GRAIN LEGUMES (LUPINS, LABLAB BEANS, COWPEAS AND NAVY BEANS) AS SUPPLEMENTS FOR SHEEP AND GOATS

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SUMMARY

A series of experiments examined intake, digestion and production with diets of roughages supplemented with grain legumes, usually at levels from 5-20 g/kg LW. For goats, lupins caused less reduction in roughage intake than insect-damaged lablab and supported greater growth rates. In a ration containing 35% maize and 35% grain legume fed to sheep, substitution of sound lablab for lupins had little effect. As a supplement to roughages for live weight gain and wool growth, the ranking was lupins, cowpeas, lablab and navy beans. Lupins supplied more ruminal NH3 than lablab without causing a dangerous level of acidity, but at high levels still caused depression of digestion of roughage. Antibiotics did not consistently improve performance of sheep fed grain legumes. Concentrations of lectins and trypsin inhibitors were highest in navy beans but were not related to digestibility in nylon bags or to absorption from the small intestine. We conclude that the differences in nutritional value of these grain legumes was not related only to their content of lectins or trypsin inhibitors.

INTRODUCTION

Lupins and peas have developed during the last decade as major crops in southern Australia, and are accepted as high protein feeds of major importance to the livestock industries. There has not been a comparable increase in production of grain legume crops suitable for dryland areas of Northern NSW and Queensland. Two crops, lablab (Lablab purpureus) and cowpeas (Vigna sinensis) grown for forage in these latter areas also have high grain yields. However anti-nutritional factors (lectins, protease inhibitors and others) are known to be present in high concentrations in the seeds of these species, and make these seeds in their uncooked form of low nutritive value or even toxic for monogastrics (Lambourne and Wood,, 1985). However, there is evidence that a large proportion of the lectins and protease inhibitors present in grain legumes is inactivated by rumen fermentation. Raw soyabeans and raw CAnavalia ensiformas seed can be fed to ruminants without difficulty at levels which would be highly detrimental for monogastrics (Dixon et al. 1983; Erickson and Barton 1987).

This paper reports the results of a series of experiments intended to evaluate the nutritive value of the three tropical grain legumes lablab (Lablab purpureus), cowpeas (Vigna sinensis) and navy beans (Phaseolus vulgaris) in comparison with lupins (Lupinus angustifolius) for sheep and goats, and to examine the concentration and rumen inactivation of the lectins and protease inhibitors known to be present.

MATERIALS AND METHODS

Experiment 1: Lablab and lupins for goats

Insect-damaged cull (Batch A) lablab grain (var Highworth) or lupins were fed as a supplement to a roughage diet to weaner dairy goats (16-28 kg) held in metabolism cages. Groups of five goats received mixed pasture hay plus straw (1:1) ad libitum, alone or supplemented with 3,6 or 12 g lablab or 12 g lupins/day/kg LW plus a mineral mix, for 42 days (Table 1). Intake, digestibility and growth were measured.

*School of Agriculture and Forestry, The University of Melbourne, Parkville, Vic. 3052. Table 1. Composition of dietary ingredients fed to sheep and goats or assayed in seven experiments studying supplementation of roughages with grain legumes (g/kg DM)

		<u>Roughages</u>				<u>Grain legumes</u>				
	Pasture hay	Wheat straw	Oaten chaff	Barley straw	Lablab (A)	Lablab (B)	Lupi Un harv	ns i- est	Cowpeas Caloona	Actolac Navy beans
Experiment	1	1	3567	3567	167	2367	12	3567	7 567	567
Dry Matter	896	892	902	911	901	887	903	905	898	883
Organic Matte	er 914	927	944	934	956	958	970	974	963	956
N x 6.25	84	55	80	59	280	247	308	345	276	260
N.D.F.	696	705	643	786	336	275	264	356	214	235
A.D.F.	354	401	364	483	152	123	227	226	73	80
Lignin	32	32	42	52	30	nd	25	2	4	2
Ether Extract	nd nd	nd	nd	nd	10	4	63	53	36	12

Lablab A, insect damaged cull Highworth seeds; Lablab B, seed grade Highworth; nd, not determined.

