATTD**) of DM, GE, and CP of the lupin-containing test diets are presented in Table 3. Variety ($P < 0.01$) and growing location ($P < 0.01$) were significant sources of variation for DE content of lupins, whereas interaction terms between variety and growing location were not significant. Likewise, dietary ATTD for DM, GE, and CP was affected by variety ($P < 0.01$) and growing location ($P < 0.05$). The DE content of lupins varied by up to 2.4 MJ/kg and ranged from 13.3 to 15.7 MJ/kg, with a mean value of 14.2 MJ/kg. However, the variation was caused predominantly by the greater DE content of cv. Coromup grown in the northern agricultural region (15.7 MJ of DE/kg). The same lupin variety grown in the southern agricultural region contained a reduced DE content (14.4 MJ of DE/kg), but was comparable with the DE content of the other test lupins grown in this region of WA.

Correlation and Prediction of DE Content

Pearson's correlation analysis showed that the proportion of hulls ($r = -0.938$, $P < 0.001$) and 1,000-seed weight ($r = 0.877$, $P < 0.01$) were highly correlated to the DE content of lupins, with the ethanol extractable fraction ($r = 0.701$, $P < 0.1$), CP ($r = 0.628$, $P < 0.1$), total P ($r = 0.630$, $P < 0.1$), and soluble arabinoxylans ($r = -0.799$, $P < 0.05$) showing a smaller proportion of the variation being explained (Table 4). Correlation analyses showed that DE content of lupins was positively correlated to the proportion of kernels and CP content, whereas most of the fiber components showed negative correlations.

Simple and multiple linear regression analyses were then conducted to develop prediction equations for DE content of lupins based on the results of step-wise regression analysis (Table 5). Results showed that the DE content of lupins could be predicted with a reasonable degree of accuracy by measuring the proportion of hull ($R^2 = 0.88$, $P < 0.001$) or 1,000-seed weight ($R^2 = 0.77$, $P < 0.01$; Figure 1). Addition of total NSP ($R^2 = 0.96$, $P < 0.01$) and soluble NSP ($R^2 = 0.95$, $P < 0.01$) to the equation improved the precision of the prediction (Table 5). Because the variety Coromup grown in the northern agricultural region showed a distinctively greater DE content, further regression analyses were conducted excluding this particular sample from the data set. Results showed that the prediction accuracy of the 1,000-seed weight for DE content of lupins was decreased ($R^2 = 0.59$; $DE = 0.0216x + 10.546$, $P = 0.051$) and the prediction accuracy of the proportion of hulls for DE content of lupins was improved ($R^2 = 0.92$; $DE = -0.318x + 21.69$, $P < 0.001$).

DISCUSSION

A previous cross-study review identified that the DE content of lupins can vary by 3.5 MJ/kg with minimum and maximum values of 12.3 (Wigan et al., 1995) and 15.8 MJ/kg (King et al., 2000), respectively (Kim et al., 2007). However, this range was attributable mainly to differences in particle size of lupins and the nature of the basal diet used with lupins (i.e, empty BW gain was significantly less when pigs fed a lupin plus barley diet compared with pigs fed either lupin plus wheat or triticale, possibly due to greater total NSP content) for determination of DE content (van Barneveld, 1999). Variety and growing environment are major sources of variation for the DE content of cereal grains and legumes (van Barneveld, 1997; Kim et al., 2005, 2007). A previous study with Australian-grown wheats demonstrated that chemical composition, especially the NSP composition and structure, was significantly different due to variety and growing location and was negatively correlated to the DE content (Kim et al., 2003, 2004). Our data are the first to show in lupins that, and with the elimination of other factors such as lupin particle size and the nature of the basal diet used with lupins, the variety and growing region can affect the DE content, which varied by up to 2.4 MJ/kg depending on variety and the location where they were grown. However, because the variation originated largely from 1 sample, our data may not provide clear justification that the large variation in DE content of lupins is useful for feed formulation. If the aberrant sample (Coromup, northern agricultural region) is removed from the data set, the DE content then varies by 1.4 MJ/kg (13.3 MJ to 14.7 MJ/kg, with a mean value of 14.0 MJ/kg). We do not have a clear explanation why this lu-

