Digestibility of lupin kernel meals in feeds for the black tiger shrimp, *Penaeus monodon*

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Abstract

In recent years, new cultivars of lupins have largely replaced the cultivars that were studied in previous research with shrimp. There was a need to establish if the breeding programs had introduced changes in the new lupin cultivars that would affect the nutritional value of the kernel meal for shrimp. We have determined the apparent dry matter digestibility (ADMD), apparent crude protein digestibility (ACPD) and apparent digestibility of energy (ADE) of the yellow lupin *Lupinus luteus* cv. Wodjil, as well as of six of the new cultivars of *Lupinus angustifolius* when used in diets for the black tiger shrimp, *Penaeus monodon*. The *L. angustifolius* cultivars represent about 80% of Australia’s lupin production. We have also determined the apparent digestibility (AD) of the amino acids of five of the new cultivars of *L. angustifolius*, and of *L. luteus*, cv. Wodjil. Ytterbium acetate was used as an inert digestibility marker at a concentration of 0.5 g kg⁻¹ in the diets. During the periods when faeces were collected, the shrimp were fed every 6 h and faeces were collected within 3 h of being passed. Six replicate tanks were assigned to each treatment. The kernel meal from *L. luteus* cv. Wodjil had the highest ADMD (70.0%) and ADE (79.9%) but its ACPD was mid-range at 93.8%. The ADMD of the *L. angustifolius* kernel meals varied between 56.5% and 66.3% with the mean (±s.e.m.) of 62.6% (±0.95%), and the ADE varied between 69.6% and 77.2% (mean ± s.e.m. = 74.0% ± 0.72%), whereas the ACPD varied between 92.7% and 96.8% (mean ± s.e.m. = 94.3% ± 0.48%). The AD of the amino acids was similar to the ACPD value. Though there were significant differences among the ADs of the new cultivars of *L. angustifolius*, their values are similar to, though slightly lower than the AD reported for the older cultivar, Gungurru. The general consistency of the *L. angustifolius* AD results suggests that nutritionists and feed formulators can confidently use mean AD values for dry matter, protein and energy for kernel meals comprising of random mixtures of cultivars.

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Keywords: Shrimp; Lupins; Digestibility; Amino acid

1. Introduction

The availability of nutrients in the feed and its component ingredients is of prime interest to nutritionists and feed formulators. Though the gross chemical composition will give an indication of the nutrients present in a feed or in an ingredient, the digestibility of the nutrients gives a much better estimate of their availability. With any new ingredient, its nutrient composition and the digestibility of its key nutrients need to be determined before it can be used with best effect in nutritionally-based feed formulations.

Fishmeal has long been the main protein source used in feeds for most aquaculture species. However, with the increasing cost and periodic shortages of fishmeal on the
global markets, the aquaculture industry is interested in reducing its dependence on fishmeal through the development of alternative protein sources (New and Wijkström, 2002). Lupins are a useful, protein-rich ingredient which can partially replace fishmeal in feeds for both fish and shrimp (Hughes, 1991; Burel et al., 1998; Smith et al., 2000). As Australia contributes about 80% of the global production of lupins, there has been a significant research effort in this country to evaluate lupin products in aquaculture feeds (Allan and Rowland, 1998; Smith, 1998; Carter and Hauler, 2000). Lupin kernel meal was found to be better digested than the whole seed meal, and its protein was found to be highly digestible (Smith, 1998; Booth et al., 2001; Glencross, 2001). Much of the early work was carried out using kernel meals derived from the narrow leaved lupin, *Lupinus angustifolius*, particularly from a variety (or cultivar) called Gungurru. During the 1990s, Gungurru was the most widely-grown cultivar in Australia. Since then, lupin breeding programs have produced new cultivars that are better suited to the soil types and climatic conditions found in the different growing regions. Gungurru has been largely replaced by these new cultivars and now represents <5% of Australian production (B. Buirchell, WA Agriculture, personal communication; Pulse Australia, 2006). In studies with rainbow trout, *Oncorhynchus mykiss*, Glencross and co-workers have determined the digestibility of the kernel meal from a number of the new cultivars of *L. angustifolius* and of the yellow lupin, *L. luteus* cv. Wodjil (Glencross et al., 2003; Glencross and Hawkins, 2004). These studies showed that the digestibility of protein in the kernel meals was generally high (85 to 90%). However, there have been no studies reported which have examined the response of any species of shrimp to these new cultivars.

In this study with the black tiger shrimp, *Penaeus monodon*, we have determined the apparent dry matter digestibility (ADMD), apparent crude protein digestibility (ACPD) and apparent digestibility of energy (ADE) of the yellow lupin *L. luteus* cv. Wodjil, and of six of the new cultivars of *L. angustifolius* which represent about 80% of Australia’s lupin production. We have also determined the apparent digestibility of the amino acids (excluding tryptophan) of *L. luteus*, cv. Wodjil and of five of the new cultivars of *L. angustifolius*.

## 2. Materials and methods

### 2.1. Lupin kernel meals

Samples of whole-seed *L. angustifolius* lupins were obtained from the Department of Agriculture — Western Australia, lupin breeding program. The lupins were grown at either of two research field stations, Katanning (33.69 S, 117.61 E) and Wongan Hills (30.89 S, 116.72 E). Both batches of seed were obtained from the 2003 crop season. A sample of the older cultivar, *L. angustifolius* cv. Gungurru, from the 2002 harvest at the Mingenew field station (29.18 S, 115.42 E) was also included. The seed was harvested and segregated by source and variety and stored at 4 °C prior to processing. In addition, a sample of *L. angustifolius* cv. Myallie and a sample of *L. luteus* cv. Wodjil, both of which had been grown in the northern growing area near Coorow (29.88 S, 116.02 E) in the 2002 season, were obtained from Coorow Seed Cleaners.

