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Lupins for poultry

Mechanical and enzymatic improvement of lupins for broiler and layers

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Lupins in poultry diets: Mechanical and enzymatic improvement of lupins for broiler and layers
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Foreword

Australia produces 87% of the world's lupins which are an excellent source of protein and energy. While the world faces a shortage in plant protein meals, feed manufacturers and poultry producers cannot use more than about 5% lupins in broiler and 7% in layer diets. The main reason is because lupins contain complex cell-wall polysaccharides (33%) that are indigestible. The main component of cell walls is pectin which varies from 33 to 71%. Pectin increases the viscosity of digesta in the bird's digestive tract, increases water intake and wet droppings and, consequently, reduces food intake and efficiency of feed utilisation. Poultry cannot digest pectin because they don't secrete the appropriate enzymes so their use of lupins is limited. However, by treatment of lupins with exogenous enzymes and mechanical heat treatment (expansion), it might be possible to increase the nutritive value of lupins for poultry. Three experiments were carried out to investigate whether exogenous pectinases and expansion might increase the nutritive value of lupins for broilers and layers.

This publication gives the data obtained from a two-year investigation on 1) the inclusion of 10 and 20% whole and dehulled lupins using pectinase (endo-polygalacturonase) for layers, 2) enhancement of pectinase activity through expansion of lupins before including them at 10 and 20% in the diets for broilers and, 3) *in vitro* breakdown of pectin in dehulled lupins by two pectinases, endo-polygalacturonase and pectin methyl esterase.

This project was funded from industry revenue which is matched by funds provided by the Australian Government, RIRDC and Agriculture Western Australia.

This report, an addition to RIRDC's diverse range of over 1200 research publications, forms part of our Chicken Meat and Egg R&D program, which aims to support sustainability and profitability in the chicken meat and egg industry by focussing research and development on those areas which enable the industry to become more efficient and globally competitive and which will assist in the development of Australian industry and product images.

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Peter O'Brien
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Abbreviations

AME	Apparent metabolisable energy
L. angus	Lupinus angustifolius
ANOVA	Analysis of variance
CWP	Cell-wall polysaccharides
Endo-PG	endo-polygalacturonase
Exo-PG	exo-polygalacturonase
LSD	Least significant difference
NSP	Non-starch polysaccharides
PG	Polygalacturonase (endo)
PME	Pectin methyl esterase
sem	Standard error of mean
var	Variety

Contents

Executive Summary

The first objective of this project was to improve the nutritive value of lupins and increase the inclusion rate of whole and dehulled lupins from below 10% up to 20% in broiler and layer diets without compromising the production performance. The second objective was to reduce the problem of wet droppings by using enzymes and expansion to treat lupins. In the main, these objectives were accomplished. Three experiments were conducted to evaluate the potential of specific enzymes and expansion (mechanical treatment) to breakdown pectin and hence the lattice that constitutes the cell walls of lupins. In the first experiment we investigated whether pectinase would allow lupins to be better used by layers knowing that pectinase would breakdown approximately 10% of the pectin present in cell walls. Next we studied whether mechanical treatment might enhance the activity of pectinase and allow a greater breakdown of pectin. Third, we studied whether a combination of two pectinases might give even greater breakdown of pectin and cell walls.

Determination of optimal dose of pectinase for laying hens

Two hypotheses were tested in this experiment. First, egg layers should benefit more than broilers from pectinase supplementation of lupins because their digestive tract is more developed and they may respond to different levels of pectinase, either higher or lower. Second, pectinase should allow feed manufacturers to use 20% whole and dehulled lupins in the diets without compromising production performance or the dry-litter condition. A 2 x 2 x 4 complete factorial experiment (whole and dehulled lupins, 10 and 20% lupin inclusion and 0, 0.6, 0.8 and 1.0g pectinase/kg diet, specifically, polygalacturonase (PG), was conducted for 10 weeks.

The lowest dose, 0.6g/kg diet, of PG was the most effective dose for reducing water intake, wet droppings, viscosity of the digesta, soiled eggs, and food conversion ratio. In addition, this dose was also the most effective for increasing the digestion of dry matter, metabolisable energy of the diet, and egg yield and egg-shell thickness. There were no interactions between the percentage of lupins in the diets, whole and dehulled lupins and the amount of pectinase. The higher doses, 0.8 and 1.0g/kg diet, improved several parameters but not all and had detrimental effects on bird performance and wet droppings.

Layers performed slightly better when the lupins were dehulled. Increasing whole and dehulled lupins to 20% in the diet slightly increased water intake, wet droppings and soiled eggs and reduced metabolisable energy of the diet with or without PG supplementation.

These results suggest that a PG dose of 0.6g/kg diet improves the nutritive value of whole and dehulled lupins for laying hens. This level of PG treatment should allow feed manufacturers to include 20% lupins in diets for laying hens and, at the same time, control wet droppings to a manageable level.

Expansion of lupins and PG treatment for broiler chickens

The hypothesis for this experiment was that expansion of lupins should improve the activity of PG by increasing the surface area available for PG to degrade the pectins in cell walls. Consequently, this should increase the metabolisable energy content of the diet and give a significant improvement of the growth of broilers. A 2 × 2 × 2 × 2 complete factorial experiment (no expansion or expansion, whole or dehulled lupins, 10 or 20% lupins, 0 or 0.8g PG/kg diet) was carried out for 11 days. The results showed that expansion had no beneficial effect on production. The combination of expansion + PG caused a greater breakdown of cell walls and pectin but this was not reflected in the performance of

the birds. Heat generated during expansion may have solubilized some of the insoluble fibres, induced Maillard reactions and increased water-holding capacity. PG on its own was beneficial as has already been shown. It also reversed the adverse effects of expansion more so for birds fed 10% whole and dehulled lupins than their corresponding 20% inclusion.

These results highlight the significance of pectinase treatment of lupins for broilers. PG allows an inclusion of 10% whole and dehulled lupins without significant losses in productivity or an increase in wet droppings. Broilers may even tolerate a 20% inclusion rate without significant losses in productivity but there is likely to be an increase in wet droppings which may or may not be tolerable depending on producer circumstances.

Treatment of dehulled lupins with two pectinases *in vitro*

The hypothesis tested was that a combination of two pectinases, polygalacturonase (PG) and pectin methyl esterase (PME), would break down much more pectin than PG on its own. In addition the enzyme combination should reduce the water-holding capacity and viscosity of dehulled lupins more than PG on its own. There was unequivocal support for these hypotheses. The combination of PG and PME broke down 50% of pectin and 27% of the cell walls compared with only PG where 21% of the pectin and 13% of the cell walls were broken down. All other measurements, viscosity, water-holding capacity, molecular weight of pectin, long chains of pectins, showed that the combination of enzymes was much more effective than PG on its own.

By using these two pectinases it should be possible to make substantial improvements in breaking down cell walls and hence improving the nutritive value of lupins for broilers and layers. In addition because water-holding capacity is reduced it is likely that wet droppings may also be reduced.

1. Introduction

Feed manufacturers, nutritionists and poultry producers in Australia are seeking cheaper ingredients that can replace expensive, imported protein meals such as soybeans. The price of soybean has increased two to three times since animal protein meals have been banned from use in ruminant diets in recent years. Lupins are most important legume produced in Australia and the annual production amounts to 1.5 million tonnes, worth over $350 million. The nutritional value of lupins appears slightly less than that of imported soybean meal, for example protein is less (32 vs 42%) but there is slightly more fat and fat (6 vs. 4%). Importantly, lupins cost one-third the price of soybean and are potentially good sources of protein and energy for poultry. However, the metabolisable energy of lupins for broilers is 30% lower than that of soybean. As a consequence, the use of lupins is limited to 5% in broiler and 7% in layer diets.

The main reason that lupins have a low metabolizable energy is that they contain a high proportion of cell-wall material, mainly pectins, which exist in both the hull and kernel. Pectins increase water intake and, consequently, wet droppings because of their high water-binding capacity. They increase viscosity of digesta in the intestinal tract and this is responsible for inhibiting the digestion of the nutrients. This leads to low growth and poor food conversion ratio and low utilisation of dietary nutrients. Increase of water intake causes a high incidence of wet droppings, which leads to wet litter, odour problems, and outbreaks of coccidiosis. However, pectin can be broken down by an enzyme, pectinase, which break downs the molecular bonds that exist between the basic units of the pectin chains. This breakdown reduces viscosity, prevents excessive water intake and hence wet droppings, and it helps in digestion of the nutrients. We have conducted three experiments in the last eight years and have shown that pectinase gives small but consistent improvements in the nutritive value of whole and dehulled lupins for broilers.