Experiment 2 : Lablab and lupins for sheep

In a switch-over design 21 rumen-fistulated Merino weaner wethers (18-31 kg) were held in metabolism cages for two periods of 49 days, with an intervening two-week recovery period on green pasture. A diet of oaten chaff and barley straw (1:1) was offered *ad libitum* supplemented with Batch B lablab or with lupins fed at 5, 10 and 20 g/kg LW/day, plus a mineral mix (Table 1). Each diet was fed to three sheep in each period. Intake, digestibility, LW gain, wool growth on midside patches, rumen NH3 and nylon bag disappearance of roughage DM and cracked legume grains were measured.

Experiment 3: Cowpeas, naw beans and lupins for sheep (digestibility)

The digestibility of four diets, roughage alone (oaten chaff plus barley straw, 1:1) or supplemented at 10 g/kg LW/day with cowpeas, navy beans or lupins was measured by total collection over seven days after a 14 day adaptation period with six sheep per diet.

Experiment 4: Cowpeas, naw beans and lupins for sheep (intake and growth)

In a switch-over design, 39 Merino ewes (eight months, 16-31 kg) were held in individual pens for two periods of 49 days. Diets offered were the roughage alone and roughage supplemented with each grain legume, each fed at 5, 10, 20g or 20g plus antibiotics/kg LW/day, plus a mineral mix (Table 1). The antibiotic consisted of 15mg Terramycin and 22mg Flavomycin/sheep/day. Each diet was fed to three sheep in each period. Intake, LW gain and wool growth on midside patches were measured.

Experiment 5 : Cowpeas and lupins

Groups of six or seven two-year old strong wool Merino rams (55-70 kg) grazed winter pasture alone or were supplemented with 5, 10 or 20g/kg LW/day of lupins or cowpeas for 11 weeks. Each week they were rotated among the paddocks. The major purpose was a study of spermatogenesis which will be reported elsewhere. Growth rate, condition score, wool growth on midside patches and fleece weight, backfat thickness and weight of pancreas at slaughter are reported here.

Experiment 6: Lablab and lupins

Sound, seed-grade (Batch B) lablab grain was compared to lupins in concentrate mixtures fed to early-weaned three months old mixed sex (Border Leicester X Merino) X Dorset lambs (21-29 kg) in metabolism cages for 49 days (Table 1). Groups of five lambs received 400 g/day mixed oaten and lucerne hays and *ad libitum* a mixture of 49% ground maize, 2% minerals and 49% mixed ground lupins and lablab in the ratios 100:0, 75:25, 50:50, 25:75 and 0:100. Intake, LW growth and wool growth on midside patches were measured over the last four weeks.

Experiment 7: Agglutination assav

Lectins were measured by the method of Liener and Hill (1953) and Jaffe (1969) in lupins (var Uniharvest), lablab (var Highworth Batch A and B and var Rongai), cowpeas (var Caloona), navy beans (var Actolac, Gallaroy and Kerman). Erythrocytes were used from sheep, goats and cattle. A suspension of enterocytes was made by incubating washed sections of ovine small intestine in collagenase (Sigma Chemical Co) for 15 minutes at 37°. Tests were performed comparing the agglutination by extracts of lupins, lablab, cowpeas and navy beans of enterocytes and erythrocytes from sheep.

Experiment 8: Disappearance of lectins and trypsin inhibitors from nylon baas

Groups of five 8-12 month old Merino wethers weighing 17-28 kg, with ruminal fistulas, were kept in metabolism cages and fed diets of barley straw and oaten chaff ad libitum, supplemented with lupins, Batch A and B lablab, cowpeas or navy beans, plus a mineral mix, for four weeks (Table 2). The nylon bag technique was used to determine the rate of disappearance of roughage DM and legume DM, lectins and trypsin inhibitors from bags containing feeds ground through a 1mm screen and incubated for 3, 6, 12, 24 and 48 hours.

Experimental and analytical techniques

The methods used to measure digestibility, intake, LW gain, ruminal NH_3 wool growth and condition score were standard in this laboratory (Doyle <u>et al</u>. 1988; Dixon <u>et al</u>. 1989). The nylon bag technique of **Orskov** and McDonald (1979) was used.