### Table 1

Proximate composition (g kg\(^{-1}\) DM, unless otherwise stated) of lupin kernel meals evaluated in the digestibility experiments

<table>
<thead>
<tr>
<th>Lupin cultivar(^1)</th>
<th>Moisture</th>
<th>Ash</th>
<th>Crude protein</th>
<th>Total lipid</th>
<th>NFE</th>
<th>Energy (MJ kg(^{-1}) DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kalya (KT)</td>
<td>101</td>
<td>36</td>
<td>418</td>
<td>96</td>
<td>451</td>
<td>20.7</td>
</tr>
<tr>
<td>Mandelup (KT)</td>
<td>101</td>
<td>30</td>
<td>416</td>
<td>94</td>
<td>456</td>
<td>20.7</td>
</tr>
<tr>
<td>Tanjil (KT)</td>
<td>101</td>
<td>30</td>
<td>413</td>
<td>105</td>
<td>449</td>
<td>21.1</td>
</tr>
<tr>
<td>Myallie (KT)</td>
<td>102</td>
<td>31</td>
<td>453</td>
<td>89</td>
<td>422</td>
<td>20.8</td>
</tr>
<tr>
<td>Wàlan 2173(KT)</td>
<td>103</td>
<td>31</td>
<td>458</td>
<td>94</td>
<td>412</td>
<td>20.9</td>
</tr>
<tr>
<td>Gungurru (MG)</td>
<td>85</td>
<td>29</td>
<td>463</td>
<td>94</td>
<td>415</td>
<td>21.0</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kalya (WH)</td>
<td>71</td>
<td>37</td>
<td>494</td>
<td>80</td>
<td>389</td>
<td>20.6</td>
</tr>
<tr>
<td>Mandelup (WH)</td>
<td>69</td>
<td>34</td>
<td>468</td>
<td>81</td>
<td>416</td>
<td>20.6</td>
</tr>
<tr>
<td>Tanjil (WH)</td>
<td>63</td>
<td>34</td>
<td>480</td>
<td>88</td>
<td>398</td>
<td>20.6</td>
</tr>
<tr>
<td>Wonga (WH)</td>
<td>66</td>
<td>33</td>
<td>470</td>
<td>88</td>
<td>409</td>
<td>20.6</td>
</tr>
<tr>
<td>Myallie (CO)</td>
<td>83</td>
<td>37</td>
<td>426</td>
<td>87</td>
<td>450</td>
<td>21.1</td>
</tr>
<tr>
<td>Wodjil (CO)</td>
<td>73</td>
<td>44</td>
<td>546</td>
<td>94</td>
<td>316</td>
<td>20.4</td>
</tr>
</tbody>
</table>

\(^1\)All kernel meals were from cultivars of *L. angustifolius*, except for Wodjil which is a cultivar of *L. luteus*. The region in Western Australia where the lupins were grown is indicated in parentheses after the cultivar name: KT=Katanning, MG=Mingenew, WH=Wongan Hills, CO=Coorow.
(Coorow, WA). For processing, the seed was graded according to seed size using round-holed 7 mm, 6 mm and 5 mm sieves, and each segregation of each variety was separately split using a disc-mill dehulling unit (Department of Agriculture, South Perth, WA, Australia). The split (dehulled) segregations of each variety were then pooled prior to aspiration (air stream mediated density classification) to remove the hulls from the kernels. Any remaining seed hull fragments were manually removed to ensure a 100% pure preparation of seed kernels of each variety. The kernels were then rotor-milled (Retsch, Haan, Germany) through a 750 μm screen.

Two experiments were carried out to determine the digestibility of lupin kernel meals from a total of seven cultivars of *L. angustifolius* and one of *L. luteus*. The first experiment examined the digestibility of *L. angustifolius* cultivars grown in the south of the Western Australian wheat belt, at Katanning. These cultivars were Kalya, Mandelp, Walan 2173, Myallie and Tanjil. This experiment also included the older cultivar Gungurru which had been grown the previous season at Mingenew (Table 1). The second experiment examined kernel meals from lupins grown in the main (northern) growing areas of the wheat belt, at Wongan Hills. These were the *L. angustifolius* cultivars Kalya, Mandelp, Tanjil and Wonga. Also included were kernel meals from the cultivar Myallie and from *L. luteus* cv. Wodjil (Tables 1 and 2).