However, pectinase only hydrolyses about 11% of the pectins because they occur within a complex cell-wall network in lupins. One way to increase the response to pectinase is to increase the surface area of the cell-wall materials by subjecting lupins to mechanical and/or thermal processes such as extrusion or expansion. Extrusion is effective at breaking down cell walls but, because of the extreme pressures and low throughput, it is a high-cost operation and is unlikely to ever become economic. Expansion is often called low-cost extrusion and, if it has a similar efficiency to extrusion for disrupting cell-wall networks, it is likely to be cost effective.

Expansion may have other benefits besides making pectinase more effective at breaking down cell walls. It may destroy anti-nutritional factors. There are at least nine anti-nutritional factors (alkaloids, saponins, tannins, trypsin and chymotrypsin inhibitors, phytate, glycosyl flavanol, oligosaccharides and uric acid) in lupins that may reduce food intake and possibly digestion. Expansion may destroy some or all of these.

Therefore, the aim of this work was to expand lupins to increase the surface area available for the pectinase to degrade the pectins in cell walls, and thereby release some of the highly digestible nutrients. In addition we expect that expansion should destroy some or all of the anti-nutritional factors and allow an increase in food intake and possibly digestion.

Another major problem in breaking down the pectins by pectinase, polygalacturonase (PG), is presence of methyl ester radicals attached to the carbon six atom along the pectin chain. The methyl esters block the binding sites of PG from breakdown the glycosidic bonds. These radicals can be only removed by a specific pectinase, pectin methyl esterase (PME). Our initial investigations were focused on whether we could achieve a more complete breakdown of pectin by a combination of two enzymes (PG + PME) rather than just PG alone.

As alluded to above, PG produces small but consistent improvements in the nutritional value of lupins for broilers. It might be expected that adult birds with better-developed digestive tracts might digest lupins better than broilers so we investigated whether treating lupins with PG would be beneficial for laying birds.

2. Objectives

To improve the nutritional value of whole and dehulled lupins so that they can replace imported and expensive soybean meal in diets for broilers and layers, with major savings in feed costs. To destroy the thick cell walls and their main anti-nutritional factor, pectic substances, by expansion and enzymatic treatment. To devise ways to increase the proportion of whole or dehulled lupins up to 20% in diets for both broilers and layers by the above treatments without compromising performance.

3. Background

The main anti-nutritional factor in whole and dehulled lupins is the thick cell walls that surround the nutrients that are highly digestible. Intact cell walls are responsible for poor growth of broilers, wet droppings and low metabolisable energy of the diet. If these cell walls can be broken down, the digestible nutrients can be released and used by the bird. Cell walls can be destroyed by three methods, mechanical, thermal, and enzymatic treatments.

Expansion of lupins under mechanical pressure and temperature followed by pectinase enzyme treatment will collapse the cell wall networks and their main content of pectic substances. Such treatments will reduce the viscosity of pectin and its high water-binding capacity and lead to a reduction of wet droppings by broilers. Ultimately these treatments will improve the metabolisable energy of the diet and lead to increases in growth and feed conversion efficiency.

Clearly, these improvements benefit feed manufacturers and poultry producers because they will be able to include more than 10% lupins in broiler and egg layer diets without compromising production or increasing the incidence of wet droppings. Replacing half of the imported soybean with lupins will save $22/tonne of diet. This will also aid lupin exports to Asian and Middle East poultry industries.

4. The Optimal Dose of Pectinase in Lupin-Based Diets for Laying Hens

4.1 Introduction

Despite lupins being a rich source of protein and energy and locally available throughout the year, feed manufacturers in Australia are unable to incorporate more than about 10% of whole or dehulled lupins in layer diets. Most feed manufacturers keep lupins to 7% or less. This is because lupins contain considerable amounts of indigestible and complex cell-wall carbohydrates known as non-starch polysaccharides (35%, Evans *et al.*, 1993; Chesson, 1993; Annison and Choct, 1993). These polysaccharides mainly consist of pectic substances that cannot be digested because poultry lack specific enzymes to hydrolyse them into simple sugars.

Furthermore, pectins increase the viscosity of the digesta in the intestine and this interferes with digestion and absorption of the nutrients. They also increase water-holding capacity of the digesta and increase water intake by the bird. This leads to poor weight gain, low metabolisable energy of the diet, wet droppings and dirty eggs. However, treating lupins with pectinase, specifically polygalacturonase (PG), can reverse most of these effects primarily by breaking down the pectin chain (Ali, 2003). A few reports have also shown that pectinases improve the nutritional value of feed for layers but the optimal dose has not been determined (Burnett, 1965; Patel and McGinnis, 1980).

The optimal dose of PG (0.8g/kg diet) has been established for lupin-based diets fed to broilers (Ali, 2003) but we need to determine the optimal dose of PG for egg layers. PG is likely to be of greater benefit in layers than broilers because the digestive system of adult hens is more developed and mature than that of young broilers. This may allow greater amounts of lupins in layer than in broiler diets.

4.2 Hypothesis

The hypotheses were:
1. Egg layers are expected to benefit from dietary pectinases (PG) in a similar way to broilers but, because their digestive tract is more developed, they may respond to different levels.
2. PG should allow feed manufacturers to use 20% whole and dehulled lupins in layer diets without compromising production performance or the dry-litter condition of the layers.

4.3 Materials and methods

4.3.1 Experimental design

Whole and dehulled lupins were treated with four concentrations of PG (0, 0.6, 0.8 and 1.0g/kg diet). The effectiveness of PG was tested by measuring food and water intake, weight gain, food conversion ratio, digestibility of dry matter, apparent metabolisable energy of the diet, digesta viscosity, moisture content of faeces, egg yield and quality (shell thickness, yolk colour and Haugh unit) and numbers of soiled eggs.

4.3.2 Animal ethics, housing, diet formulation, feeding and data collection

The animal ethics committees of the University of Western Australia and Agriculture Western Australia have approved this study. Health and husbandry practices compiled with the *Code of Practice for the Welfare of the Domestic Fowl* issued by the *Australian Bureau of Animal Health in 1983.*

Two hundred and forty hens (20-weeks old) of brown Hy-Line were sourced from Swan Valley Egg Farm and housed in metabolism cages in the layer shed at Medina Research Centre. The hens were randomly distributed to individual cages (0.4 x 0.4 x 0.4m^3 per hen). During the experimental period, the temperature was maintained at 22°C, the relative humidity at 55% and photoperiod was provided from 04.00 to 20.00h to give 16h light. The water misters (foggers) became operational for 45 second every 5 minutes when the temperature exceeded 26°C. All the cages were fitted with individual feeders and drinkers. Feed and water were supplied *ad libitum* throughout the experiment and the diets were fed in mash form.

The experimental diets were fed to the hens for 11 weeks (1 week adaptation to the cages + 10 weeks experimentation). The diets were formulated to be isocaloric and isonitrogenous to meet all nutrient requirements of laying hens on the basis of the standards of Standing Committee on Agriculture, Australia (SCA, 1987) (Table 4.1).

Table 4.1. Composition of the experimental diets

Ingredients	Whole lupins (%)		Dehulled lupins (%)	
	10	20	10	20
Wheat 13%CP	64.3	58.0	66.8	61.5
Lupins	10.0	20.0	10.0	20.0
Soybean meal 46%CP	4.7	3.0	3.0	1.0
Meat & bone meal 48%CP	10.4	8.0	10.1	5.5
Vegetable oil	1.3	1.9	0.5	1.05
Limestone fine	8.7	8.5	9.0	9.7
Di-calcium phosphate	0.0	0.0	0.0	0.6
Salt (iodised)	0.1	0.1	0.1	0.2
Sodium bicarbonate	0.1	0.1	0.1	0.1
DL-Methionine	0.2	0.2	0.2	0.2
Choline chloride 75%	0.1	0.1	0.1	0.1
Commercial layer premix[#]	0.3	0.3	0.3	0.3
Calculated analysis of dietary nutrients and energy				
AME (MJ/kg)	11.6	11.6	11.6	11.6
Crude protein	17.0	17.4	17.0	17.0
Calcium	4.3	4.0	4.4	4.3
Available phosphorus	0.5	0.4	0.5	0.4
Methionine	0.4	0.4	0.4	0.4
Lysine	0.8	0.8	0.8	0.7

Pectinase was added to each diet at levels 0, 0.06, 0.08 and 0.1%. Xylanase was added 0.02% to each experimental diet. Vitamin-mineral premixes provided per kg diet: Vitamin A, 9,000 IU, vitamin D_3, 2,750 IU; vitamin E, 50 IU; menadione 2.5 mg, vitamin B_1, 2.5 mg; vitamin B_2, 6.6 mg, vitamin B_{12}, 0.025 mg; niacin 45 mg choline chloride, 500 mg; d-pantothenic acid, 12 mg; pyridoxine, 5 mg; biotin 0.2 mg; folic acid, 2 mg; ethoxyquin, 100 mg; manganese oxide, 62.6 mg; zinc oxides, 5 mg; farrous sulfate 68.2 mg; copper sulfate, 4.4 mg; potassium iodine, 1.1 mg sodium selenite 0.10 mg.