pin variety contained a significantly greater DE content when it was grown in the northern agricultural region. However, because other lupins grown in the northern agricultural region also contained greater DE contents compared with the same varieties grown in the southern agricultural region, environmental factors such as rainfall, temperature, and soil type could be responsible. This was reported in a wheat study conducted in WA (Kim et al., 2004). The significantly greater DE content of Coromup grown in the northern agricultural region compared with other varieties suggests that Coromup could be a superior variety in terms of digestible protein and energy contents for pigs. Therefore, further genetic development of lupin seeds should be monitored and evaluated for nutritive value if newer varieties bred for greater protein, fat, and reduced hull content be available for the pig industry. Other factors, apart from the processing effect, affecting the DE content of grains and legumes are season (Cowling and Tarr, 2004) and the duration of storage (Kim et al.,

Table 2. Physical characteristics and chemical composition of different varieties and growing regions of *Lupinus angustifolius* L.

	Belara		Coromup		Mandelup		Tanjil				
Item	North	South	North	South	North	South	North	South	Mean	SD	CV
Physical property											
1,000-seed weight, g	170	160	204	170	181	155	158	124	165	23	14
Hull, % of DM	24.4	26.2	21.4	23.1	22.8	23.2	23.3	26.4	23.9	2	7
Kernel, % of DM	75.7	73.8	78.6	76.9	77.2	76.8	76.7	73.6	76.2	2	2
WEF,[1] g/kg of DM	46	62	56	85	54	57	52	77	61	13	22
EEF,[2] g/kg of DM	161	153	164	159	161	155	145	141	155	8	5
Chemical composition, g/kg (air-dry basis)											
DM	912	921	916	911	914	914	926	919	917	5	1
GE, MJ/kg	18.4	18.5	18.4	18.3	18.3	18.3	18.4	18.3	18.4	0.1	<1
CP	313	275	319	319	294	269	294	275	295	21	7
Crude fat	66	66	62	70	61	66	64	67	65	3	4
NDF	261	273	244	263	247	232	300	306	266	26	10
ADF	257	236	222	226	236	218	223	229	231	12	5
Lignin	39	18	19	21	29	26	23	16	24	7	31
Total NSP[3]	512	427	370	384	393	411	461	411	421	46	11
Rhamnose (Rha)	2.2	2.2	2.8	2.6	2.3	2.0	2.3	2.3	2.3	0.3	11
Fucose (Fuc)	1.0	0.9	1.1	0.9	0.9	0.8	1.0	1.0	1.0	0.1	10
Ribose (Rib)	0.2	0.8	0.9	0.9	1.0	1.0	0.7	0.9	0.8	0.3	33
Arabinose (Ara)	57	53	54	54	52	54	56	52	54	2	3
Xylose (Xyl)	60	46	41	39	42	38	45	44	44	7	16
Mannose (Man)	13	11	8	7	9	9	11	11	10	2	20
Galactose (Gal)	170	186	159	176	166	185	186	157	173	12	7
Glucose (Glu)	267	177	146	149	164	169	214	192	185	40	22
Insoluble NSP[3]	479	388	346	359	366	374	432	384	391	44	11
Rha	1.8	1.9	2.5	2.2	2.0	1.7	2.0	1.9	2.0	0.3	13
Fuc	1.0	0.9	1.1	0.9	0.8	0.8	1.0	1.0	1.0	0.1	10
Rib	—	0.5	0.6	0.6	0.7	0.6	0.4	0.6	0.5	0.2	44
Ara	51	45	49	49	47	47	49	46	48	2	4
Xyl	56	40	37	35	40	34	41	39	40	7	17
Man	5.7	8.0	4.7	5.0	4.1	4.5	7.6	8.8	6.1	2.0	32
Gal	153	161	146	161	150	161	168	141	155	9	6
Glu	266	176	146	148	164	168	213	191	184	40	22
Soluble NSP[3]	33	39	24	25	27	37	30	28	30	6	18
Rha	0.4	0.4	0.3	0.4	0.3	0.3	0.3	0.4	0.4	0.1	15
Fuc	—	—	—	—	—	—	—	—	—	—	—
Rib	0.2	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.1	18
Ara	6.6	7.6	5.1	5.3	4.7	7.0	6.3	6.6	6.2	1.2	19
Xyl	4.9	6.3	4.1	3.9	2.5	4.1	4.7	5.1	4.5	0.9	21
Man	7.7	2.8	3.4	2.2	5.3	4.3	3.1	2.5	3.9	2.0	53
Gal	17	26	13	15	16	25	18	16	18	5	26
Glu	0.5	0.4	0.5	0.5	0.6	0.5	0.5	0.4	0.5	0.1	13
Total P	2.5	2.8	3.5	3.7	3.4	2.4	3.2	2.7	3.0	0.5	16
Phytate P	1.5	1.8	2.2	2.9	2.4	1.3	2.6	1.9	2.1	0.6	27
% phytate P	60	63	62	77	71	55	81	70	67	9	13