### Table 2
Amino acid composition of lupin kernel meals (g 100g⁻¹ DM) evaluated in Experiment 2 of the digestibility study (WH=Wongan Hills; CO=Coorow)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>1.63</td>
<td>1.64</td>
<td>1.54</td>
<td>1.49</td>
<td>1.49</td>
<td>1.84</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.92</td>
<td>5.20</td>
<td>5.44</td>
<td>5.40</td>
<td>4.66</td>
<td>6.11</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>5.52</td>
<td>5.06</td>
<td>5.19</td>
<td>5.06</td>
<td>4.54</td>
<td>5.85</td>
</tr>
<tr>
<td>Cysteine*</td>
<td>0.81</td>
<td>0.80</td>
<td>0.70</td>
<td>0.66</td>
<td>1.86</td>
<td>3.90</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>11.50</td>
<td>10.11</td>
<td>10.44</td>
<td>10.27</td>
<td>9.08</td>
<td>13.45</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.95</td>
<td>1.95</td>
<td>1.83</td>
<td>1.79</td>
<td>1.70</td>
<td>2.16</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.06</td>
<td>0.86</td>
<td>0.96</td>
<td>0.86</td>
<td>1.03</td>
<td>1.39</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.90</td>
<td>1.80</td>
<td>1.76</td>
<td>1.85</td>
<td>1.60</td>
<td>2.00</td>
</tr>
<tr>
<td>Leucine</td>
<td>3.58</td>
<td>3.31</td>
<td>3.23</td>
<td>3.30</td>
<td>2.88</td>
<td>4.26</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.29</td>
<td>2.21</td>
<td>2.00</td>
<td>2.14</td>
<td>1.24</td>
<td>1.68</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.22</td>
<td>0.33</td>
<td>0.27</td>
<td>0.28</td>
<td>0.20</td>
<td>0.30</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.86</td>
<td>1.79</td>
<td>1.77</td>
<td>1.81</td>
<td>1.58</td>
<td>2.08</td>
</tr>
<tr>
<td>Proline</td>
<td>3.08</td>
<td>3.26</td>
<td>3.71</td>
<td>2.40</td>
<td>2.56</td>
<td>3.62</td>
</tr>
<tr>
<td>Serine</td>
<td>2.59</td>
<td>2.31</td>
<td>2.42</td>
<td>2.36</td>
<td>2.15</td>
<td>2.86</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.88</td>
<td>1.77</td>
<td>1.82</td>
<td>1.80</td>
<td>1.70</td>
<td>1.68</td>
</tr>
<tr>
<td>Valine</td>
<td>1.70</td>
<td>1.80</td>
<td>1.66</td>
<td>1.68</td>
<td>1.58</td>
<td>1.86</td>
</tr>
</tbody>
</table>

* Determined as cysteic acid derived from the conversion of each molecule of cysteine to one molecule of cysteic acid and each molecule of cysteine to two molecules of cysteic acid.

### 2.2. Experimental animals

Juvenile shrimp, *P. monodon*, were obtained from commercial shrimp farms in northern Queensland, Australia. They were reared in the laboratory from about 2 g until they were >12 g before being used in the digestibility experiments. During the grow-out period, the shrimp were maintained in 2500 L holding tanks with flow-through seawater (salinity 32 to 36‰ and temperature 28±0.5 °C) and fed twice daily with a commercial shrimp feed (CP # 4004, CP Feeds, Samut Sakorn, Thailand).

### 2.3. Digestibility tanks

The tanks used in the digestibility studies were circular, white polyethylene (100 L capacity, 600 mm diam.), fitted with a central standpipe drain. They were supplied with filtered (10 μm), heated seawater flowing at a rate of 500 mL min⁻¹ to maintain tank temperatures at 29.0±0.4 °C, and with continuous aeration from a single air-stone. In-flowing seawater was used to create a gentle circular current within the tank to aid the concentration of waste in the centre.

### 2.4. Feed formulation and preparation

The reference diet used in this study (Table 3) was formulated to be nutritionally-adequate and attractive to the shrimp, with 390 g kg⁻¹ crude protein and 100 g kg⁻¹ total lipid, on DM basis. Micro-nutrients were included at twice the minimum rate in the reference diet to ensure that they were not deficient when diluted with the test ingredients in the test diet formulations. The test diets comprised 50% by weight of the kernel meal (‘as used’ basis) and 50% by weight of the reference diet mash (‘as used’). The test diets had a similar crude protein content as the Reference diet (range: 380 to 425 g kg⁻¹) but slightly less total lipid (∼90 g kg⁻¹). Ytterbium acetate tetrahydrate (99.9%, Aldrich, Sydney, Australia) was included in the feeds as an inert digestibility marker at a rate of 0.5 g kg⁻¹.

Water was added to the mixed ingredients to form a dough containing 40 to 50% moisture. The dough was extruded twice through a 3 mm die of a meat mincer (Hobart Corporation, Troy, OH, USA) to form spaghetti-like strands which were air dried in a forced-draft cabinet at 40 °C, and then re-ground to pass through a 0.500 mm screen. Additional water was added to the re-ground material and the ‘feed’ mixed to form a dough again. This dough was extruded twice through the mincer, steamed for 5 min and air dried again before
being broken-up into 5 to 10 mm pellets and stored at −5 °C until used. This process was found to significantly improve the homogeneity of the feed pellets (Smith and Tabrett, 2004).

2.5. Experimental

The two digestibility experiments involved the feeding of the reference diet and six lupin kernel meal diets to groups of shrimp (mean weight±SD: Experiment 1 = 23.5±3.8 g, Experiment 2 = 16.6±2.4 g). In both experiments, six tanks, each containing two randomly-selected shrimp, were allocated to each dietary treatment. The shrimp were placed in the tanks 7 days prior to the start of the faecal collection periods, to adapt to their allocated diet. During the adaptation period the shrimp were fed twice daily and no faeces were collected. After the adaptation period, and commencing on a Monday at 06:00 am, the shrimp were fed every 6 h, with a 30 s interval between feeding successive tanks.