The xylanase of 0.2g (Rovabio^{TM} xylanase Excel AP, 2000units/g) obtained from Bio-John Pty Ltd Perth, was included in all the diets containing wheat as a main ingredient of the diet according to the recommendation of Chicken Egg sub-committee of RIRDC. The powder pectinase (PECLYVE CP) was obtained from Lyven Enzyme Industries, Caen, France. The manufacturer assayed the pectinase

for main and side-enzyme activities before the dispatch. The pectinase product mainly consisted of polygalacturonase, PG, of activity of 2200 unit/g, with traces of pectin methyl esterase and pectin lyase.

Each hen was weighed at the commencement of the experiment and weekly thereafter. After the first week of adaptation, feed and water intake, egg and egg-shell weights, shell thickness, Haugh unit and number of soiled eggs were measured weekly. The egg production was recorded daily. Total excreta were collected twice daily each week by placing a tray with plastic sheet beneath each individual cage. The fresh excreta samples were collected, weighed and stored at –20°C after each collection and then freeze-dried for determination of water content of faeces (wet droppings). The freeze-dried samples of excreta and diets were ground to pass through a 0.7mm screen for the determination of metabolisable energy of the diet. The faecal and feed samples were compressed into a pellet form. The pellet was ignited using a ballistic oxygen bomb calorimeter (Gallenkamp-CB 330) for measurement of the amount of energy released (kilo calorie/gram sample).

4.3.3 Viscosity

At end of the experiment, the birds were killed by the cervical dislocation and the ileal digesta were extracted and frozen at –20°C for the measurement of viscosity. After collection, ileal digesta were pooled and approximately 1.5g was centrifuged at 6000×*g* (gravity) for 15 minutes at 15°C. The supernatants were collected for measurement of the viscosity.

Prior to the measurement, the supernatants were filtered through cheese cloth to remove any floating particles. Absolute viscosity was determined using a viscotester (HAAKE, PK 100, VT 550) of cone plate PK5 at shear rate 4802/second and the speed rate 800/minute at temperature 22°C, as described in the manufacturer's handbook. The supernatant of samples did not exhibit Newtonian flow behaviour, i.e. shear thinning (viscosity decreases with increasing the shear rate). Hence, the viscosity of the solution was a function of the shear rate.

4.3.4 Statistical design

The experiment consisted of a 2 × 2 × 4 complete factorial of two levels of lupins (10 and 20%), two types of lupins (whole and dehulled) and four doses of pectinase (0, 0.6, 0.8 and 1.0 g/kg diet). Each experimental treatment had 15 replicates, the number calculated to detect a significant difference of 5% between the treatments.

Data were analysed by Analysis of Variance (ANOVA) for a complete factorial design. Sixteen experimental treatments were analysed for each measured trait using the statistical package Genstat (5 release 4.1, Lawes Agricultural Trust, IACR Rothamsted). Prior to ANOVA, transformation of the data to logarithmic, square root, arcsinic or reciprocal values were carried out where they were required. If the ANOVA revealed significant effect of treatments, differences among two means were tested using Tukey's Honestly Significant difference (HSD).

4.4 Results

4.4.1 Viscosity

PG reduced the viscosity of the digesta of hens fed all diets (10 and 20% whole and dehulled lupins) by 11% ($P<0.05$). There were no differences in the viscosity between the levels of PG ($P>0.05$) (Table 4.2).

4.4.2 Water intake and faecal moisture

PG reduced water intake of hens on all diets by the same extent (7%) ($P<0.05$). Faecal moisture was reduced by a similar extent (6%) but this only happened on the two lower doses of PG (0.6 and 0.8 g).

When the amount of lupins in the diet was increased, faecal moisture increased. However, PG reduced the faecal moisture of 20% lupin diets back to the same level as the 10% lupin diets and this response was consistent for both whole and dehulled lupins.

4.4.3 Soiled eggs

The lowest level of PG reduced soiled eggs by 8% (Table 4.2). Again there were no significant interactions.

Table 4.2 Responses of laying hens to lupin-based diets supplemented with PG

PG dose	Whole lupins		Dehulled lupins		Mean
g/kg diet	10%	20%	10%	20%	
Viscosity (m.Pas/sec.)					
0	4.48	5.91	4.21	5.88	5.12[a]
0.6	4.02	5.28	3.77	5.29	4.59[b]
0.8	3.98	5.23	3.72	5.26	4.55[b]
1.0	3.87	5.23	3.72	5.27	4.52[b]
s.e.m.	0.15	0.19	0.15	0.22	0.18
Water intake (ml/hen/day)					
0	206	218	184	226	209[a]
0.6	188	203	167	209	193[b]
0.8	189	210	174	209	195[b]
1.0	192	211	169	211	195[b]
s.e.m.	4	7	6	7	6
Faecal moisture (%)					
0	60.9	63.6	57.5	61.6	60.9[a]
0.6	56.7	59.1	51.6	57.9	56.3[b]
0.8	57.4	60.9	54.6	58.7	57.9[b]
1.0	58.0	61.3	55.9	59.2	58.6[ab]
s.e.m.	1.8	1.9	2.1	1.1	1.4
Soiled eggs (%)					
0	5.53	5.80	5.11	5.53	5.49[a]
0.6	5.07	5.35	4.59	5.21	5.05[b]
0.8	5.10	5.45	4.82	5.33	5.18[ab]
1.0	5.21	5.54	5.02	5.25	5.26[ab]
s.e.m.	0.18	0.19	0.21	0.12	0.18

Means within columns with different superscripts differ significantly ($P<0.05$).

4.4.4 Food intake and food conversion ratio (FCR)

PG had no effect on food intake of hens fed lupins-based diets. Only the lowest level of PG reduced FCR by 4% and, again, there were no interactions (Table 4.3).

4.4.5 Digestibility of dry matter and apparent metabolisable energy (AME)

The lowest dose of PG (0.6g) increased digestibility of dry matter by 7% and increased AME by 4%. Both the higher doses had no effect and there were no interactions.

Table 4.3 Performance of laying hens fed lupin-based diets supplemented with PG

PG dose	Whole lupins		Dehulled lupins		Mean
g/kg diet	10%	20%	10%	20%	
Food intake (g/hen/day)					
0	121	122	114	119	119
0.6	119	121	112	118	118
0.8	120	121	113	118	118
1.0	119	120	113	117	118
s.e.m.	2	2	2	2	
Food conversion ratio (g food : g egg)					
0	2.02	2.04	1.89	1.95	1.97[a]
0.6	1.94	2.01	1.82	1.92	1.92[b]
0.8	1.96	2.00	1.85	1.89	1.93[ab]
1.0	1.96	1.99	1.87	1.90	1.93[ab]
s.e.m.	0.03	0.02	0.02	0.02	0.02
Digestibility of dry matter (%)					
0	57.4	55.4	59.6	57.7	57.5[a]
0.6	61.2	59.5	63.8	60.5	61.3[b]
0.8	60.7	57.6	60.8	59.3	59.6[ab]
1.0	60.0	56.8	59.4	59.0	58.8[ab]
s.e.m.	1.7	1.8	2.2	1.4	1.8
Apparent metabolisable energy (MJ/kg DM)					
0	10.4	9.7	10.8	10.1	10.4[a]
0.6	11.0	10.1	11.4	10.5	10.8[b]
0.8	10.8	10.0	11.2	10.3	10.6[ab]
1.0	10.8	10.0	11.1	10.3	10.5[a]
s.e.m.	0.1	0.1	0.1	0.1	0.1

Means within columns with different superscripts differ significantly ($P<0.05$).

