CHAPTER 5

EXPERIMENT 4 : THE EFFECT OF SUGAR, WHEAT, DEGREE OF GRINDING AND METHODOLOGY ON THE DETERMINATION OF DIGESTIBLE ENERGY IN LUPINS AND WHEAT

5.1 INTRODUCTION

Experiments 2 and 3 demonstrated that some variability was associated with the use of chromic oxide for determining nutrient digestibility in diets containing lupins and kernels. Furthermore, the results of experiment 3 demonstrated that despite a highly significant ($P<0.001$) interaction between dietary energy source and retention of ileal digestible lysine. The techniques used to measure sites and extent of nutrient disappearance of lupins were unable to detect any difference in either ileal (lysine or energy) or faecal (energy) disappearance of nutrients to help explain the interaction.

Experiment 1 demonstrated the precision with which the total collection method could identify smaller changes in nutrient digestibility compared with chromic oxide. It was concluded that aspects of energy digestibility could be effectively explored using the current method of total collection, and experiment 4 was developed using this rationale and the recognition that lupins contain energy-rich compounds which are digested by microbes in the large intestine, leading to a lower efficiency by which digestible energy is utilised.

It was hypothesised that the interactions reported in experiment 3 could, at least in part, be induced by changes in the relative extent and efficiency of energy digestibility when lupins are included in wheat- vs sucrose-based treatments.

Experiment 4 was designed to consolidate the findings of experiments 1 and 3 by studying the following aspects of digestibility:

- The effect of dietary energy source on estimates of digestible energy using methods of total collection

- To compare total collection vs indigestible marker methods for determining digestible energy, so as to further assist in interpretation of the digestibility data generated by experiment 3 where chromic oxide was used.

- To finely crush lupin and wheat in all dietary treatments, allowing for a cross experiment study of the effect of fineness of grinding on gross energy digestibility in wheat and lupins, and to determine whether this has any effect on estimates of gross energy digestibility in sugar- or wheat-based diets.

- To permit a comparison of two dietary levels of lupins to assess the effect of inclusion level on gross energy digestibility.

5.2 SPECIFIC MATERIALS AND METHODS

5.2.1 Animals and Management

The experiment was of a randomised block design, containing 4 dietary treatments and 6 replications in part A (7-day total collection of faeces) and 2 dietary treatments with 5 replications of diet 1 and 6 replications of diet 2 in part B (assessment of digestible energy using chromic oxide and partial collection of faeces).

All pigs were selected and prepared, and part A of the experiment was conducted as documented in Chapter 3.

At the conclusion of the 7-day total collection, pigs offered dietary treatments 1 and 2 were moved to individual grower accommodation as documented in Experiment 3. The Part A dietary treatments were offered in the grower accommodation for two days after completion of Part A, at which time chromic oxide was added(2g/kg), and the dietary treatments were continued on an *ad libitum* feeding regime for five complete days prior to sampling of faeces.

Calculations, laboratory assays and other procedures are documented in Chapter 3.

5.2.2 Feed Ingredients and Diet Formulation

The dietary treatments used in this experiment contained finely crushed lupins in both sucrose-and wheat-based diets. A wheat treatment was included to permit re-definition of the digestible energy content of this ingredient when finely crushed (Table 17)

Table 17. Composition (g/kg, air-dry basis) of diets used in experiment 4

	Diet number			
Components	1	2	3	4
Lupin	-	360	500	500
Soyabean	-	-	-	-
Wheat	942.5	587.5	447.5	-
Sugar	-	-	-	446.9
Soyabean oil	15	15	15	15
Dicalcium phosphate	30	30	30	30
Mineral premix	3.5	3.5	3.5	3.5
Vitamin premix	1.5	1.5	1.5	1.5
L-Lysine HCl	5.0	-	-	-
Dl-Methionine	2.0	2.0	2.0	2.6
Fuzone 200	0.5	0.5	0.5	0.5
Composition				
GE (MJ/kg)	6.10	16.79	17.05	17.04
Dry matter	904	914	918	963
Total alkaloids	-	0.06	0.09	0.09

Diets 1 and 2 included chromic oxide when used in Part B of experiment 4 and are referred to from this point as diets 1 and 2 (Cr) respectively

Chromic oxide was added to diets 1 and 2 prior to commencement of Part B of the experiment.