Thirty minutes after the feed was put in the tanks, all the uneaten feed pellets and fragments were removed from the tanks by siphoning and discarded. Thereafter, faeces from individual tanks were collected by siphoning 3 h after feeding and again immediately before feeding. This process ran continuously for 5 d each week until Saturday mornings at 06:00 am. Between Saturday and Monday mornings, the shrimp were fed twice daily and no faeces were collected.

The faeces siphoned from the each tank were collected into a 10 L bucket and within 30 min were transferred into a 10 mL centrifuge tube using a wide mouth pipette tip and bulb. The excess water was decanted from the centrifuge tubes after a short settling time. Distilled water was added to the tubes to make the volume up to 10 mL and the tubes centrifuged at 2000 rpm (700 rcf) for 30 s. The supernatant was decanted off, and the tubes capped and placed in a freezer. Once frozen, the faecal pellet was transferred to a pre-weighed sample vial and stored at −20 °C.

This routine was maintained for about 10 weeks in both experiments until at least 2 g dry weight of faecal material (~30 g of wet faeces) had been collected from each tank. This was the amount required for the intended chemical analyses for dry matter (DM), crude protein, energy and ytterbium, and additionally in the second experiment, for hydrolysis and amino acid determination. At the end of the experiment, faeces were freeze-dried, ground and stored at −20 °C.

2.6. Chemical analyses

Samples of faeces, finely ground feed and lupin kernel meals were analysed using standard laboratory methods essentially in accordance with AOAC International (1999) recommendations. DM was determined gravimetrically after drying at 105 °C to constant weight, generally for 16 h, and ash by heating and ignition at 600 °C for 6 h. The total N content was determined using a modified Kjeldahl digestion (Bradstreet, 1965) followed by colorimetric analysis (Searle, 1984) in a Technicon segmented flow autoanalyser (Technicon Instruments Corporation, Tarrytown, NY, USA) (Varley, 1966). Crude protein (CP) was calculated by multiplying total N by 6.25. Total lipid was determined gravimetrically following extraction with chloroform–methanol (ratio 2:1) (Folch et al., 1957). The concentration of Yb was determined using a Varian Vista Pro axial CCD simultaneous ICP-OES (Varian Techtron, Mulgrave, Victoria, Australia) after digestion in nitric acid/perchloric acid mixture (McQuaker et al., 1979). Gross energy (GE) was determined by isothermal bomb calorimetry using a
microprocessor-controlled Leco AC 200 automatic bomb calorimeter (Leco Corp. St Joseph, MI, USA). Amino acids, including methionine and cysteine, were determined after hydrolysis using 6M HCl with 0.5% phenol and DTDP for 24 h at 110 °C (Barkholt and Jensen, 1989). Amino acids were analysed by HPLC as the OPA and FMOC derivatives using a C18 column.

2.7. Calculations

The ADMD, ACPD, and ADE of the reference and test diets were calculated using the following equation:

\[
ADN_{\text{in Diet}}(\%) = \frac{100}{C2} \left( \frac{M_D}{N_D} - \frac{M_F}{N_F} \right)
\]

where \(M_D\) and \(M_F\) is the concentration (on a DM basis) of the marker in the diet and faeces, respectively, and \(N_D\) and \(N_F\) are the concentration (on a DM basis) of the analyte of interest (nutrient, DM or energy) in the diet and faeces, respectively.

The test ingredient (lupin kernel meal) ADMD, ACPD and ADE were calculated using the respective digestibility of the test feed and of the reference diet in the equation described by Pfeffer et al. (1995):

\[
ADNI = \frac{1}{\alpha} [ADNTD - (1-\alpha)ADNRD]
\]

where \(ADNI\), \(ADNTD\) and \(ADNRD\) are the apparent digestibility of the analyte in the ingredient, in the test diet and in the reference diet, respectively, and where \(\alpha\) is the proportion of the analyte in the test diet that is contributed by the test ingredient.

\[
\alpha = \frac{i \times DM_I \times N_I}{(i \times DM_I + (1-i)DM_{RD}) N_{TD}}
\]

where \(i\) is the inclusion level of the test ingredient in the test diet (as mixed), \(DM_I\) and \(DM_{RD}\) are the DM (as

Table 4

Derived apparent digestibility (%) of dry matter (ADMD), crude protein (ACPD) and energy (ADE) of lupin kernel for the black tiger shrimp, *P. monodon*