RESULTS

Experiment 1

All kids consumed all of the grain legume offered. Intake of roughage was depressed by one third at the highest intake of lablab which made up 39% of the diet (P<0.01) and total digestible organic matter intake (DOMI) was unaltered. At the same intake of lupins roughage intake was not changed and DOMI was significantly increased (Table 2). LW gain was significantly reduced by the 12 g/kg lablab diet below that achieved with lupins and below that on similar DOM intakes of 0 and 3 g/kg LW of lablab.

digestibility	and	growth	ΟĬ	KIdS	(n	5)			
							Lablab	Lupins	SEM
Measurement		Nil		3g/}	(g I	W	6g/kg LW 12g/kg LW	12g/kg LW	~

595b

713^{bc}

632^{ab}

576b

508b

76b

117

390a

638ab

674^b

732^C

389ª

32a

248

513b

261

774^C

663^b

690C

543^b

77b

28

na

29

17

20

27

11

517b

55

571^a

619^{ab}

549b

382ª

66ab

0

600a

585^a

378^a

396^a

64ab

Roughage intake g/d 600^b

Legume intake g/d

Total intake g/d

OM dig g/kg

N dig g/kg

LW gain g/d

DOMI g/d

Table 2. Experiment 1. DM intake of roughage and legume supplement, digestibility and growth of kids (n 5)

Experiment 2

Supplementation with 10g/ kg LW of lupins or either grain legume at 20 g/ kg LW depressed roughage intake, but total DM intake, OM digestibility and DOMI increased with all supplements (Table 3). OM digestibility and DOMI were higher for lupins than lablab at high levels of feeding. LW loss on the roughage diet was unaltered by the lowest .level of lablab; all other supplements supported LW gain. Wool production was enhanced more by lupins, particularly at the highest level of feeding. Lupin DM disappeared more rapidly from nylon bags than lablab DM but none of the supplements at any level accelerated disappearance of roughage and the highest level of lupins significantly depressed roughage digestion. Both legumes caused ruminal pH to fall to a minimum of 5.8-6.1 nine hours after feeding. At all times, . lupins resulted in higher NH3 concentrations than the equivalent level of lablab.

Table 3. Experiment 2. Intake, digestion and production of sheep fed roughage and Lablab or lupins (n 6)

Measurements			lablab			<u>lupins</u>		SEM	p
	nil	5g	10g	20g	5g	10g	20g		
Roughage intake (g.	/d)545	513	646	373	560	444	323	45	**
Legume intake (g/d) 0	114	251	424	120	237	483	na	na
Total intake (g/d)	545	628	897	797	680	681	806	48	* *
OM digestibility(g	/kg)536	616	627	690	593	676	737	17	* *
DOM intake (g/d)	271	351	525	518	376	433	560	24	* *
LW change (g/d)	-36	-39	37	64	59	85	105	21	* *
Wool growth (g/d)	74	88	120	103	95	125	142	10	**

Experiment 3

OM and NDF digestibility of the lupin diet tended to be lower than with other legumes, but N digestibility was significantly higher (Table 4).

Measurement		Nil	Lupins	Cowpeas	Navy Beans	Р
Digestibility of	DM (g/kg)	535a	641 ^b	656 ^b	643 ^b	* * *
	OM	539 ^a	634 ^b	659 ^b	652 ^b	* * *
	NDF	520a	556ab	602 ^b	578 ^b	*
	ADF	489	559	534	536	ns
	N	421 ^a	704 ^C	634 ^b	620 ^b	**

Table '4. Experiment 3. Digestibility of roughage alone or with 10g/kg LW of grain legumes (n 6)

<u>Experiment 4</u>

At 5 g/kg LW the three grain legumes did not alter roughage intake, but higher levels reduced roughage intake Table 5). Supplements of lupins and cowpeas at 10 g/kg LW increased total DM intake, LW gain and wool growth (Table 5) but increasing the intake of navy beans beyond the lowest level had little effect and some sheep developed diarrhoea and reduced appetite. At 20 g/kg LW lupins were superior to the other grain legumes for wool production. Antibiotics increased total intake and LW gain with lupin feeding, but not wool growth. With cowpeas, intakes of roughage and grain were reduced; antibiotics increased intake of roughage with navy beans but with neither grain was there a production response to antibiotics.