4.4.6 Egg production and egg weight

The lowest level of PG increased egg output by 3% ($P<0.05$). The higher levels of PG had no effect.

4.4.7 Haugh unit, yolk colour and shell weight and thickness

PG had no effect on Haugh units, yolk colour or shell thickness but the lowest level (0.6g) increased the weight of the egg shell by 2% (Table 4.4).

Table 4.4 Egg characteristics of laying hens fed lupin-based diets supplemented with PG

PG dose	Whole lupins		Dehulled lupins		Mean
g/kg diet	10%	20%	10%	20%	
Egg production (egg/100 hens/day)					
0	89.2	89.0	89.2	87.5	88.7[a]
0.6	91.8	91.5	91.8	89.7	91.2[b]
0.8	90.8	89.9	91.0	89.2	90.2[ab]
1.0	90.5	88.7	91.0	89.2	89.8[ab]
s.e.m.	0.9	0.9	0.8	1.0	0.9
Egg weight (g)					
0	60.3	60.4	61.0	61.4	60.8
0.6	61.5	60.9	62.2	61.9	61.6
0.8	61.6	61.0	61.3	62.4	61.6
1.0	61.4	60.9	61.1	62.0	61.3
s.e.m.	0.7	0.9	0.9	0.8	0.8
Haugh unit					
0	87.3	86.7	87.7	87.8	87.4
0.6	87.5	86.8	88.1	87.7	87.5
0.8	87.5	86.9	87.2	88.1	87.4
1.0	87.3	86.8	87.1	87.6	87.2
s.e.m.	0.6	0.7	0.6	0.6	0.6
Yolk colour (Roche scale)					
0	5.8	5.8	5.8	5.7	5.8
0.6	5.9	5.8	5.9	5.8	5.9
0.8	5.9	5.9	5.8	5.8	5.9
1.0	5.9	5.8	5.8	5.8	5.8
s.e.m.	0.1	0.1	0.1	0.1	0.1
Shell weight (g)					
0	6.07	6.09	6.11	6.02	6.07[a]
0.6	6.22	6.14	6.25	6.08	6.18[b]
0.8	6.22	6.15	6.15	6.11	6.16[ab]
1.0	6.20	6.14	6.12	6.08	6.14[ab]
s.e.m.	0.05	0.06	0.05	0.05	0.05
Shell thickness (μm)					
0	358	355	358	356	357
0.6	359	357	359	356	358
0.8	359	357	356	358	358
1.0	358	356	357	357	357
s.e.m.	3	3	2	4	3

Means within columns with different superscripts differ significantly ($P<0.05$).

4.5 Discussion

The first hypothesis of the experiment was accepted because treatment of diets containing whole or dehulled lupins with PG significantly reduced water intake by 7%, wet droppings by 6%, viscosity of the digesta by 11% and soiled eggs by 8%. PG had no effect on food intake but reduced food conversion ratio slightly by 4%. PG also slightly improved digestibility of dry matter and

metabolisable energy of the diet. There was a small increase in egg yield (3%) and egg-shell weight (2%) but no effect on egg weight, shell thickness, yolk colour or Haugh unit score.

The second hypothesis was also accepted because 0.6g PG/kg diet allowed layers to handle 20% whole or dehulled lupins without compromising their production performance or increasing their wet droppings to levels that would be considered too high. PG reduced wet droppings (faecal moisture) of layers eating 20% lupins to levels normally seen on diets containing 10% lupins.

By breaking down pectins of the lupins, PG was able to reduce the viscosity of digesta. This breakdown released nutrients confined within the cell-wall lattices and allowed them to be digested and absorbed. As a consequence, digestibility of dry matter and metabolisable energy of the diet were increased and food conversion ratio (g food: g egg) decreased. The increase in egg production and egg-shell weight were most likely due to the increase in digestibility of dry matter and metabolisable energy of the diet. The decrease in percentage of soiled eggs was likely due to the decrease in water intake and wet droppings.

The most effective dose of those tested was the lowest one, 0.6g PG/kg diet. This dose produced all the significant results outlined above. The highest level, 1.0g PG/kg diet, was also effective but, in some cases, did not give the anticipated results. For example, both the lowest and highest levels reduced viscosity and water intake significantly but it was only the lower level that significantly reduced faecal moisture and soiled eggs. Several studies have confirmed that low levels of enzyme outperform higher levels in both layers and broilers (Petersen & Sauter, 1968; Patel & McGinnis, 1985; Boling *et al*, 2000; Lazaro *et al*, 2003) but several studies have been less conclusive (Francesch *et al*, 1995; Scott *et al*, 1999).

Dose level is particularly important and there is likely to be a compromise between sufficient breakdown of pectin to release nutrients but not too much to release large numbers of small, indigestible molecules, mono-galacturonic acid units. For example, 0.6g PG/kg diet significantly reduced faecal moisture from 60.9 to 56.3% but higher doses of PG reduced it less. The 1.0g PG/kg diet reduced faecal moisture to 58.6. Small units of mono-galacturonic acid are poorly metabolised by poultry and other monogastrics (Longstaff *et al*, 1988; Longstaff & McNab, 1986; Yule & Fuller, 1992). They are also hydrophilic which increases their water-holding capacity (Kertesz, 1951; Gupta, 1962; McCready, 1970; Griffiths & Kennedy, 1988; Schejter & Marcus, 1988; Sakai *et al*, 1993). Breakdown of the pectic chains that releases excessive numbers of galacturonic acid units will increase the osmotic pressure in the gut. This will increase water in the digestive tract and lead to an increase in wet droppings (Carter, 2001, pers. Comm.).

As anticipated layers performed slightly better when the lupins were dehulled but the difference was not great. This suggests that lupin hulls do not act simply as a diluent because they are responsible in their own right for increases in viscosity, water intake, faecal moisture and soiled eggs. So the dehulling process may improve the nutritive value of lupins for layers.

4.6 Implications

- PG improves the nutritive value of whole and dehulled lupins for laying hens. Of the doses tested 0.6g PG/kg diet is required in a layer diet containing either 10 or 20% whole and dehulled lupins.
- 0.6g PG/kg should allow feed manufacturers to include 20% lupins in layer diets. It is possible that does even lower than 0.6g might elicit an even better response.
- Increasing the dose of PG above 0.6g/kg diet might be detrimental and increase wet droppings and hence soiled eggs.
- Enzyme supplementation can reduce the production costs because more lupins can be substituted for expensive soybean while allowing the wet dropping problem to be managed.

4.7 Recommendations

By using commercially available pectinase (polygalacturonase) feed manufacturers should be able to use up to 20% whole lupins in layer diets and keep wet droppings within manageable limits.

5. Expansion and pectinase to improve the nutritional value of lupins for broilers

5.1 Introduction

The feed manufacturers and grain industry in Western Australia are seeking a new strategy to break down the indigestible cell-wall polysaccharides of lupins to increase their nutritional value for poultry. Lupins are locally grown, cheap and have the potential to become nutritionally comparable to that of imported soybean meal which is expensive.

The nutritional value of lupins can be improved by treatment with pectinase, an enzyme that is capable of degrading the cell-wall polysaccharides. By treating lupins with pectinase growth performance of broilers can be increased and the incidence of wet droppings reduced. However the extent to which pectinase can break down the cell walls is governed by several factors such as the complexity of the cell-wall polysaccharides, the retention time of digesta, and extremes of pH throughout the digestive tract that may inactivate the enzyme. As a consequence treatment of lupins with only pectinase can increase nutritional value but the response can be variable.

One way to reduce the variability and increase the response to enzyme treatment is to subject lupins to mechanical and thermal processes such as extrusion. While extrusion can effectively destroy the cell-wall network by shear force, pressure and temperature, the process by itself only marginally improves the nutritional value and wet droppings remain high (Bishop, 1989). So, for best results, extrusion must be combined with enzyme treatment. However, extrusion is a high-cost operation because of the extreme pressures and the low throughput hence an alternative must be sought. Expansion is often called low-cost extrusion and has several advantages over extrusion. It requires less energy and maintenance input, has a large throughput of feed, allows inclusion of high levels of fat and other liquid ingredients without compromising the quality of the pellet. Expanded feed is easy to pellet and, this less aggressive process, causes less damage to heat-sensitive vitamins and amino acids than extrusion.