5.3 RESULTS

The gross energy digestibility (GED) and the digestible energy (DE) content of each experimental ingredient contained in treatments of Part A (treatments 1,2,3 and 4) and Part B (treatments 1(Cr) and 2(Cr) are reported in Table 18. Columns showing SEM and significance are relevant to either part A or part B of the experiment and superscripts in this table should not be used to make comparisons between Part A and Part B.

Table 18. Faecal gross energy digestibility (GED)[1] and digestible energy (DE)[2] of finely crushed lupin-seed meal (lupin)(in sucrose- and wheat-based diets) and wheat when assessed by total collection and by indigestible marker

Diet number	1	2	3	4			1(Cr)	2(Cr)		
Diet description	wheat	36% lupin+ wheat	50% lupin+ wheat	50% lupin+ sugar	SEM	Sig	wheat	36% lupin+ wheat	SEM	Sig
GED (test ingredient)[1]	0.82^b	0.80^b	0.79^b	0.73^a	0.020	*	0.86^a	0.69^b	0.022	***
DE (MJ/kg air-dry)[2]	14.2^b	14.5^b	14.4^b	13.2^a	0.29	*	14.0^a	12.5^b	0.40	*

GED (test ingredient) = digestibility of gross energy in the experimental protein ingredient, calculated by difference using GED of sugar = 1.0, GED of wheat as found for diet 1 and GED of other ingredients = 0.9

*Sig = Significance of differences between means: NS=P>0.05, *=0.01<P<0.05, **=0.001<P<0.01, ***=P<0.001.*
Different superscripts in the same row denote diet differences; (P<0.05)
SEM = LS Mean SE within experiment
Statistical references in the table above address part A and B treatments separately

Both the GED and DE of finely crushed lupins was significantly ($P<0.05$ and $P<0.01$ respectively) improved when assessed by total collection (part A methodology) in wheat- compared to sucrose-based diets.

There was no effect ($P>0.05$) of lupin inclusion level on the determination of either GED or DE in wheat-based diets by total collection.

The standard errors for part A and part B treatment means are similar indicating that a equivalent confidence can be given to the results derived from each technique.

In order to make comparisons between experiment 4 (parts A and B) and experiment 1, the treatment in which wheat was the test ingredient was adopted as a positive control. The statistical comparison of the GED of wheat in these three instances is reported in Table 19. No difference ($P>0.05$) was found in the GED of wheat between experiment 1 (0.86) and experiment 4 part A (0.82), indicating that degree of grinding had no effect on determination of GED by total collection. There was no difference ($P>0.05$) between methods of total collection and indigestible marker between parts A (0.82) and B (0.86) of experiment 4 for determination of GED in wheat.

Table 20 is a statistical comparison of the GED of lupin-based treatments from experiment 1 and parts A and B of experiment 4. Determination of GED of finely crushed lupins in wheat-based diets was found to be significantly ($P<0.01$) lower when assessed by indigestible marker (0.69) than when assessed using total collection (0.80).

The effect of particle size was examined by comparing the GED of lupins in sucrose-based diets by total collection as reported in experiments 1 (treatment 2)(0.68) and 4 (treatment 4)(0.73). This represents a 7% improvement in digestibility by decreasing the particle size of the same sample of lupins and almost reached statistical significance ($P=0.07$). This may be an important finding and its implications are worthy of discussion.