Table 5. Experiment 4. DM intake, LW gain and wool growth of sheep fed roughage and legume supplements (n 6)

Diet _		Intake (g	/d)	LW gain	Wool growth
R	oughage	Legume	Total	(g/d)	(mg/p/d)
Roughage alone	628	0	628	16	79
" + lupins 5 g/kg	655	132	787	60	104
" " 10 g/kg	527 .	· 261	788	75	122
" " 20 g/kg	341	545	886	136	168
"+ cowpeas 5 g/kg	654	1	775	50	98
" " 10 g/kg	590	278	868	109	134
" " 20 g/kg	384	532	916	127	114
"+ navy beans 5 g/kg	580	113	693	60	96
" 10 g/kg	462	233	695	32	94
" 20 g/kg	350	437	787	87	114
"+ lupins + a'biotic	s 450	540	990	179	160
" cowpeas "	325	373	698	116	na
" navy beans "	453	377	830	90	86
LSD	113	na	132	38	37
Significance	**	na	**	**	**

<u>Experiment 5</u>

While all levels of both supplements increased growth rate, condition score, wool growth and **backfat** thickness, **cowpeas** were slightly less effective on a DM basis for increasing condition score, wool growth or **backfat** thickness than lupins (Table 6). The weights of the pancreas of rams declined with increasing supplementation with either grain legume (P<0.001) and were smaller in animals fed lupins (P<0.05).

Table 6. Experiment 5. Response of rams to supplementation with legumes for 11 weeks (n 6 or 7) $\,$

Supplement	LW gain	Condition	Backfat	Wool growth	Pancreas
	(g/d)	score 1-5	thickness	s (mg/p/d)	(g/kg LW)
Grazing only	80 ^C 163 ^{Cd} 121 ^{de} 256 ^a 108 ^e 205 ^{bc} 230 ^{ab}	2.50 ^C 3.83 ^a 3.75 ^a 3.50ab 3.17 ^b 3.33ab 3.64 ^{ab}	2.00 ^d 4.83 ^{bc} 4.50 ^c 6.86 ^a 4.83 ^{bc} 3.33 ^{cd} 5.00 ^b	86 ^{cd} 106 ^{ab} 110 ^{ab} 111 ^a 83 ^d 91 ^{bcd} 109 ^{ab}	n a 1.14 ^a 0.98 ^b 0.90 ^c 1.19 ^a 1.14 ^a 0.96 ^{bc}

Experiment 6

There were no differences between diets in concentrate intake (mean 722 se 48 g DM/d), total intake (mean 1070 se 48 g DM/d), LW gain (mean 229 se 32) or clean wool growth (mean 145 se 16 mg/p/d) over a range of substitution of lablab for lupins from 0 - 34% of total DM intake.

<u>Experiment 7</u>

Haemagglutination activity of the legumes tested did not vary with the origin of the erythrocytes and were highest for navy beans, lowest for lupins (Table 10). The agglutination activity did not differ between erythrocytes and enterocytes from the small intestine of sheep, confirming that the assay done with blood cells does measure the capacity of lectins to bind to the mucosal cells of the gut.

Table 7. Experiment 7. Lectin (haemagglutinating) activity (HU/mg DM) of grain legumes tested with erythrocytes from three species and enterocytes from sheep

Legume species and variety	RBCs of Sheep	RBCs of Goat	RBCs of Cattle	Enterocytes of sheep	SEM
L. angustifolius var.Unihar	vest 14	12	. 11	22	7.4
L. purpureus var.Highworth	A 28	28	26	23	19.7
<pre>var.Highworth</pre>	B 23	23	22	22	9.1
" var. Rongai	22	22	21	nd	9.1
Vigna unguiculata var. Calo	ona 21	21	20	17	8.3
P. vulgaris var. Actolac	167	167	159	88	67.9
" var. Gallaroy	162	162	157	nd	85.0
" var. Kerman	162	162	157	nd	85.0
Phaseolus lunatus	42	42	42	nd	0
Glycine max	47	47	47	nd	0
Vicia faba	44	44	43	nd	16.9

The means within rows were not significantly different.