Like extrusion, expansion enhances feed intake by 4.4%, feed conversion ratio by 2.3%, nitrogen retention by 6.8%, and metabolisable energy by 11% of wheat- and barley-based diets (Plavnik & Sklan, 1995; Fancher *et al*, 1996; Scott *et al*, 1997; Fasina *et al*, 1997). Expansion effectively eliminates harmful microbes and destroys anti-nutritional factors (ANFs) that exist in many foods. This is an important factor because lupins contain toxic fungi such as phomopsins and several ANFs, namely, alkaloids, saponins, tannins, trypsin and chymotrypsin inhibitors, phytate, C-glycosyl flavanol, oligosaccharides and uric acid (Sipsas, 1994; Petterson & Mackintosh, 1997). These ANFs can depress food intake so it would be an advantage to destroy them. Edwards & Tucek (2000) have suggested that expansion of lupins may prove beneficial but this has yet to be investigated.

Most important, expansion destroys the cell-wall lattices of wheat and barley and might do the same in lupins. For example, Chesson *et al*, (2002) have shown that expansion reduces the β-glucan content of barley by 12 fold and the arabinoxylan content of wheat by 5 fold. Destruction of cell walls reduces particle size and increases the surface area of the feed. This allows a) greater release of the digestible nutrients that were previously encapsulated within the cell-wall matrices and makes them available to the bird's own digestive enzymes, b) better access for exogenous enzymes like pectinase to better target their substrates in lupins, and c) greater breakdown of indigestible cell-wall structures which may help to reduce ANFs. Overall, there should be a reduction in viscosity and water-holding capacity of expanded lupins and, as a consequence, wet droppings should be reduced while growth and metabolisable energy are improved.

If expansion was used on its own without pectinase, it could be detrimental because viscosity and water-holding capacity might increase if too much insoluble non-starch polysaccharide was solubilized (Fancher *et al*, 1996; Liebert & Wecke, 1998; Chesson *et al*, 2002). For example, there are two reports that demonstrate that expansion *per se* has little effect in improving growth and metabolisable energy (1.5 – 4.0%; Vest, 1996; Plavnik & Sklan, 1995). And there are several reports where expansion of wheat, wheat bran, and barley has increased the viscosity of the digesta.

However, where expansion has been used in combination with enzyme treatment improvements have been forthcoming. For example expansion + enzyme treatment (eg. xylanase or β-glucanase) counteracted the increase in viscosity (34 vs. 14.3%) and gave increased weight gain (7.1 vs. 4.4%), better feed conversion ratio (3.3 vs. 2.3%) and higher metabolisable energy (11 vs. 8.5%) than either expansion or enzyme treatment on their own (Scott *et al*, 1997; Liebert & Wecke, 1998). Chesson *et al*, (2002) reported that thermal-mechanical treatment such as expansion perforated plant-cell walls which allowed the release of readily digested proteins, oils and polysaccharides contained within them, and the entry of host enzymes caused a reduction in viscosity and digestion to take place within the cells. Therefore, it seems that enzyme treatment must be used in concert with expansion to counteract detrimental effects such as an increase in viscosity of digesta.

In reviewing the literature there are only two papers that show an interaction between expansion and enzyme treatment. Complete degradation of cell-wall structures of lupins, in particular the pectins, by combined expansion (thermal and mechanical process) and enzymatic treatments has not been studied but deserves close examination as this may be one way to improve the nutritional value of lupins for broilers.

5.2 Hypothesis

The hypothesis tested was that a combination of expansion and pectinase has a synergistic effect on the breakdown of cell-wall polysaccharides of lupins and, consequently, will increase the metabolisable energy content of the diet and give a significant improvement in the growth of birds.

5.3 Materials and methods

5.3.1 Experimental Design

Whole and dehulled lupins were expanded, added into the diet at either 10 or 20%, treated with PG (0.8g/kg diet) and then fed to broilers for 11 days. The effectiveness of expansion and PG and their combination were tested by measuring food and water intake, weight gain, food conversion ratio, digestibility of dry matter, apparent metabolisable energy of the diet, viscosity of digesta in the intestine, wet droppings, cell-wall polysaccharides, and pectin.

5.3.2 Animal ethics, housing, diet formulation, feeding and data collection

The animal ethics committees of the University of Western Australia and Agriculture Western Australia approved this study. Health and husbandry practices compiled with the *Code of Practice for the Welfare of the Domestic Fowl* issued by the *Australian Bureau of Animal Health in 1983*.

Five hundred and seventy six mixed sex broilers (Cobb strain) 3-weeks old were sourced from Inghams Pty Ltd. The birds were randomly distributed into the metabolism cages (3 birds per $0.6x0.5x0.8m^3$ cell) in broiler-growing shed. During the experimental period, the temperature was maintained at 24 – 26°C and the relative humidity was kept at 55%. The lighting regime was kept at 23 hours light : 1 hour dark throughout the experiment.

Each of the experimental treatments included 12 replicates with 3 birds per replicate. The diets were fed to the broilers for 11 days (26 – 37 days old) plus 3 days for adaptation to the cages and environment. The diets were formulated to be isocaloric and isonitrogenous to meet all nutrient requirements of broiler chicks on the basis of the standards of Standing Committee on Agriculture, Australia (SCA, 1987, Table 5.1). The birds had free access to the feed and water (*ad libitum*) throughout the experiment and the diets were fed in pellet form.

Table 5.1 Composition of the experimental diets for broiler chicks (4-5 weeks old)

Ingredients	Whole lupins (%)		Dehulled lupins (%)	
	10	20	10	20
Wheat 11%CP	65.0	59.4	68.3	66.0
Lupins	10.0	20.0	10.0	20.0
Soybean meal 47% CP	15.8	10.5	13.6	6.2
Meat & bone meal 48%CP	3.8	3.8	4.0	3.9
Canola oil	4.4	5.3	3.1	2.8
Salt (iodised)	0.3	0.3	0.3	0.3
Sodium bicarbonate	0.35	0.35	0.35	0.35
Lysine	0.1	0.2	0.2	0.2
Choline chloride 75%	0.05	0.05	0.05	0.05
Commercial broiler premix[1]	0.1	0.1	0.1	0.1
Calculated analysis of dietary nutrients and energy				
AME (MJ/kg)	13.2	13.2	13.2	13.2
Crude protein	20.0	20.0	20.0	20.0
Calcium	0.6	0.6	0.6	0.6
Avail. phosphorus	0.3	0.3	0.4	0.4
Methionine	0.2	0.2	0.2	0.2
Lysine	0.9	0.9	0.9	0.9

[1]The premix provided vit. A 8,000 IU, vit. D_3 2,400 IU, vit. E 8 mg, vit. K 0.3 mg, niacin 20 mg, riboflavin 4 mg, calcium pantothenate 6 mg, vit. B_{12} 10 μg, pyridoxine 0.5 mg, folic acid 0.5 mg, biotin 30 μg, cobalt 0.2 mg, iodine 1 mg, copper 12 mg, iron 20 mg, manganese 75 mg, selenium 0.1 mg and zinc 50 mg.

During pelleting, the temperature of pelleting machine was kept below 50°C to avoid the enzyme denaturation by the pelleting heat. The enzyme pectinase consisted mainly of polygalacturonase, PG, of 3,500 units/g. This enzyme was assayed for the activity by the manufacturer and found to have 3,740 units/g before addition to the experimental diets. Xylanase of 0.2g (Rovabio™ xylanase Excel AP, 2000units/g) obtained from Bio-John Pty Ltd Perth, was included in all experimental diet diets.

Feed intake, weight gain, water intake and faecal output were recorded seven times throughout the experiment. Excreta were collected by placing a tray with plastic sheet beneath each individual cage. It was collected twice a day and stored at -20°C. Each sample was freeze-dried and reweighed for determination of water content of faeces (wet droppings).

The freeze-dried samples of excreta and diets were ground to pass through 0.7mm screen to determine the content of cell walls and pectin described in a previous *in vitro* experiment (Ali *et al*, 2001) and metabolisable energy of the diet. The faecal and feed samples were compressed into a pellet form. The pellet was ignited using a ballistic oxygen bomb calorimeter (Gallenkamp-CB 330) for measurement of the amount of energy released (kilo calorie/gram sample).

