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New grain legume for layers

Evaluation of *Lathyrus cicera* as a feed ingredient for layers

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by Colin Hanbury and Bob Hughes

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In submitting this report, the researcher has agreed to AECL publishing this material in its edited form.

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Foreword

Lathyrus species are new grain legumes being trialed by CLIMA in farming systems of southern Australia. They are adapted to low-to-medium rainfall areas and are seen as having a potential area of 100 000 to 300 000 ha. Indications from historical use are that they are a good quality animal feed, however, a neurotoxin ODAP in the seed has been linked with a paralysis known as "lathyrism".

The egg industry is seen as a potential market for *Lathyrus* grain, both *L. cicera* and *L. sativus*. The *L. cicera* cultivar "Chalus" was available in sufficient volume for animal feed testing. Chalus has low levels of ODAP and was developed by CLIMA for commercial production.

In order to demonstrate the safety of Chalus long term feeding trials were necessary. It was also necessary to show that production and egg quality were not adversely affected by Chalus.

The present study found that hens were not affected by the ODAP in the diet. Trace amounts of ODAP were detected mainly in brain tissue. These amounts of ODAP were so little that they will not affect consumers of eggs or bird tissue.

In comparison to field peas Chalus showed equal or marginally better egg production and egg quality characters. Yolk colour was significantly improved by the use of Chalus. Since the price of Chalus is likely to be considerably less than field pea and slightly less than lupins it is highly likely to be a useful ingredient in layer diets. It is concluded that in quality Chalus is at least equal to field pea and it also has higher levels of protein.

Future cultivars of *Lathyrus* species are being developed by CLIMA. The ODAP levels will be as low or lower than Chalus. Accordingly the safety issue of *Lathyrus* for layers will not be an issue in future and the increased adoption of a low priced grain legume will stabilise a supply of grain suitable and competitively priced for the egg industry. Increased interest from the feed industry is likely to influence many growers to consider growing *Lathyrus* species as one of the initial impediments in establishing an industry has been the lack of established markets for the grain.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report is an addition to AECL's range of research publications and forms part of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Tables and figures

Abbreviations

CLIMA	Centre for Legumes in Mediterranean Agriculture, University of Western Australia
ODAP	3-(-*N*-oxalyl)-L-2,3-diamino propionic acid

Executive Summary

Recent research at CLIMA has indicated that *Lathyrus cicera* and *Lathyrus sativus* (grasspea) have potential as grain legumes on 100 000 to 300 000 ha of neutral to alkaline soils in low-to-medium rainfall areas of southern Australia. They are adapted to similar regions to field peas and are quite drought tolerant. They do not have serious disease problems and are envisaged as low maintenance/low cost crops for the purposes of green manure, managing herbicide resistant weeds, forage, hay and grain principally for animal feed. Particularly *Lathyrus sativus* is a human food in large parts of the Indian sub-continent and Ethiopia.

Both of these *Lathyrus* species have been used extensively in the past for animal feed. The principal drawback with *Lathyrus* species is the presence, principally in the grain, of the neurotoxin 3-(-*N*-oxalyl)-L-2,3-diamino propionic acid (ODAP). When consumed in large amounts ODAP can produce a paralysis of the hind legs known as "lathyrism". ODAP was identified in the 1960's and since that time plant breeding has produced lines with low toxin levels. Due to the presence of ODAP *Lathyrus* species have almost disappeared from many regions where they were once cultivated extensively, such as Europe. The newer lines with low toxin levels have not been widely evaluated for animal feed and the present study is the only one to our knowledge that has evaluated low toxin lines in poultry.

Since one of the goals of establishing *Lathyrus* cultivation in Australia was to develop animal feed markets it was decided to investigate the use of *Lathyrus cicera* cultivar Chalus in trials with laying hens. Chalus was released by CLIMA in 1998 and was shown in extensive studies to have low levels of ODAP, about 70% lower than what is found in fields in India for example. Chalus has shown good adaptation across southern Australia and is the first in a series of cultivars that are to be released by CLIMA.

One part of the study was to establish Chalus as safe for both the laying hens and for any consumers of eggs or bird tissue. Since little was known about the fate of ODAP in hens, one aim was to investigate whether after feeding with Chalus that ODAP could be found in eggs or body tissue of hens. The second aim was to demonstrate that Chalus was a good quality feed capable of replacing, for example, field peas in laying hen diets without penalty in egg production.