<table>
<thead>
<tr>
<th>Lupin cultivar</th>
<th>ADMD(^2)</th>
<th>ACPD(^2)</th>
<th>ADE(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalya (KT)</td>
<td>59.6(^e)</td>
<td>93.0(^bc)</td>
<td>71.9(^e)</td>
</tr>
<tr>
<td>Mandelup (KT)</td>
<td>60.8(^ec)</td>
<td>93.8(^de)</td>
<td>72.2(^c)</td>
</tr>
<tr>
<td>Tanjil (KT)</td>
<td>65.3(^c)</td>
<td>95.5(^ab)</td>
<td>76.3(^bc)</td>
</tr>
<tr>
<td>Myallie (KT)</td>
<td>64.3(^bc)</td>
<td>95.7(^ab)</td>
<td>75.0(^cd)</td>
</tr>
<tr>
<td>Walan 2173 (KT)</td>
<td>62.5(^cd)</td>
<td>94.1(^cd)</td>
<td>73.8(^de)</td>
</tr>
<tr>
<td>Gunugurru (MG)</td>
<td>66.3(^b)</td>
<td>96.8(^a)</td>
<td>77.2(^b)</td>
</tr>
<tr>
<td>Kalya (WH)</td>
<td>64.6(^c)</td>
<td>95.0(^bc)</td>
<td>75.8(^bc)</td>
</tr>
<tr>
<td>Mandelup (WH)</td>
<td>62.4(^cd)</td>
<td>93.3(^bc)</td>
<td>73.4(^de)</td>
</tr>
<tr>
<td>Tanjil (WH)</td>
<td>61.3(^c)</td>
<td>92.8(^c)</td>
<td>72.8(^c)</td>
</tr>
<tr>
<td>Wonga (WH)</td>
<td>64.9(^c)</td>
<td>94.6(^bc)</td>
<td>75.9(^bc)</td>
</tr>
<tr>
<td>Myallie (CO)</td>
<td>56.5(^f)</td>
<td>92.7(^g)</td>
<td>69.6(^f)</td>
</tr>
<tr>
<td>Wodjil (CO)(^1)</td>
<td>70.0(^a)</td>
<td>93.8(^ab)</td>
<td>79.9(^a)</td>
</tr>
</tbody>
</table>

\(^1\)S.E.M. = ±0.95

Table 5

Comparisons, following 2-way ANOVA, of apparent digestibility data for three cultivars of *L. angustifolius* grown at two locations, Katanning and Wongan Hills

<table>
<thead>
<tr>
<th>Location</th>
<th>Kalya</th>
<th>Mandelup</th>
<th>Tanjil</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Apparent dry matter digestibility (%)</strong>(^1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Katanning</td>
<td>59.6(^d)</td>
<td>60.8(^b)</td>
<td>65.3(^a)</td>
<td>61.9</td>
</tr>
<tr>
<td>Wongan Hills</td>
<td>64.6(^a)</td>
<td>62.3(^b)</td>
<td>61.3(^a)</td>
<td>62.8</td>
</tr>
<tr>
<td>Mean</td>
<td>62.1</td>
<td>61.6</td>
<td>63.3</td>
<td>(±1.03)*</td>
</tr>
<tr>
<td><strong>Apparent crude protein digestibility (%)</strong>(^1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Katanning</td>
<td>93.0(^b)</td>
<td>93.8(^c)</td>
<td>95.5(^a)</td>
<td>94.1</td>
</tr>
<tr>
<td>Wongan Hills</td>
<td>95.0(^b)</td>
<td>93.3(^c)</td>
<td>92.8(^a)</td>
<td>93.7</td>
</tr>
<tr>
<td>Mean</td>
<td>94.0</td>
<td>93.5</td>
<td>94.1</td>
<td>(±0.42)*</td>
</tr>
<tr>
<td><strong>Apparent digestibility of energy (%)</strong>(^1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Katanning</td>
<td>71.9(^b)</td>
<td>72.2(^b)</td>
<td>76.3(^a)</td>
<td>73.4</td>
</tr>
<tr>
<td>Wongan Hills</td>
<td>75.8(^b)</td>
<td>73.4(^b)</td>
<td>72.8(^a)</td>
<td>74.0</td>
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<tr>
<td>Mean</td>
<td>73.9</td>
<td>72.9</td>
<td>74.6</td>
<td>(±0.74)*</td>
</tr>
</tbody>
</table>

\(^1\)Within each digestibility parameter (dry matter, crude protein and energy), cell mean values with the same superscript letter do not differ significantly (\(P<0.05\)).

For example: apparent dry matter digestibility of Kalya from Katanning is significantly different from Kalya from Wongan Hills, and Kalya from Katanning is significantly different from Tanjil from Wongan Hills, but Mandelup from Wongan Hills is not significantly different from any of the cultivars from either location.

\(^*\)S.E.M. = ±0.95; refers to (cultivar×location) interaction term.
mixed) of the test ingredient and reference diet, respectively. $N_i$ is the concentration of the analyte in the test ingredient (DM basis), and $N_{TD}$ is the concentration of the analyte in the test diet (DM basis).

The concentration of nitrogen-free extractsive (NFE) in the kernel meals was derived to include fibre and all other carbohydrate material:

$$\text{NFE} = 1000 - (\text{Ash} + \text{Crude Protein} + \text{Total lipid}), \text{ on a g kg}^{-1} \text{ DM basis}. $$

The apparent digestible protein content of a kernel meal was calculated as:

$$\text{AD protein content (g kg}^{-1}) = \frac{\text{ACPD} (\%)}{\text{CP} (\text{g kg}^{-1})} \times 100$$

Similarly, the total amount of the digestible amino acids was calculated as:

$$\text{AD total amino acids (g kg}^{-1}) = \sum (\text{AD}_j (\%) \times \text{AA}_j (\text{g kg}^{-1})/100$$

where $j$ represents each of the amino acids.