Table 19. Statistical comparison of the effect of methodology and degree of grinding on determination of faecal gross energy digestibility (GED) of wheat within and across experiments

Experiment number ⇨		1	4	4
⇩	**Diet number**	4 (wheat)	1 (wheat)	1(Cr) (wheat)
1	4 (wheat)	-	NS	-
4	1(wheat)	NS	-	NS
4	1(Cr)(wheat)	-	NS	-

*Sig = Significance of differences between dietary treatments within and across experiments: NS $P>0.05$, * $0.01<P<0.05$, ** $0.001<P<0.01$, *** $P<0.001$.*

Table 20. Statistical comparison of the effect of methodology and degree of grinding and dietary energy source on determination of faecal gross energy digestibility (GED) of lupins within and across experiments

Experiment number ⇨		1	4	4	4	4
⇩	**Diet number**	2	4	3	2	2(Cr)
1	2	-	NS	-	-	-
4	4	NS	-	**	-	-
4	3	-	**	-	NS	-
4	2	-	-	NS	-	**
4	2(Cr)	-	-	-	**	-

Experiment 1, Diet 2 = 50% coarse lupins (in a sugar-based diet) Experiment 4. Diet 4 = 50% fine lupins (in a sugar-based diet)
Experiment 4. Diet 3 = 50% fine lupins (in a wheat-based diet) Experiment 4. Diet 2 = 36% fine lupins (in a wheat-based diet)
*Experiment 4. Diet 2(Cr) = 36% fine lupins (in a wheat-based diet) Sig = Significance of differences between dietary treatments within and across experiments: NS=$P>0.05$, *=$0.01<P<0.05$, **=$0.001<P<0.01$, *** =$P<0.001$.*

5.4 DISCUSSION

Each of the four aims of Experiment 4 were fulfilled by the chosen protocols. Dietary energy source did affect the overall digestibility of energy in lupins, a finding which is contrary to that normally anticipated. This is particularly true in view of the findings of Just (1982), who reported that a 1% increase in dietary fibre depressed gross energy digestibility by 3.5% and who also documented an increase in the proportion of energy disappearing from the hindgut as dietary fibre increases. These results are also contrary to the findings of Experiment 3 in which the faecal digestibility of energy in soyabeans was lower on the wheat-based treatment. Studies using sucrose-based diets have been reported (Beech *et al.*, 1990), in which both energy and fat retention increased with greater addition of sucrose to wheat diets.

The primary difference between the current observations and those reported by Just (1982) (and others) is that in the current experiments, sucrose diets are used as the control rather than the cereal-based diets. It is hypothesised that the viscosity of digesta produced by the ingestion of polysaccharide materials in lupins increases the viscosity of digesta in sucrose- more than in wheat-based diets and that this hampers the efficacy of enzymatic and bacterial action on the energy-containing components of lupins.

There was no effect of varying lupin inclusion from 36% to 50% of the experimental diets on energy digestibility. It is concluded that in practical terms this sample of lupins was either free of anti-nutritional factors which may hamper digestibility of energy or that the inclusion at which digestibility is hampered is below 36% or greater than 50%.

Comparing wheat across experiments by statistical means was a useful method for identifying the influence of technique and fineness of grinding on gross energy digestibility in lupins. Using this approach, it was found that chromic oxide produced a lower estimate of energy digestibility than total collection. This may be due to variability in either marker recovery or sampling, but in either case, indicates that the method of total collection is preferable for determining digestible energy in lupins.

In line with expectations, finely ground lupins tended to yield a higher digestible energy value than those more coarsely crushed, and whilst this difference was not statistically significant ($P=0.07$), the magnitude of the difference is important for feed formulators. Owsley *et al.*, (1981) reported improved ileal and faecal energy digestibility by pigs offered sorghum as particle size was reduced. The effect was significant ($P<0.05$) between all tested levels of particle size at the terminal ileum and a significant difference prevailed between the most and least coarse treatments throughout the whole digestive tract despite the effect of hindgut activity. A simlar finding was reported by Sauer *et al.*, (1977) for amino acid digestibility of wheat, and Hermann (1987) found that digestibiltiy was improved in wheat and barley as particle size decreased but reported no improvement under the same circumstances for field beans, lupins or soyabean meal.

With reports such as these in the literature, it is difficult to explain the basis for current Australian recommendations that lupins are suitable for pigs when coarsely crushed. The present study shows clearly that improved energy and most probably amino acid digestibility are the benefits of finely crushing lupins. The improvement in GED reported in this experiment may add several dollars per tonne to the value of lupins when their value is assessed relative ot other feedstuffs for use in commercial pig production.