Experiment 8

Disappearance of roughage (barley straw and **oaten** chaff) DM from nylon bags was slightly slower in those incubated in sheep fed on navy beans than on other legumes. Disappearance of **lablab** DM was significantly slower than that of other legumes (Table 8).

				Incuba	tion	time (h)
Legume in diet	Material incubated	0	3	6	12	24	. 48
Lupins	Roughage	21	nd	nd	34	47	58
Lablab A	н		н		33	46	43
Lablab B	"			10	33	47	57
Cowpeas			н		28	46	57
Navy Beans		"	H	u	30	43	52
Lupins	Lupins	12	34	50	59	87	96
Lablab A	Lablab A*	9	28	35	48	75	88
Lablab B	Lablab B	13	33	51	66	84	92
Cowpeas	Cowpeas	12	37	48	66	88	94
Navy Beans	Navy Beans	14	36	42	53	90	99

Table 8. Experiment 8. Disappearance (%) of roughage and grain legumes DM from nylon bags incubated in the rumens of sheep on diets containing the legumes

*Significantly different from other legumes

Haemaglutinating activity declined slowly for six hours, except for lupins, but by 12 hours 80%-90% had gone from all except navy beans and at 24 hours no detectible activity remained (Table 9). Activity per mg protein fell indicating that lectins disappeared faster than proteins in general. Trypsin inhibitor activity fell to about 10% of initial levels in six hours and similarly activity fell faster than the decline of total protein (Table 10).

Table 9. Experiment 8. Haemaglutination activity (HU/mg remaining DM or remaining protein) of grain legumes incubated in nylon bags in the **rumens** of sheep fed the same legumes

Treatment	Incubati	.on		Grain	leaume	
	Time	Lupins	Lablab A	Lablab B	Cowpeas	Navy Beans
Activity in DM						
Raw legume	0	14	28	23	21	167
Soaked in saline	≥ 0	13	27	21	19	145
Incubated	3	10	22	21	18	133
	6	6	17	14	13	108
	12	3	4	3	2	48
	24	0	0	0	0	1
<u>Activity in protei</u>	n					
Raw legume	0	37	99	94	78	679
Soaked in saline	e 0	34	96	85	69	573
Incubated	6	22	78	72	46	447

Table 10. Experiment 8. Trypsin inhibitor activity (TUI/mg DM remaining and /mg protein remaining) of grain legumes incubated in the rumen of sheep fed the same legume (n 5) \cdot .

	Incubati	on		Grain legume				
	Time	Lupins	Lablab A	Lablab B	Cowpeas	Navy Beans		
Activity in DM								
Raw legume	n.a.	0.95	1.80	1.58	2.59	3.22		
Incubated	3	0.53	1.01	0.91	1.07	1.30		
	6	0.10	0.20	0.20	0.30	0.30		
Activity in prot	ein							
Raw legume	n.a.	26.6	64.1	63.9	94.2	127.4		
Incubated	6	3.5	3.9	3.2	8.0	7.0		

DISCUSSION

The lower growth of goats fed Batch A lablab than of lupins (Experiment 1) was partly due to reduced DM intake and OMD, partly due to reduced efficiency of utilisation of DOM. Although lectin and trypsin inhibitor (TI) concentrations were twice those in lupins, they are still quite low compared to those in beans known to cause detrimental effects. Additionally, the activities of both anti-nutritional factors were reduced rapidly in nylon bags; however it is possible that this may represent solution rather than destruction by fermentation. When fed in a concentrate diet to sheep, Batch B lablab approached the value of lupins; the difference from Experiment 1 may be due to the species of experimental animal, the batch of lablab or the characteristics of fermentation of proteins in a starch-rich rumen. The limited amount of Batch A prevented experimentation to resolve this.

The nylon bag data in Experiment 2 indicated that cracked lupins ferment faster than lablab while there was no difference when the grains were ground in Experiment 7. The cracked material is likely to resemble more closely the ingesta entering the rumen. This indicates that physical structure of the grains may be important.