[1]WEF = water extractable fraction.
[2]EEF = ethanol extractable fraction.
[3]Total NSP (nonstarch polysaccharide) = insoluble NSP + soluble NSP; sum of insoluble and soluble NSP was calculated using the following polymerization factor: sum of insoluble and soluble NSP = (Rha + Fuc + Rib) × 0.89 + (Ara + Xyl) × 0.88 + (Man + Gal + Glu) × 0.90 (see text for details of calculations).

Table 3. Effects of lupin variety and growing region on apparent total tract digestibility (ATTD; %) and the DE content[1,2]

		Belara		Coromup		Mandelup		Tanjil				*P*-value[3]		
Item	Wheat	North	South	North	South	North	South	North	South	Mean	SEM	V	G	V × G
ATTD of the experimental diets, %														
DM	81.9	76.3	76.1	79.4	77.6	77.7	77.1	76.6	74.9	77.0	0.74	0.002	0.050	0.595
GE	81.5	75.8	75.1	79.1	77.1	77.3	76.4	76.0	76.5	74.4	0.80	0.002	0.024	0.840
CP	72.6	76.8	75.1	81.2	78.3	78.1	75.5	76.3	75.1	77.1	0.91	0.001	0.002	0.783
DE, g/kg	13.3	13.9	13.4	15.7	14.4	14.7	14.2	14.1	13.3	14.2	0.41	0.005	0.009	0.762

[1]The DE content of wheat used in this experiment was 13.3 ± 0.3 MJ/kg (determined using 50-kg pigs, n = 7).
[2]The DE value used in the calculation for canola oil was 36.7 MJ/kg (NRC, 1998).
[3]V = variety; G = growing region.

2005), which were not addressed in this study but could be a significant source of variation similar to that which occurs in wheats (Kim, 2003).

Pearson's correlation analysis clearly showed that 1,000-seed weight, the proportion of kernels, and protein content are positively associated with lupin DE content, whereas most fiber fractions had negative effects on DE content. Although other substances might be included, the water extractable and ethanol extractable fractions were used for crude quantification of alkaloids and oligosaccharides, respectively (Martinez-Villaluenga et al., 2006), which are known to be antinutritional when fed to pigs (van Barneveld, 1999). One unexpected result from the present study was the positive relationship between ethanol extractable fraction and the DE content of lupins. This positive relationship was unexpected because oligosaccharides, especially the raffinose family of oligosaccharides, acting osmotically in the small intestinal lumen (Wiggins, 1984) and increasing the flux of water and electrolytes, would increase fluid retention and flow rate and cause an increase in DM entering the large intestine (Wiggins, 1984; Coon et al., 1990). An Australian study, feeding grower pigs a diet containing 350 g/kg of dehulled lupin meals without or with ethanol extraction, demonstrated that the ethanol extraction removed 73% of oligosaccharides from the lupins and improved energy and AA digestibility in the pigs fed ethanol-extracted lupins (Hansen et al., 1991; van Barneveld et al., 1996, 1997). In contrast, a Canadian study reported that feeding diets containing 20 g of galactooligosaccharides or glucooligosaccharides to weaner pigs did not affect DM, CP, and AA digestibility (Gabert et al., 1995). Similarly, feeding diets containing either 371 g/kg of conventional or reduced oligosaccharide soybean meal (dietary oligosaccharide levels of 31 vs. 17 g/kg, respectively) to dogs did not affect ileal and fecal nutrient digestibility (Zuo et al., 1996). The discrepancy between these studies may be caused by the source of oligosaccharides (lupin vs. soybean meal), or the ethanol extraction process for removal of oligosaccharides that may have altered chemical composition or structure of kernel endosperms. For example, Martinez-Villaluenga et al. (2006) reported significant structural and compositional changes in lupin proteins such as a decrease of some polypeptides and reduction in bands corresponding to conglutin γ, which can cause an allergenic reaction in humans. Collectively, the literature and data from the present study indicate that the effects of oligosaccharides on digestibility of nutrients in pigs fed lupins may not be as significant as