### 2.8. Statistical analysis

Faecal samples from each tank were kept separate as replicate samples. Hence six estimates of AD were made for each diet and for each test ingredient. AD data were analysed for homogeneity using Bartlett’s test for homogeneity of variances prior to analysis to ensure valid use of ANOVA. Differences in the apparent digestibility of DM, CP, energy and individual essential amino acids of the kernel meals derived from the lupin cultivars were analysed using a one-way analysis of variance. Differences between treatment effects were examined a-posteriorily using Fischer’s protected ‘t’ test, wherein differences between means were examined only where the $F$-test of the ANOVA was significant ($P<0.05$) (Steel and Torrie, 1980). Data from the two experiments were combined and each sample of kernel meal was treated as an independent sample (Snedecor and Cochrane, 1967). Combining the data for analysis was considered appropriate as there was no significant difference between experiments in the ADMD of the reference diet, or in its ACPD or ADE. The same reference diet was used in both experiments providing the link between them. The means of AD, standard deviations and number of replicates of the reference diet for Experiments 1 and 2 were as follows: ADMD 74.59% (1.2450, 6) and 75.72% (0.9412, 6); ACPD 82.50% (0.6651, 6) and 82.36% (0.5412, 6); ADE 82.03% (0.8954, 6), 82.84% (0.6808, 6), respectively.

Where the same cultivar was grown at Katanning and Wongan Hills (Kalya, Mandelup, Tanjil), the AD data were also analysed using a two-way analysis of variance to determine the influence of different growing conditions on the digestibility.

### Table 6

Apparent digestibility (%) of amino acids of kernel meals from cultivars of narrow leafed lupin (Lupinus angustifolius) and yellow lupin (L. luteus cv. Wodjil) used in Experiment 2 of the digestibility study

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Kalya (WH)</th>
<th>Mandelup (WH)</th>
<th>Tanjil (WH)</th>
<th>Wonga (WH)</th>
<th>Myallie (CO)</th>
<th>Wodjil (CO)</th>
<th>±s.e.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>93</td>
<td>93</td>
<td>92</td>
<td>94</td>
<td>90</td>
<td>90</td>
<td>0.6</td>
</tr>
<tr>
<td>Arginine</td>
<td>99</td>
<td>98</td>
<td>98</td>
<td>98</td>
<td>97</td>
<td>96</td>
<td>0.6</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>95</td>
<td>92</td>
<td>92</td>
<td>94</td>
<td>91</td>
<td>92</td>
<td>0.4</td>
</tr>
<tr>
<td>Cysteine*</td>
<td>94</td>
<td>86</td>
<td>87</td>
<td>90</td>
<td>79</td>
<td>80</td>
<td>0.7</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>98</td>
<td>97</td>
<td>97</td>
<td>97</td>
<td>96</td>
<td>96</td>
<td>0.2</td>
</tr>
<tr>
<td>Glycine</td>
<td>93</td>
<td>93</td>
<td>91</td>
<td>94</td>
<td>90</td>
<td>89</td>
<td>0.6</td>
</tr>
<tr>
<td>Histidine</td>
<td>96</td>
<td>96</td>
<td>93</td>
<td>98</td>
<td>91</td>
<td>92</td>
<td>0.6</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>97</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>92</td>
<td>93</td>
<td>0.4</td>
</tr>
<tr>
<td>Leucine</td>
<td>96</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>93</td>
<td>94</td>
<td>0.4</td>
</tr>
<tr>
<td>Lysine</td>
<td>93</td>
<td>90</td>
<td>95</td>
<td>92</td>
<td>91</td>
<td>93</td>
<td>0.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>100</td>
<td>86</td>
<td>88</td>
<td>92</td>
<td>88</td>
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<td>2.4</td>
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<td>Phenylalanine</td>
<td>95</td>
<td>94</td>
<td>95</td>
<td>96</td>
<td>94</td>
<td>94</td>
<td>0.5</td>
</tr>
<tr>
<td>Proline</td>
<td>100</td>
<td>94</td>
<td>96</td>
<td>96</td>
<td>90</td>
<td>90</td>
<td>1.4</td>
</tr>
<tr>
<td>Serine</td>
<td>95</td>
<td>92</td>
<td>92</td>
<td>94</td>
<td>91</td>
<td>92</td>
<td>0.4</td>
</tr>
<tr>
<td>Threonine</td>
<td>92</td>
<td>91</td>
<td>89</td>
<td>91</td>
<td>87</td>
<td>88</td>
<td>0.6</td>
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<td>93</td>
<td>91</td>
<td>90</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Determined as cysteic acid derived from the conversion of each molecule of cysteine to one molecule of cysteic acid and each molecule of cystine to two molecules of cysteic acid.

The agricultural region in Western Australia where the lupins were grown is indicated in parentheses below the cultivar name: WH=Wongan Hills, CO=Coorow.
3. Results

There were significant differences in the ADMD, ACPD and ADE among the cultivars of *L. angustifolius* (Table 4). The AD of the older cultivar, Gungurru, was the highest of the of *L. angustifolius* cultivars. The mean ADMD of the new cultivars of *L. angustifolius* was 62.2% (range: 56.5% to 65.3%), while that of Gungurru was 66.3% (s.e.m. ± 0.95%). The mean ACPD of the new cultivars was 94.0% (range: 92.7% to 95.7%) while that of Gungurru was 96.8% (s.e.m. ±0.48%) and the mean ADE of the new cultivars was 73.7% (range: 69.6% to 76.3%) with Gungurru at 77.2% (s.e.m. ±0.72%). The ADMD and the ADE of *L. luteus* cv. Wodjil (70.0% and 79.9%, respectively) was significantly greater than that of all of the samples of *L. angustifolius*, though the ACPD was similar (93.8%) (Table 4).

Though overall there was not a significant difference in ADMD, ADCP and ADE between growing regions/conditions (location) or between cultivars, among the three cultivars that were grown at Katanning and Wongan Hills, there was a significant interaction between location and cultivar (Table 5). Kalya from Wongan Hills had greater ADs than the Kalya from Katanning, Tanjil from Wongan Hills had lower ADs than the Tanjil from Katanning, while Mandelup from Wongan Hills was not different from Mandelup from Katanning (Table 5).