Since the roughage, the sheep and the design of the experiments were very similar, the results from Experiment 2 and 4 were pooled. Supplementation had a variable small effect on roughage intake at 5 and 10 g/kg LW, but at 20 g/kg LW, roughage intake was reduced to 54-68% of the unsupplemented level. Increased digestibility. generally resulted in greater DOMI with increasing amount of legume in the diet and this was associated with more rapid growth and wool production. However, diminishing responses were seen at higher levels with legumes other than lupins. For diets containing lupins the regression of LW gain above maintenance on DOMI was :-(r = 0.993, P < 0.001). For cowpeas, data are close Y = -104 + 0.366 Xto this line while lablab and navy beans are generally below. For lupins the regression of wool growth on DOMI was : - Y (mg/day/patch) = 3.4 + 0.242 X (r = 0.948, P < 0.001). Data for all other legumes at all levels lay below this line, with navy beans lowest. This indicates that for LW gain and even more for wool growth, DOM of lupins was utilised more efficiently than DOM of the other legumes.

However, for lupins the regression of LW gain above maintenance on N. . intake was: - Y = -9.1 + 4.36 X (r = 0.971, P < 0.001) while the equivalent regression for other grain legumes was: - Y = -1.8 + 4.37 X (r = 0.80, P < 0.05), almost identical. The regression of wool growth on N intake was, for lupins: - Y = 57.4 + 3.303 X (r = 0.97, P < 0.001) and for other legumes: - Y = 71.4 + 1.94 X (r = 0.75, P < 0.01). Thus N from all these legumes seemed to be utilised with the same efficiency for LW gain but not for wool growth, where lupins were superior.

While no satisfactory method could be found to enable direct comparison of the 2-year old rams of Experiment 5 with the weaners of Experiments 3 and 4, the results were similar in that cowpeas supported LW gains almost as rapid as did lupins, although condition score and backfat thickness were inferior, but were less effective for wool production. For all legumes, the differences were quite small when they were fed at 5 g/kg LW, a level close to what might be fed on farms and were most significant at 20 g/kg LW, at which roughage intake was seriously depressed. However this latter level of feeding is unlikely in practice.

The reduction in weight of the pancreas of the rams (Experiment 5) is the reverse of the result usually observed in laboratory animals. In animals in which normal pancreas weight is more than 3 g/kg LW TI causes hypertrophy, while in those where the pancreas weighs less than 3 g/kg LW, the reverse may occur. There is no explanation for this relationship, to which these sheep conform. The poor wool production is likely to be associated with low supply of sulphur amino acids as the result of several factors : (i) grain legumes are often low in sulphur (ii) much of the sulphur present is found in the relatively poorly digested TI (iii) TI causes increased secretion of trypsin, also a sulphur-rich protein and the trypsin-TI complex is poorly digestible.

The reduced intake of DOM by goats fed high levels of Batch A labab (Table 2) may have been due to slow digestion in the rumen (Table 8). This effect on digestion was less with Batch B. Both batches resulted in lower growth /g DOM intake than lupins, suggesting either an imbalance of absorbed nutrients or a systemic effect of anti-nutritional factors other than lectins or TI since these were at low levels. With navy beans the high level of lectins and TI did not reduce digestibility (Table 4), but may have been responsible for reduced intake and diarrhoea at higher supplementation The reduced conversion of DOM intake to liveweight gain or wool levels. also suggests an imbalance of nutrients or anti-nutritional factors acting Although lectin and TI contents of navy beans were higher systemically. than in the other legumes examined, the values were much lower than those found in some Phaseolus vulgaris cultivars of known toxicity, e.g. black bean (2450 Haemagglutination units/g and 2050 units TI /g) and kidney beans (3560 HU and 1552 units TI, Domingo, 1990).

In conclusion, there may be other anti-nutritional factors operating or the differences between these grain legumes may be due to differences in the balance or amounts of nutrients absorbed. However the inefficiencies of digestion in nylon bags and lower production as LW gain and wool growth cannot be attributed to either TI or lectins measured by the procedures described.

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