5.3.3 Expansion of lupins

Specifications for expansion including the configuration of the screw were as follows. The barrel temperature of the expander was approximately 110°C throughout the length of the chamber (47.5cm). The temperature at four heating and cooling zones of the barrel was 90, 100, 110 and 120°C, respectively, and 126°C through the 2mm die hole (exit gap). The residence time of lupins in the expander was approximately 30 seconds. The moisture level of the expanded lupins in the barrel was approximately 36% due to the high water-holding capacity of lupins. The die pressure was approximately 118 – 122psi, speed of the screw was 175rpm and screw motor torque was 32%. Expanded lupins were immediately cooled and dried by spreading as a thin layer on a metal table in a cooling room for 2.5 hours. After drying at 60°C, the expanded lupins were ground and mixed with other dietary ingredients. These conditions were designed to cope with small amounts of starch (approximately 1%) in lupins.

5.3.4 Viscosity

At end of the experiment, the birds were killed by carbon dioxide and intestinal digesta were collected from the ileum for the measurement of viscosity according to the procedures mentioned in section 5.3.3.

5.3.5 Statistical design

The experiment consisted of a 2 × 2 × 2 × 2 complete factorial (no expansion or expansion, whole or dehulled lupins, 10 or 20% lupins, 0 or 0.8g PG/kg diet). Based on previous experience of the known variation in each parameter, twelve replicates per treatment were calculated as necessary to detect a significant difference of 5% between the treatments.

The data were analysed by ANOVA in a complete factorial design. Differences between treatments were analysed using the statistical package Genstat (5 release 4.1, Lawes Agricultural Trust, IACR Rothamsted). Prior to ANOVA, transformation of the data to logarithmic, square root, arcsinic or reciprocal values were carried out where they were required (which parameters did you transform?). If the ANOVA revealed significant effect of treatments, differences between two means were tested using Tukey's Honestly Significant difference (HSD).

5.4 Results

5.4.1 Food intake, digestibility, weight gain and food conversion ratio

PG on its own had no effect on food intake but it increased digestibility by 10%, increased weight gain by 5% and improved food conversion efficiency by 5% across all lupin diets. By contrast, expansion increased food intake by 3% across all lupin diets. But it had no effect on weight gain, digestibility, or food conversion ratio. The combination of expansion and PG was the same as expansion on its own (Table 5.2).

Increasing lupins from 10 to 20% reduced food intake by 4%, reduced digestibility by 5%, reduced weight gain by 11% and reduced the efficiency of food conversion by about 8%. Broilers consuming dehulled lupins ate 5% more food, digested them 5% better, grew 16% faster and converted food into liveweight gain 10% better than broilers consuming whole lupin diets.

Table 5.2 Responses of broilers to expansion of lupins and PG supplementation

Treatment	Whole lupins	Dehulled lupins	Mean

	10%	20%	10%	20%	
Food intake (g/bird/day)					
Control	130	123	137	130	130[a]
PG	131	124	137	131	131[a]
Expansion	133	129	140	135	134[b]
Expansion+PG	133	130	140	134	134[b]
s.e.m.	1	2	1	1	1
Weight gain (g/bird/day)					
Control	47.3	42.3	55.7	49.6	48.7[a]
PG	50.2	44.1	58.9	51.8	51.3[b]
Expansion	47.4	42.7	55.7	48.9	48.7[a]
Expansion+PG	48.7	43.2	56.7	48.7	49.3[a]
s.e.m.	1.1	0.9	1.2	1.1	1.1
Food conversion ratio (food g : gain g)					
Control	2.75	2.93	2.46	2.64	2.70[a]
PG	2.61	2.81	2.34	2.53	2.57[b]
Expansion	2.83	3.02	2.53	2.78	2.79[a]
Expansion+PG	2.75	3.01	2.47	2.77	2.75[a]
s.e.m.	0.03	0.04	0.03	0.04	0.04
Digestibility of dry matter (%)					
Control	51.7	49.6	53.5	51.6	51.6[a]
PG	57.9	52.6	60.7	55.0	56.6[b]
Expansion	50.1	48.3	51.6	51.0	50.3[a]
Expansion+PG	51.3	49.4	55.0	52.2	52.0[a]
s.e.m.	1.1	1.1	1.2	0.9	1.1

Means within columns with different superscripts differ significantly ($P<0.05$).

5.4.2 Apparent metabolisable energy (AME)

This measurement has yet to be determined owing to breakdown of the bomb calorimeter.

5.4.3 Viscosity, water intake, faecal moisture and breakdown of cell-wall polysaccharide (CWP) and pectin

PG on its own reduced the viscosity of digesta by 14%, water intake by 6% and faecal moisture by 6% across all lupin diets. Expansion worked in the opposite direction and increased viscosity by 10%, water intake by 3% and faecal moisture by 3%. So expansion and PG cancelled each other out and the combination of the two had little effect on any of these parameters (Table 5.3).

PG had a large effect on cell walls. It more than doubled the breakdown of cell-wall polysaccharides and increased the breakdown of pectin 4 fold. Expansion was even more effective than PG because it increased the break down of cell wall polysaccharides by 3 fold. However, it only increased pectin breakdown by 66%.

There was a synergistic effect of PG plus expansion on the breakdown of cell walls. When applied together PG and expansion increased breakdown of cell-wall polysaccharides by 3.5 fold and pectin by 5.5 fold.

Increasing lupins from 10 to 20% increased viscosity by 32%, water intake by 10% and faecal moisture by 5%. In addition, breakdown of cell-wall polysaccharides was reduced by 20% and pectin by 28% when the proportion of lupins in the diet was increased. But PG reduced the faecal moisture of 20% lupin diets to the same amount as diets with 10% lupins. Dehulled lupins induced a higher

viscosity (16%), increased water intake by 6% and faecal moisture by 3% compared with whole lupins. There was also more breakdown of cell-wall polysaccharides by (26%) and pectin (5%) in dehulled lupin diets than diets with whole lupins.

Table 5.3. Response of broilers to expansion of lupins and PG supplementation.

Treatment	Whole lupins		Dehulled lupins		Mean
	10%	20%	10%	20%	
Viscosity (m.Pas/sec.)					
Control	5.11	6.91	6.30	7.93	6.56[a]
PG	4.08	6.42	4.73	7.45	5.67[b]
Expansion	5.72	7.95	6.71	8.57	7.24[c]
Expansion+PG	5.16	7.02	6.43	8.04	6.66[a]
s.e.m.	0.2	0.2	0.2	0.2	0.2
Water intake (ml/bird/day)					
Control	305	330	321	350	327[a]
PG	283	319	299	336	309[b]
Expansion	314	342	333	363	338[c]
Expansion+PG	311	337	328	356	333[d]
s.e.m.	2	2	2	2	2
Faecal moisture (%)					
Control	65.4	68.7	68.5	70.3	68.2[a]
PG	60.1	66.6	62.7	68.2	64.4[b]
Expansion	67.4	71.1	70.6	72.9	70.5[a]
Expansion+PG	65.7	69.8	67.5	71.7	68.7[a]
s.e.m.	1.2	1.1	1.0	1.1	1.1
Breakdown of cell-wall polysaccharides (%)					
Control	3.2	2.1	4.6	3.0	3.2[a]
PG	7.3	5.8	9.2	7.3	7.4[b]
Expansion	9.7	7.4	12.4	9.6	9.8[c]
Expansion+PG	10.6	9.4	13.6	10.3	11.0[c]
s.e.m.	1.2	1.3	1.3	1.4	1.3
Breakdown of pectin (%)					
Control	2.5	2.2	2.5	2.3	2.4[a]
PG	11.1	8.1	12.1	8.9	10.1[b]
Expansion	5.3	3.0	4.6	3.1	4.0[a]
Expansion+PG	15.0	11.2	16.3	11.2	13.4[c]
s.e.m.	1.2	1.3	1.4	1.3	1.3

Means within columns with different superscripts differ significantly ($P<0.05$).

5.5. Discussion

The hypothesis was rejected because expansion did not appear to increase the activity of PG and increase performance of the birds as was anticipated. When lupins were expanded and then treated with PG, growth performance and values for wet droppings were similar to the control. Yet expansion was very effective at breaking down both cell walls and pectin. Therefore, if expansion on its own was so effective at breaking down cell walls and pectin, why wasn't this reflected in better activity of PG and better performance of the broilers?

Expansion may have had a negative influence on nutritive value of lupins and there are three possibilities. First, the heat of expansion may have solubilised the insoluble fractions of the cell-wall

polysaccharides which in turn may have increased viscosity (Fadel *et al.*, 1988; Vukic-Vranjes *et al.*, 1994). Any increase in viscosity reduces digestion, growth and food conversion efficiency. This effect was more pronounced with whole than dehulled lupins because whole lupins contain 25% fibrous hulls of which 85-95% is insoluble fibre (Harris and Jago, 1985; Cheung, 1991; Evans *et al.*, 1993).