Table 4. Pearson's correlation coefficients (r) between the physical and chemical properties of lupins and their DE content

Item[1]	DE	TSW	Hulls	Kernels	WEF	EEF	CP	NDF	Total P	TNSP	INSP	SNSP
TSW, g/kg	0.877**											
Hulls, % DM	−0.938***	−0.794*										
Kernels, % DM	0.937***	0.798*	−1.000***									
WEF, % DM	−0.226	−0.386	0.276	−0.286								
EEF, g/kg of DM	0.701†	0.876**	−0.639†	0.647†	−0.286							
CP, g/kg	0.628	0.703†	−0.592	0.601	−0.018	0.632†						
NDF, g/kg	−0.615	−0.657†	0.616	−0.619	0.297	−0.842**	−0.201					
Total P, g/kg	0.630†	0.570	−0.577	0.570	0.321	0.333	0.638†	−0.048				
TNSP, g/kg	−0.507	−0.266	0.370	−0.354	−0.532	−0.190	−0.022	0.313	−0.585			
INSP, g/kg	−0.461	−0.240	0.327	−0.311	−0.518	−0.184	0.057	0.346	−0.518	0.994***		
SNSP, g/kg	−0.606	−0.369	0.525	−0.522	−0.324	−0.202	−0.668†	0.064	−0.779*	0.480	0.377	
SAx,[2] g/kg	−0.799*	−0.586	0.825**	−0.822**	−0.069	−0.513	−0.546	0.445	−0.729*	0.562	0.495	0.783*

[1]TSW: 1,000-seed weight; WEF: water extractable fraction; EEF: ethanol extractable fraction; TNSP: total nonstarch polysaccharide; INSP: insoluble NSP; SNSP: soluble NSP; SAx: soluble arabinoxylans.
[2]Calculated as sum of soluble arabinose and soluble xylose multiplied by polymerization factor 0.88 (see text for details of the calculation).
†$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 5. Linear regression equations for prediction of lupin DE content[1]

Linear regression equation[2]	R^2	RSD[3]	*P*-value
Simple linear regression			
DE = 24.2 – 0.417 Hull%	0.88	1.116	0.001
DE = −17.6 + 0.417 Kernel%	0.88	1.117	0.001
DE = 9.38 + 0.0292 TSW	0.77	1.092	0.004
DE = 17.5 – 0.350 SAx	0.64	1.072	0.017
DE = 7.32 + 0.0234 CP	0.40	1.094	0.095
DE = 11.2 + 0.984 Total P	0.40	1.082	0.104
Multiple linear regression			
DE = 19.2 – 0.291 Hull% + 0.0119 TSW	0.93	1.192	0.001
DE = 24.7 – 0.386 Hull% − 0.00307 TNSP	0.91	1.099	0.001
DE = 19.6 – 0.253 Hull% + 0.0124 TSW − 0.00327 TNSP	0.96	1.187	0.003
DE = 18.6 – 0.24 Hull% + 0.0127 TSW − 0.0253 SNSP	0.95	1.200	0.004

[1]Regression equations were developed based on step-wise regression analyses.
[2]Abbreviations used: Hull% and Kernel% = proportions of lupin hulls and kernels; TSW = 1,000-seed weight; SAx = soluble arabinoxylans; TNSP and SNSP = total and soluble nonstarch polysaccharides, respectively.
[3]RSD: residual SD.

originally thought, at a dietary level less than 40 g/kg. However, studies by van Barneveld et al. (1996, 1997) and Martinez-Villaluenga et al. (2006) suggest that the positive relationship between ethanol extractable fraction and DE content of lupins reported in the present study might be due to a fraction or fractions other than oligosaccharides.