CHAPTER 6
GENERAL DISCUSSION AND CONCLUSIONS

The current studies were motivated to explore a possible interaction (Fernandez and Batterham, 1992 and Batterham, unpublished) between dietary energy source and lupins relative to soyabean meal on pig performance. The hypothesis was made that digestible nutrient utilisation and retention by pigs offered these protein ingredients should be closely reflected by their digestible nutrient intake, particularly because the ileal digestibility and availability of lysine in soyabean meal are similar (0.89 and 0.90 respectively) (Batterham, 1994). Because lupins are an unheated protein source the potential for absorption of denatured and unavailable lysine (as shown by Van Barneveld *et al.*, 1991 for field peas) is removed.

The fundamental value in accepting this hypothesis, is that a rapid and comparatively inexpensive method for assessing the relative availability of nutrients in protein ingredients could be adopted. In this instance lupins and kernels were assessed relative to soyabean meal, however numerous protein sources have been assessed at Wollongbar using techniques similar to the method stated in section 4.4 of this thesis. The current experiments have demonstrated that using sucrose as a dietary energy source does influence the efficiency with which lysine from lupins and kernels is utilised and implies that the results of other experiments which have assessed ingredients in surcrose-based diets may be influenced by a similar mechanism.

The sucrose technique relies upon the initial assumption that lysine is the first nutrient limiting growth, yet it was revealed in Experiment 4 that both the site and overall extent of energy digestibility can be affected by energy source. It was considered likely that differences in available energy between treatments were likely to have contributed to the interaction. This may have been resolved had resources permitted additional treatments containing higher lysine levels to clarify that lysine rather than energy was in fact the first nutrient limiting utilisation.

Simplicity and relatively low costs are virtues of comparative nutrient availability studies using sucrose-based diets. However this study demonstrates a weakness in the practical value of the technique when used for lupins and kernels. The source of the reported interaction must be the subject of further studies if the sucrose technique is to be used with confidence for a wide range of ingredients under practical conditions. If the source of the interaction is found to be related to compounds in lupins such as non-starch polysaccharides, a greater understanding of how they interact with sucrose will assist in determining which protein ingredients may be confidently assessed in sucrose-based diets.

No interactions were found to indicate that amino acid digestion was altered by dietary energy source, despite a significant depression in retention of ileal digestible lysine recorded for lupins and kernels in the wheat- rather then the sucrose-based diets. Experiment 4 was devised on the hypothesis that the lower lysine retention observed in pigs offered wheat-based lupin treatments may in some way have been related to a difference in the extent of energy digestibility of lupins as a result of differing dietary energy source.

Experiment 4 revealed that dietary energy source does affect energy digestibility in lupins, and that determination of energy digestibility of lupins in sugar-based diets is inappropriate for subsequent application to commercial-style wheat-based diets. The implications of this concern are that other ingredients may be similarly influenced by dietary energy source, and care must be exercised in adoption of estimates for which verification in conventional diets is absent. A simple calculation in which the estimated digestible energy content of lupins assessed in wheat-based diets (converted back to a coarsely-crushed equivalent), is applied to diet 5 in Experiment 3, adds almost 1.1 to the efficiency with which digestible energy intake is utilised for empty body weight gain (DEI/EBW) by pigs. This effectively removes the improved performance of pigs offered the wheat-based treatments for this parameter.

Experiment 4 also indicated improvements in energy digestibility by finely crushing lupins, and this topic is suggested by the author for subsequent studies. This is particulary applicable to the current use of lupins in Australia, in which coarse crushing is the predominant practice.

Despite expectations to the contrary, dehulling lupins was of no benefit and may even be slightly detrimental to both retention of digestible nutrients and the efficiency of conversion of digestible nutrients to empty body weight gain. The implication that hull components are of no detriment to the nutritive value of lupins and possibly other ingredients has important commercial ramifications and enhances the economic value of these ingredients to commercial pig production.