To investigate the possible residue of ODAP in the hens they were fed up to 30% Chalus in a wheat based diet for a period of 32 weeks from 26 weeks of age. Three hens each were given one of the following diets with an inclusion of Chalus at 0, 5, 10, 15, 25 and 30%, basically as a substitute for field peas. No ODAP was detected in egg white at any time. Egg yolk, breast meat and liver showed trace levels of ODAP, but this was not consistent. After the 32 weeks of feeding the brain tissue showed the most consistent traces of ODAP but levels were 20 times less than that shown in rats when lathyrism symptoms were evident. The trace levels detected in hens and egg yolk were 300 times less than the ODAP levels in the Chalus grain they were fed. Any consumption of these low levels of ODAP in the hens and the eggs would be too low to affect humans or animals. Studies of human consumption of ODAP have shown that regular consumption of grain at levels 3000 times the levels found in this study are sufficient to cause lathyrism symptoms, only if the grain is consumed exclusively and under circumstances of malnourishment. Hence the possibility that consuming eggs or hen tissue as shown here is extremely unlikely to pose any problem. The hens showed no signs of neurotoxicity and deaths in the experiment were minimal and not related to feed type.

Egg production and quality was measured in a flock of 760 hens over an intensive feeding period of 8 weeks at levels of inclusion of Chalus at 0, 5, 10, 15, 25 and 30% and compared to field peas in a wheat based diet. Egg production and quality was as good as the field pea based diet and in some cases showed small improvements and was always at a level expected for the age and breed of the hens. Feed intake was marginally greater for Chalus than field pea. Feed conversion was also

marginally better for Chalus than field pea. Soiling of eggs was shown to be slightly better in Chalus. Egg shell thickness and proportion were unaffected by inclusion of Chalus in the diet, as was the egg weight. However the yolk colour was significantly improved by the inclusion of Chalus.

The conclusion was that in comparison to field pea Chalus was as good a feed ingredient, with no detrimental effects evident. One significant advantage of Chalus is however the likely grain price. Due to the low maintenance nature of the crop it is expected that grain price is likely to be around $140 per tonne. This compares very favourably with field peas at $220 per tonne and even lupins at $190 per tonne. Chalus certainly seems to be a better quality ingredient for layers than lupins are currently. Composition-wise Chalus is almost identical to field peas but has about 2% higher protein levels at about 26%.

The very pleasing results with laying hens bode well for the continued adoption of *Lathyrus* species in low-to-medium rainfall farming systems of southern Australia, especially as the initial problem of establishing cultivation has been the lack of existing markets. Other cultivars are planned with greater adaptation and better agronomic characteristics, the adoption of these cultivars will lead to a stable supply of grain for the egg industry and thus ensuring access to a low cost, high quality feed ingredient.

Wide dissemination of the results of the layer feeding trial will go a long way to establishing *Lathyrus* species as a choice for farmers wishing to grow a grain legume in their crop rotation system. Other pleasing feeding results with pigs and sheep will further stabilise as supply of grain for the egg industry.

1. Introduction

1.1 Background

Recent research at the Centre for Legumes in Mediterranean Agriculture (CLIMA) has shown that two closely related winter grain legume species new to Australia, *Lathyrus cicera* and *L. sativus*, have considerable potential in low to medium rainfall (250-500 mm p.a.) areas of southern Australia (Siddique *et al.*, 1996; Hanbury *et al.*, 1999). They are suited to fine textured alkaline soils in areas where field pea is often grown in rotation with cereals and on-farm yields have generally been in the range 1-2 t/ha. In contrast to field pea they do not have serious diseases which limit sowing times (eg. black spot in field pea), they are more tolerant to waterlogging and are tolerant to a wider range of herbicides. Consequently crop management is relatively simple and low cost. *Lathyrus sativus*, in particular, has a long history of cultivation for human consumption, and remains important in Ethiopia and on the Indian sub-continent. Both *L. sativus* and *L. cicera* have been used widely in the past for animal feed both as fodder and seed but little reliable information is published, most being anecdotal. Both species are seen as having a role in southern Australia as multi-purpose crops providing feed grain, fodder, hay and green manure. However, the presence in the seed of the neurotoxin 3-(-*N*-oxalyl)-L-2,3-diamino propionic acid (ODAP) restrains potential commercial releases to only lines having low ODAP levels. ODAP has been identified as the causal agent of lathyrism (Spencer *et al.*, 1986), a paralysis of the lower limbs, in humans and also in animals. Lathyrism occurs following consumption over an extended period of high levels of ODAP, and is most often associated with *L. sativus*. Recent studies have shown that ODAP concentrations in *L. cicera* (mean 0.18%) are typically lower than in *L. sativus* (mean 0.39%; Hanbury *et al.*, 1999). There are currently no regulations that relate to ODAP levels in foods in Australia or in any other countries, research in this area is due to duty-of-care concerns.