Thousand-seed weight and the proportion of kernel had significant positive relationships with the DE content of lupins. In lupins, larger seed size is associated with less proportion of hulls per unit of weight due to reduced surface area compared with a smaller seed size. In addition, dehulling is known to significantly increase DE content (Wigan et al., 1993; Flis et al., 1996). Therefore, a significant correlation was evident between 1,000-seed weight and the proportion of hulls. The impact of the proportion of lupin hulls on DE content was greater than any other components because the hulls contain mostly insoluble and less fermentable lignified fibers. The present study showed clearly that the proportion of hulls has potential as a screening tool for rapid assessment of the DE content of lupins. A recent Chilean study demonstrated that rapid and accurate (R^2 = 0.94) scanning of hull contents was possible in lupin seed samples using near infrared spectroscopy (**NIRS**) with a SE of 0.73% (Alomar and Mera, 2008). Use of NIRS technology for the prediction of DE content directly or via the prediction of hull content offers a sound opportunity for industrial application. Nevertheless, previous grain studies used 1,000-seed weight in barley (Fairbairn et al., 1999) and reported no significant relationship with DE. Also, Zijlstra et al. (1999) suggested that physical variables, such as hectoliter weight of wheats, could not accurately predict nutritional value. This apparent discrepancy between lupins and cereals (e.g., barley and wheat) in the ability to predict the DE content using physical variables might be due to a greater contribution of hulls (approximately 25%) in lupin seeds compared with cereal grains, which has a reduced energy contribution (van Barneveld, 1999) in lupins per unit weight than cereal grains. The less significant correlation between DE content and the NSP constituent might be due to the fact that the cell wall materials, which comprise about 30% of the endosperm of lupin kernels, are highly fermentable in the gastrointestinal tract (Kim et al., 2007). Therefore, these cell wall materials variably contribute to the total DE content depending on the ability of the animal to ferment the cell wall materials. Nevertheless, the significant negative relationship between the proportion of hulls and the DE content of lupins suggests there is a need to develop new lupin varieties having a decreased proportion of seed coat. Alternatively, dehulling may be necessary to improve energy utilization efficiency of lupins.

To account for the greater DE content of one sample (Coromup, grown in the northern agricultural region) on the accuracy of the prediction, this sample was excluded and regression analysis was reanalyzed. The prediction accuracy of the 1,000-seed weight was decreased, but the proportion of hulls was increased and accounted for 92% of the DE variation. This result reinforces the concept that the proportion of hulls is a potentially useful measurement for the prediction of DE content in lupins.

Multiple regression analyses attempted to establish prediction equations with better predictability by including additional characteristics. Although including the NSP content in the equation improved accuracy, the extent of the improvement compared with using single characteristics such as the proportion of hulls is not warranted. Therefore, the use of proportion of hulls and 1,000-seed weight together could potentially predict the DE content of lupins.

In summary, the DE content of lupins grown in WA varied by up to 2.4 MJ/kg in response to genetic and environmental variation. The fiber fraction generally reduced the DE content of lupins, and variability of the DE content could be predicted with reasonable accurately by measuring the proportion of hulls. However