Accurate estimates of nutrient digestibly within and between feedstuffs is essential for practical diet formulation. In this respect it is particularly difficult to establish precise estimates for lupins as there is high variation both within and between cultivars. It is known that wide variation in oligosaccharide conentration also exists within and between cultivars, and it is hypothesised that this may influence energy digestibility, particulary in experiments containing young pigs with immature fermentation capacity.

Many methods for determining nutrient digestibility and by extension nutritive value of feedstuffs are reported in the literature. These variations are essential for expanding our awareness and knowledge of factors influencing nutrient digestibility, and in the opinon of the author should be actively encouraged at a research level. The negative consequence of this diversity is most evident at the interface between research and applied nutrition, in which it is often difficult to identify estimates which provide representative comparative measures of nutritive value between feedstuffs. As an initial step in producing applied comparative estimates of nutritive value, there is a need for a standardised method of comparing nutrient digestibility between feedstuffs. The need for this interface has been recognised by Rhône-Poulenc Animal Nutrition and a dedicated on-going program has been developed using identical methodology for amino acid digestibility by pigs across many feedstuffs.

In conclusion, it is apparent that whilst the use of sucrose-based diets does provide a simplified method for assessing the nutritive value of feedstuffs, the technique requires verification with a wide variety of ingredients. To date this verification has not occurred, and the present study has provided evidence of interactions between both retention of ileal digestible lysine and digestibility of energy in lupins and dietary energy source. It is suggested that this issue may be further clarified by;

a) comparative assessment of nutrient digestibility of ingredients in sucrose- and wheat-based diets. It is recommended that until this has been achieved, digestible energy estimates should only be adopted from experiments in which the experimental diets resemble practical application.

b) assessment of ileal digestibility of amino acids using techniques associated with low variability

c) developing growth experiments which compare dietary energy source and in which identical digestible nutrient levels are adopted across treatments. Furthermore, addititional treatments are required to confirme that the nutrient which is assumed to be limiting performance is that actually doing so.

Appendix 1

Acid detergent fibre (ADF) and Neutral detergent fibre (NDF) digestibility for each experimental protein in dietary treatments from experiment 3

The ileal and faecal digestibility of ADF and NDF in wheat was calculated using the results of experiments 1 and 2

Treatment number	ileal ADFD	faecal ADFD	ileal NDFD	faecal NDFD
1	45.1	88.1	58.6	94.9
1	36.0	83.2	54.0	95.5
1	27.5	93.6	30.2	98.5
1	missing plot	85.5	49.8	95.5
1	35.7	82.8	61.5	95.8
2	0	67.1	0	80.8
2	0	missing plot	0	54.7
2	0	61.7	0	70.2
2	0	51.8	5.4	71.0
2	0	42.0	2.6	64.7
2	missing plot	59.9	missing plot	75.5
3	0	82.7	71.7	97.1
3	8.7	88.2	80.2	98.0
3	0	89.7	75.1	98.5
3	0	86.9	73.8	98.0
3	0	missing plot	70.5	missing plot
3	missing plot	56.7	88.3	93.2
4	47.1	98.4	58.4	97.5
4	-35.8	87.0	missing plot	72.8
4	44.3	98.2	60.7	90.2
4	missing plot	75.2	100	60.4
4	23.0	62.3	missing plot	62.8
4	38.4	75.4	51.7	69.8
5	-5.9	32.8	-7.3	47.8
5	-5.9	30.7	-0.9	48.3
5	28.7	56.7	52.5	64.2
5	-5.9	34.1	-3.1	49.5
5	27.9	31.0	36.7	50.0
6	-29.8	[illegible]5.4	-30.0	82.1
6	-29.8	29.7	-30.0	83.1
6	-29.8	38.7	-12.3	80.6
6	32.2	38.9	89.3	86.2
6	-13.8	25.8	73.2	84.9
6	-29.8	missing plot	9.6	81.4

In assigning mean values to dietary treatments, negative digestibility coefficients were considered "0" and values greater than 100% were considered 100%. Obvious outlying values are presented as missing plot statements

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