There was a significant, inverse relationship (*P*<0.05) between NFE content of the kernel meals and ADMD (*R*²=0.63) and ADE (*R*²=0.66), but it was not significant for ACPD (*R*²=0.07) (Fig. 1). The mean amino acid digestibility across cultivars and amino acids was about 93%. Arginine generally had the highest AD (mean=98%) and cystine the lowest (mean=86%), (Table 6). The LSD (*P*=0.05) for the estimates of amino acid digestibility were about 0.5% except for methionine (2.4%), proline (1.6%) and tyrosine (1.9%). There was close agreement between average AD of all amino acids of a cultivar and its ACPD (*Y*=0.9851*X*+1.9507; *R*²=0.98). There was also a strong linear relationship between the amount of digestible protein in a kernel meal and the total amount of the digestible amino acids (*R*²=0.98) (Fig. 2).

4. Discussion

Formulating cost-effective feeds for the aquaculture industry, which have low inclusion levels of fishery-sourced feed ingredients such as fishmeal, relies on the provision of sound data showing the effectiveness of alternative protein sources. Information about the digestibility of these ingredients is a vital component of this data. In earlier digestibility and growth response studies with older cultivars of lupins, lupin kernel meals have been shown to be a useful protein source in feeds for both fish and shrimp. The cultivars chosen for this study are currently the most widely grown lupin cultivars in Australia, representing >80% of the national production.

The digestibility of the old cultivar of *L. angustifolius*, Gungurru determined in this study, was similar to that reported previously by Smith (1998): ADMD, 66.3% and 67%, respectively; ACPD, 96.8% and 94%, respectively; and ADE, 77.2% and 71%, respectively. Studies with silver perch using the same cultivar, Gungurru, were also similar, with ADMD, ADCP and ADE values of: 68%, 100% and 74% respectively (Allan et al., 1998). However, the digestibility of the new cultivars were generally slightly lower than that determined for Gungurru in this study, though the values were generally similar to those reported for Gungurru in the earlier study (Smith, 1998). The mean ADMD of the new cultivars was 62.2%; the ACPD was 94.0% and the ADE was 73.7%. Glencross and co-workers, working with rainbow trout, *O. mykiss*, have assessed the digestibility of 60 samples of lupin kernel meal derived from a range of new and old cultivars grown in different locations and growing seasons, including the kernel meals used in this study. Their results indicated that the digestibility of the lupin kernel protein could vary between 70% and 100% (Glencross et al., 2006). However, data identifying which cultivars had the low digestibility has not been reported at this stage. Such a large variation in the protein digestibility is in contrast with the observations of this study with *P. monodon* where the range in ACPD of all *L. angustifolius* cultivars was only 4.1%.

Generally, the ADs of the amino acids closely match the average ACPD of the cultivars in Experiment 2.
The AD of arginine was consistently greater, having an average AD of 98% while cysteine had the lowest (86%). The variability in the estimates of AD of the amino acids tended to be strongly influenced by the markedly higher estimates of AD from the Kalya sample. There was also greatest variability in the estimates of AD of methionine. Whether this was an analytical issue or a feature of the methionine peptide linkages remains to be resolved. However, there appears to be a close relationship between the digestible crude protein content of the kernel meals and the digestible amino acid content (Fig. 2), suggesting the robustness of this data. The general close equivalence of ACPD and average AD of amino acids seen in this study was also noted by Akiyama et al. (1989), who reported that with the Pacific white shrimp Penaeus (=Litopenaeus) vannamei, the average digestibility of amino acids in a soybean meal test diet was ~90% while that of crude protein was 89.9%. However, Akiyama et al. (1989) did not report on the AD of methionine or cysteine. In a study with silver perch, Allan et al. (2000) found the digestibility of amino acids in L. angustifolius whole seed meal was high with an average apparent digestibility of about 98%. As with the current study, they also found that cysteine had the lowest apparent digestibility (79.5%). Again, the reason for this low digestibility has not been explained.

The NFE component in the kernel meals comprises mainly carbohydrates. The carbohydrate is comprised predominantly of soluble and insoluble non-starch polysaccharides (oligosaccharides and dietary fibre, respectively) and negligible amounts of starch (reviewed by van Barneveld, 1999). The non-starch polysaccharides, such as dietary fibre, are poorly digested by monogastric animals (van Barneveld, 1999), fish (Glencross et al., 2003), shrimp (Akiyama et al., 1989; Smith, 2002) and freshwater shrimp (González-Péña et al., 2002). As the protein in the kernel meal is highly digestible, and as the lipid is also likely to be highly digestible (Merican and Shim, 1995; Glencross et al., 2002), the relatively low ADMD of the kernel meals (56.5% to 70.0%) is a reflection of the low digestibility of the NFE. This appears to be supported by the significant trend of decreasing ADMD with increasing NFE content (Fig. 1). It is interesting to note that the NFE did not appear to have any affect on ACPD (Fig. 1). However, the concentration range of NFE in the samples of L. angustifolius was quite narrow: 404 g kg⁻¹ to 469 g kg⁻¹ (as used), and this would also have been reflected in the NFE content of the test diets.