Second, expanded lupins have a higher absorptive capacity for water because of the "puffing" effect which increases porosity of final product. Expanded lupins swell more than unexpanded lupins and this would be expected to lead to increased water intake by the birds and increased wet droppings.

Third, the heat generated during expansion could have damaged some of heat-sensitive nutrients such as lysine due to the Maillard reaction. For example, Fadel *et al.*, (1988) found extrusion increased Maillard products by 63% in barley-extruded diets fed pigs. In addition perhaps of the endogenous enzymes (amylase and proteases) may have been destroyed (Batterham *et al.*, 1986a,b).

There are several reports indicating that broilers fed expanded/extruded food grow slower, use food less efficiently and have digesta of higher viscosity than birds fed unprocessed food (Adams and Naber, 1969; Vukic-Vranjes *et al.*, 1994; Nissinen, 1994; Vukic-Vranjes and Wenk, 1995; Edwards *et al.*, 1999). There are some reports that indicate otherwise (Scott *et al.*, 1997; Fasina *et al.*, 1997). In addition, most reports suggest that a combination of extrusion/expansion and enzyme supplementation do not improve broiler growth more than that of the enzyme on its own (Vukic-Vranjes *et al.*, 1994; Vukic-Vranjes and Wenk, 1995; Scott *et al.*, 1997).

However, Chesson *et al.* (2002) believes that expanding or extruding feeds may be beneficial. They suggest that pre-conditioning of a feedstuff before expansion significantly lowers the viscosity compared to conditioning with water during expansion. This is an interesting concept and might be worthy of further investigation.

PG offset the negative effects of expansion and, on its own, was very effective at reducing viscosity of digesta, water intake and wet droppings. It increased the digestibility of dry matter and, as anticipated, increased growth rate by 5% but only at the 10% inclusion rate of lupins not the 20% rate. The reason why there was no improvement at the 20% level was that PG reduced food intake of birds fed 20% lupins but improved the digestibility of the diets. The net result was that birds received similar amounts of useable energy. Metabolizable energy would be expected to follow the digestibility of dry matter.

All the above improvements were greater with dehulled than with whole lupins except for a slight increase in water intake and wet droppings of birds fed dehulled lupins. This suggests that dehulling improves the nutritive value of lupins with/without PG and may reduce any detrimental effects of hulls on growth and efficiency of food conversion for broilers (Brenes *et al.*, 1993; Ali, 1997; Hughes *et al.*, 1998).

Although PG did not increase growth rate of birds fed 20% lupins it did reduce wet droppings to a similar level to that seen in birds eating 10% lupins without enzyme treatment.

5.6. Implications

- There does not appear to be any synergistic interaction between PG and expansion as hypothesised. Although expansion seems to break down cell walls this benefit is not translated into improved growth rate. Perhaps expansion exerts negative effects on nutritive value such as increased viscosity and Maillard reactions.

- PG on its own can improve the nutritive value of lupins for broilers and reduce wet droppings, particularly on diets containing 10% whole or dehulled lupins.

- PG seems unable to increase weight gain when lupins make up 20% of the diet. However they can lower wet droppings to levels seen in birds consuming 10% lupins.

- Dehulling improves the nutritive value of lupins and growth performance and efficiency of food conversion for broilers.

- Enzymatic treatment by PG alone can lift the level of lupins from the current 5% in diets to 10% without increasing wet droppings. This level can be lifted to 20% without much loss in productivity if it is considered that a suitable level of wet droppings is that seen at the10% inclusion rate.

5.7 Recommendations

- Expansion is not recommended for broiler diets because the positives such as breakdown of cell walls do not outweigh the negatives such as increased viscosity.

- Lupins could successfully substitute soybean up to 10% without significant losses in productivity or an increase in wet droppings. This level can be lifted to 20% with only small losses in productivity and levels of wet dropping normally seen at 10% inclusion rate of lupins.

- In view of the improvements achievable a commercial preparation of pectinase should be developed for future application to broiler diets.

6. Complete Hydrolosis of Lupin Pectin by Pectinases (*In Vitro*)

6.1 Introduction

A major stumbling block for the degradation of cell walls by any pectinase is the existence of pectin methyl ester radicals attached to the C_6 atom of galacturonic acid units along the pectin chain. The pectinase, polygalacturonase (PG), targets specifically the glycosidic bonds that join the galacturonic acid units together. But when cell walls are treated with PG alone, only 11% of the bonds are broken because the methyl ester radicals block the binding sites of PG to the glycosidic bonds along the pectin chain (Endo, 1964a,b; English *et al.*, 1972; Ali *et al.*, 2001).

In addition, the methyl esters through their cross links with neighbouring polymers via divalent ions such as Ca^{++} and Mg^{++} are directly responsible for the properties of water-holding capacity and viscosity (Northcote, 1958; Grant *et al.*, 1973; Jarvis, 1984). Increased water-holding capacity is the main cause for an increase in water intake and hence an increase in wet droppings. Higher viscosity is also undesirable as it is implicated in poor digestion of nutrients and, consequently, depressed weight gain and poor food utilization (Erdman *et al.*, 1986; Langhout and Schutte, 1996; Langhout *et al.*, 1999, 2000).

However, these radicals can be removed by a specific enzyme, pectin methyl esterase (PME) which strips off these radicals along the pectin chain and hydrolyses them into methanol and hydrogen ions. When PME does this, many of the branches are destroyed, leaving mainly smooth, linear chains of galacturonic acid units that are 4 to 10 times more susceptible to attack from PG (Jansen and McDonnell, 1945; Endo, 1961; Christgau *et al.*, 1996).

As a consequence, PME improves the hydrolytic activity of PG for complete degradation of pectin and reduces the viscosity and water-holding capacity of feedstuffs containing pectin. Lupin seems a particularly good target for the action of PME because lupin kernel contains 8 – 11% pectin within cell-wall lattices, and the majority of this, between 80 – 90%, is methyl esterified (Konovalov *et al.*, 1999).

6.2 Hypothesis

The hypothesis was that incubation of dehulled lupins with combination of two pectinases, PG and PME, should give a more complete breakdown of pectin and reduction of water-holding capacity and viscosity than PG on its own.

6.3 Materials and methods

6.3.1 Experimental design

Dehulled lupins were incubated with polygalacturonase (PG) and pectin methyl esterase (PME). The extent of degradation of pectin was estimated by measuring changes in the viscosity, water-holding capacity, filtration rate, cell-wall materials, content of pectin and its molecular weight, chain length and methyl ester content and methanol.

6.3.2 Incubation of dehulled lupins with PG and PME

Ten grams of dehulled lupins of narrow leaf (*Lupinus angustifolius*, var. Quilinock) were incubated with 1,400 units of either PG, PME, PG+PME or without (no enzyme). The incubation mixture was prepared by dissolving ground, dehulled lupins in 70ml acid buffer (42g citric acid + 280ml 1*M* NaOH + 200ml 1*M* $CaCl_2$, pH = 3.9) according to the assay method described by the enzyme manufacturer. The analytical procedures and measurements described in a previous *in vitro* experiment (Ali *et al.*, 2001) were followed in this experiment. The determinations of methyl ester and methanol were carried out according to the method of Wood and Siddiqui (1971).

6.3.3 Statistical design

Analyses of the data were carried out by using an ANOVA in a complete randomised design. Four experimental treatments (control, PG, PME and PG+PME) and 12 replicates per treatment were analysed for each measured trait using statistical package Genstat (5 release 4.1, Lawes Agricultural Trust, IACR Rothamsted). Transformation of the data was carried out where it was required. If ANOVA of any treatment was statistically significant, orthogonal contrasts were used to compare the differences among their means as follows: 1) control vs enzymes, and 2) PG+PME vs PG and PME.

6.4 Results

The combination of enzymes, PG+PME, reduced ($P<0.05$) cell walls by 27%, pectin nearly 50%, molecular weight of pectin by 56% and the length of pectin chains by 65% more than PG, PME and control treatments. Also, the combination reduced ($P<0.05$) methyl esters by a similar amount (64%) and increased methanol by 116%. This combination reduced viscosity by 7% and water-holding capacity by 15% (Table 6.1).