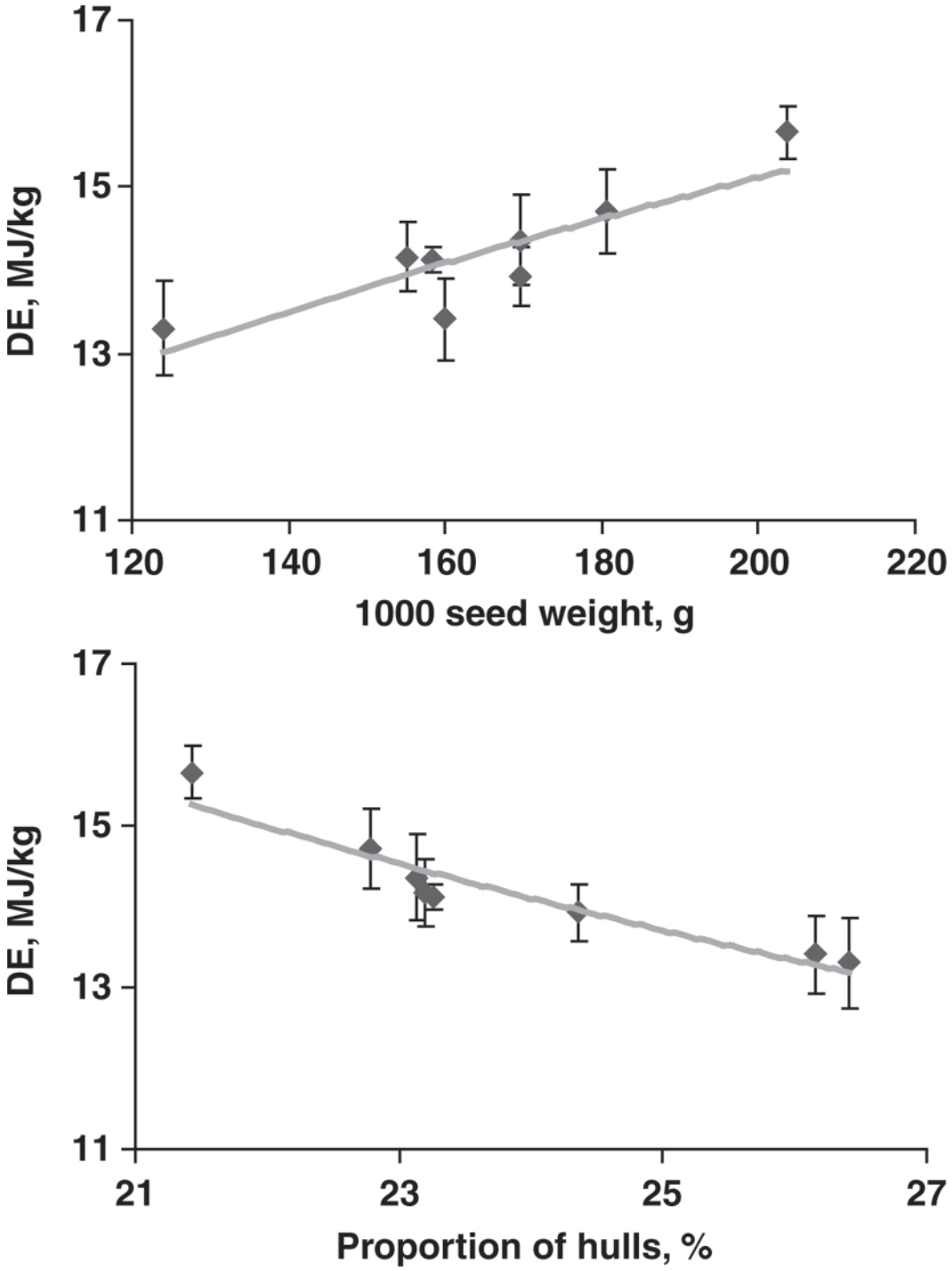

Figure 1. Linear relationships between DE content of lupins (MJ/kg as-fed basis) and 1,000-seed weight (DE = 0.0292 × 1,000-seed weight + 9.3838, $R^2 = 0.77$, residual SD = 1.092, $P = 0.004$) and proportion of hulls (DE = −0.4107 × proportion of hulls + 23.998, $R^2 = 0.88$, residual SD = 1.116, $P < 0.001$).

measuring the proportion of hulls is time- and labor-intensive, and the development of a NIRS calibration to predict the proportion of hulls in lupin seeds could be a solution for rapid and practical prediction of DE content.

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