To estimate the AD of the NFE, the difference between the digestible energy (ADE × GE/100) of the kernel meals and the calculated DE derived from crude protein and total lipid was calculated. As the digestibility of lipid was not measured in this study, nor was it separated into its lipid classes, several assumptions were made: (a) total lipid in lupin kernel meal was comprised

<table>
<thead>
<tr>
<th>Lupin cultivar¹</th>
<th>NFE (g kg⁻¹)</th>
<th>Determined DE of LKM</th>
<th>Calculated DE from CP+lipid²</th>
<th>DE of NFE (difference)</th>
<th>Digestible NFE (g kg⁻¹)</th>
<th>AD of NFE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Kalya (KT)</td>
<td>451</td>
<td>14.9</td>
<td>12.5</td>
<td>2.4</td>
<td>141</td>
<td>31</td>
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<tr>
<td>Mandelup (KT)</td>
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<td>2.5</td>
<td>147</td>
<td>32</td>
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<tr>
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<td>449</td>
<td>16.1</td>
<td>12.9</td>
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<td>41</td>
</tr>
<tr>
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<td>422</td>
<td>15.6</td>
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<td>2.3</td>
<td>136</td>
<td>32</td>
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<tr>
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<td>2.1</td>
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<td>34</td>
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<td>Experiment 2</td>
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<td></td>
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<td></td>
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<td>416</td>
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<td>13.1</td>
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<td>87</td>
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<td>Wonga (WH)</td>
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<td>2.1</td>
<td>124</td>
<td>30</td>
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<td>Myallie (CO)</td>
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<td>14.7</td>
<td>12.3</td>
<td>2.4</td>
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<td>31</td>
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<td>15.3</td>
<td>1.0</td>
<td>59</td>
<td>19</td>
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</table>

¹All kernel meals were from cultivars of L. angustifolius, except for Wodjil that is a cultivar of L. luteus. The agricultural region in Western Australia where the lupins were grown is indicated in parentheses after the cultivar name: KT=Katanning, MG=Mingenew, WH=Wongan Hills, CO=Coorow.

²Calculated values are based on the following energy values: protein, 23.4 MJ kg⁻¹; lipid, 39.8 MJ kg⁻¹; NFE (=carbohydrate), 17.2 MJ kg⁻¹ (Cho et al., 1982), and assumed AD of lipid = 87% (Merican and Shim, 1995). Energy is reported on a MJ kg⁻¹ DM basis.
of both triacylglycerides (67%) and phospholipids (33%) (van Barneveld, 1999) and (b) that the digestibility of the triacylglycerides was 98% and phospholipids was 64% (Merican and Shim, 1995), giving a total lipid digestibility of 87%. The estimates of DE from NFE were found to be between 1.0 and 3.2 MJ kg$^{-1}$ of kernel meal (Table 7). It is interesting to note that the lowest value was for *L. luteus* cv. Wodjil. Assuming the NFE was all carbohydrate, and using a conversion factor of 17.2 MJ kg$^{-1}$ for carbohydrate (Cho et al., 1982), this equates to the energy provided by between 59 and 187 g of carbohydrate. Using these estimates, the AD of the NFE in *L. angustifolius* cultivars was calculated to vary between 22% and 41% (mean±s.e. = 31%±1.4%), while that of *L. luteus* cv. Wodjil was 19%. In *L. luteus* cultivar Wodjil, the NFE content and its AD appear to differ quite markedly from the *L. angustifolius* cultivars, suggesting its NFE composition might be substantially different.

The method for calculating ingredient digestibility has been the subject of discussion in recent literature (Forster, 1999; Bureau et al., 1999; Bureau and Hua, 2006). The equation that we have used is based on that reported by Pfeffer et al. (1995). All these equations are equivalent as they are derived from the same base equation proposed by Kleiber (1975). The difficulty in using them appears to be in incorporating into the calculation the contribution of the test ingredient to the concentration of a particular nutrient (or analyte) in the test diet. To be able to do this, the DM content of the test ingredient and that of the mixed ingredients (mash) of the reference diet need to be known. Another difference between the equation reported by Pfeffer et al. (1995) and the one advocated by Bureau and Hua (2006) lies in the use of either of two alternative parameters: the concentration of nutrient in the test diet (Pfeffer et al., 1995) or the concentration of nutrient in the reference diet (Bureau and Hua, 2006). Any differences in the estimates of ingredient AD are due to the errors inherent in the analysis of either of these diets. In addition to calculating the ADs using Pfeffer’s equation, we have also calculated the ADs using the equations proposed by Forster (1999) and by Bureau and Hua (2006), and we have obtained closely similar results.

In conclusion, there were a number of significant differences among the ADs of the new cultivars of *L. angustifolius* when used in diets for *P. monodon*. However, their values were broadly similar and similar to the AD reported for the older cultivar, Gungurru. The ACPD was uniformly high, with the average of 94.3% across 12 samples, and the AD of the amino acids was of a similar value. The sulphur amino acid methionine, showed the most variability, with most of the variability due to the AD of one particular cultivar (Kalya). Whether this was a hydrolysis/analytical artefact or a feature of the methionine peptide linkages remains to be resolved. The AD of cysteine, another of the sulphur amino acids, was the lowest of the amino acids at 86%. The general consistency of the AD results across the range of cultivars, which represent over 80% of the production of narrow leafed lupins in Australia, suggest that nutritionists and feed formulators can confidently use mean AD values for dry matter, protein and energy for kernel meals comprising random mixtures of the new cultivars.

**Acknowledgements**

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