Table 6.1. Effect of PG, PME and PG+PME on physical-chemical properties of dehulled lupins *in vitro* (mean±sem)

Parameters	Control	PG	PME	PG+PME	LSD[#]
Cell-wall materials (% DM)	23.4±0.9	20.3±1.2	23.5±1.0	17.1±0.9	2.8
Pectin (% DM)	10.9±0.8	8.6 ±0.3	9.1±1.0	5.8±0.3	1.9
MW of pectin (kilodaltons)	135±5	101±5	128±6	59±3	14
Length of pectin chain[§]	63.0±2.8	56.1±2.6	61.2±2.2	22.3±1.6	6.7
Methanol (µg/ml incubation media)	8.8±0.8	9.6±0.8	14.6±1.5	19.0±1.8	3.7
Methyl esters of pectin chain (%)	20.1±0.9	18.6±1.0	9.7±0.6	7.3±0.5	2.2
Viscosity (m.Pas/sec)	1.69±0.01	1.48±0.01	1.91±0.03	1.57±0.03	0.09
Water-holding capacity (g:g)	3.56±0.17	3.19±0.15	4.10±0.21	3.04±0.13	0.48
Filtration rate (µl/sec)	69.4±2.6	79.0±3.6	47.0±1.6	63.2±2.7	7.9

Least significant differences (LSD) were applied to the single degree of freedom orthogonal contrasts at the 5% level of probability as follows: 1) control vs enzymes, and 2) PG and PME vs PG+PME. § Number of galacturonic acid units per pectin chain.

When PME was used alone, methyl esters were reduced and methanol was increased ($P<0.05$) more than the PG and control. However, PME increased both the viscosity and water-holding capacity ($P<0.05$) and decreased filtration rate of the supernatant (Table 4.1).

PG used alone induced similar changes ($P<0.05$) compared with the combination of pectinases but the extent of change was much less with the exception of viscosity and filtration rate. PG had no effect on methanol or methyl esters.

6.5 Discussion

The evidence was unequivocal that the hypothesis was supported because the combination of pectinases was superior to either pectinase alone. PG and PME together were very effective at breaking down pectin (50% reduction) and reducing cell walls (27%). Using just PG alone, we expect a breakdown of between 10 and 20%. Other parameters measured confirmed the effectiveness of the combination of enzymes. For example, molecular weight of pectin was more than halved and the length of pectin chains was reduced 3-fold. As anticipated, the methyl esters on the pectin were reduced by a similar magnitude (3-fold) and they were converted to methanol and hydrogen which more than doubled.

Surprisingly, the breakdown of pectins and cell walls did not change filtration rate and the reductions in viscosity and water-holding capacity were smaller than anticipated. There are at least four possible reasons. First, removal of methyl esters from the cell-wall matrix by PME gives more non-methylated galacturonic acid chains and these can be cross-linked by Ca^{++} and Mg^{++}. In turn this leads to gel formation and an increase in water-holding capacity and viscosity (Penn *et al.*, 1966; Nichols and Deese, 1966; Gupta and Nichols, 1962; Willats *et al.*, 2001).

Second, PME itself requires large amounts of water to convert methyl esters (CH_3COO) into methanol (CH_3OH) and H^+. This also increases water-holding capacity of the pectin. Third, PME converts insoluble to soluble pectin, causing the coagulation of soluble pectin to form a gel. This leads to a large retention of water for formation of the gel network (Kertesz, 1951; Oi and Satomura, 1965; Ben-Arie and Lavee, 1971).

Third, the result could be dose dependent. Just enough PME is required to expose the right number of sites on pectin so that PG can break down the maximum number of glycosidic bonds. Too much PME could be detrimental if it produces too many radicals because the radicals themselves are responsible for water-holding capacity (Endo, 1964ab; Pressey and Avants, 1982). Therefore, had we chosen a lower dose of PME, the reductions in viscosity and water-holding capacity may have been greater because the production of methyl ester radicals may have been less. Before this *in vitro* work is tested in broilers the appropriate combination of PG and PME needs to be determined more precisely.

The combination of PG and PME used did not achieve complete breakdown of pectin which was contrary to expectations. There is a PME that can be extracted from plants and it removes blockwise nearly all of the methyl esters from the pectin chain. The common PME comes from microorganisms and this only removes a portion of the radicals because it acts randomly (Sajjaanantakul and Pitifer, 1991; Kester *et al.*, 2000; van Alebeek *et al.*, 2000). We used the commercial PME from microbial origin which can only hydrolyse about half of the methyl esters. Complete breakdown of pectin requires other pectinases such as exo-PG, pectin lyase and rhamnogalacturonase for cleavage of the terminal, methylated and rhamnogalacturonan glycosidic bonds of the pectin chain, respectively (Rombouts and Pilnik, 1980; Sakai *et al.*, 1993).

6.6 Implications

- The two pectinases are much more effective than a single pectinase for breaking down cell walls. First, PME is required to remove the methyl ester radicals to expose the glycosidic bonds of pectin. Second, PG is then required to break down the glycosidic bonds to destroy the pectin lattice.

- This synergistic action between PME and PG could allow greater inclusion of dehulled lupins, up to 20%, into broiler diets without an increase in wet droppings.

6.7 Recommendations

- The present *in vitro* findings should be applied to live broilers.

- If *in vivo* work provides positive results a commercial-pectinase preparation of PG and PME needs to be developed.

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Plain English Compendium Summary

Project Title:	**Mechanical and enzymatic improvement of lupins for broiler and layer diets**
RIRDC Project No.:	WAU-1A
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Objectives	• To improve the nutritional value of whole and dehulled lupins so that they can replace soybean meal in diets for broilers and layers, with major savings in feed costs. • Breakdown of thick cell walls of whole and dehulled lupins by expansion and enzymatic treatments. • To increase the inclusion rates of whole and dehulled lupins up to 20% in broiler and layer diets by the above treatments without significant losses in productivity.
Background	Australia produces 87% of the world's lupins which are an excellent source of protein and energy. While the world faces a shortage in plant protein meals, feed manufacturers and poultry producers cannot use more than about 5% lupins in broiler and 7% in layer diets. The main reason is because lupins contain complex cell-wall polysaccharides (33%) that are indigestible. The main component of cell walls is pectin which varies from 33 to 71%. Pectin increases the viscosity of digesta in the bird's digestive tract, increases water intake and wet droppings and, consequently, reduces food intake and efficiency of feed utilisation. Poultry cannot digest pectin because they don't secrete the appropriate enzymes so their use of lupins is limited. However, by treatment of lupins with exogenous enzymes and mechanical heat treatment (expansion), it might be possible to increase the nutritive value of lupins for poultry.
Research	Three experiments were carried out to investigate whether exogenous pectinases and expansion might increase the nutritive value of lupins for broilers and layers. 1) the inclusion of 10 and 20% whole and dehulled lupins using pectinase (endo-polygalacturonase) for layers, 2) enhancement of pectinase activity through expansion of lupins before including them at 10 and 20% in the diets for broilers and, 3) in vitro breakdown of pectin in dehulled lupins by two pectinases, endo-polygalacturonase (PG) and pectin methyl esterase (PME).
Outcomes	Low levels of PG (0.6 g/kg diet) improved the nutritive value of both whole and dehulled lupins for laying hens while higher doses appeared detrimental to performance. Higher levels (0.8 g/kg diet) of PG improved the nutritive value of whole and dehulled lupins for broilers. Expansion was effective in breaking down cell walls of lupins but this was not reflected in improved performance of birds. PG on its own broke down about 20% of the pectin in cell walls but a combination of pectinases broke down 50% of the pectin. Initially, PME removed the methyl ester radicals to allow PG to break down more of the glycosidic bonds of pectin.
Implications	Treatment of lupin-based diets with PG should allow feed manufacturers to incorporate at least 10% lupins in both broiler and layer diets and, depending on the level of wet droppings that can be tolerated, may allow up to 20% inclusion. Layers can tolerate higher levels of lupins than broilers. Although expansion breaks down the cell walls of lupins it cannot be recommended as a process for improving nutritional value because of negative effects. On the *in vitro* evidence, a combination of two pectinases, PG and PME, offers much greater potential to improve the nutritive value of lupins and, at the same time, control the problem of wet droppings than PG on its own.
Publications	Ali, A., Williams, I. H., Martin, G. B. and Sipsas, S. (2005). Hydrolysis of lupin

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Ali, A., Williams, I. H., Martin, G. B. and Sipsas, S. (2006). Expansion and pectinase to improve the nutritional value of lupins for broilers. *Proceedings of Australian Poultry Science Symposium*. **18**. (*In preparation*).