A *L. cicera* cultivar "Chalus" selected by Dr C.D. Hanbury and Dr K.H.M. Siddique was commercially released in 1998 through CLIMA. Chalus was selected due to good yields and consistently low levels of ODAP (0.09%) in comparison to many other *L. cicera* lines tested (range 0.08 -0.34%; Hanbury *et al.*, 1999). Approximately 1.5 t of Chalus seed was distributed to several commercial growers in WA, SA and Vic. in time for the 1998 season, about 25 ha was sown. Technical information on production recommendations as a farmnote for growers was also published (Hanbury and Siddique, 1998) and widely disseminated across southern Australia at field days and other personal contact with growers. In the 1999 growing season some 250 ha was sown in WA, SA and Vic., further increases are expected as more seed is produced and available for sale. In addition 1.5 t of a *L. sativus* cultivar is bulked for commercial release in 2000, awaiting official permission from the Indian Agricultural Research Council (breeders of the original seed). It is expected that the potential area for *Lathyrus* spp. will be 100,000-300,000 ha across the southern Australian dryland cropping regions, with a distribution of 50% in WA, 10% in NSW, 30% in SA/Vic Mallee and Wimmera and 10% in SA Lower Midnorth and Yorke and Eyre Peninsulas.

Following the successful outcomes of the RIRDC project (UWA 21A, 1993-1998) entitled "Development of *Lathyrus* species as new grain legumes" it was apparent that establishing the role of *Lathyrus* spp. in dryland farming systems was necessary. Accordingly a new project was developed entitled "On-farm use and industry development of *Lathyrus* in Australia", which was funded for 1998-2001 by GRDC (UWA 287). This project involved extensive collaborative work in WA, SA, Vic. and NSW with grower groups, other research agencies, feed manufacturers and marketing bodies. Dr C.D. Hanbury was employed on this project.

The aims of this project were:
i. in cooperation with grower groups, to identify the role of *Lathyrus* in southern Australian dryland farming systems and fine tune production packages.
ii. to develop animal feed and/or human food markets for *Lathyrus* both within Australia and overseas.

iii. to further select *Lathyrus* cultivars with improved yield and quality from existing early generation breeding lines.

1.2 Evaluation of *Lathyrus* as animal feed.

Lathyrus cicera and *L. sativus* are closely related species and seed chemical composition is very similar. Protein content of both species is high (26-28%; Petterson *et al*., 1997), Chalus has a protein content of 26% and higher lysine content per g of seed than either lupin or field pea (Table 1). Composition of Chalus is very similar to field pea, except that Chalus has a higher protein concentration. Currently sufficient quantity of Chalus seed is available to assess its use as animal feed. A comprehensive review of the use and potential of *L. cicera* and *L. sativus* as grain for animal feed has been published (Hanbury *et al*., 2000).

Table 1: Composition of *Lathyrus cicera* cv Chalus grain compared to field pea (*Pisum sativum*) and lupin (*Lupinus angustifolius*).

		Chalus	Field pea*	Lupin*
Protein	(% a.r.)	25.9	23.2	32.0
Lysine	(% a.r.)	1.59	1.58	1.49
	(g/16g N)	6.14	6.81	4.66
Fat	(% a.r.)	0.6	1.1	5.9
Fibre crude	(% a.r.)	6.0	5.9	15.4
Lignin	(% a.r.)	0.20	0.53	0.86

*mean data from Petterson *et al*. (1997).

In order to achieve the aim of developing animal feed markets the animal feeding rate recommendations must be established experimentally. Due to the presence of ODAP it is essential to establish confidence in *Lathyrus* spp. as feed amongst potential users. Anecdotal evidence suggests that monogastrics are more susceptible to effects of excessive ODAP consumption than ruminants. Consequently, as part of the current GRDC funded project there were included a range of animal feeding studies:

- A preliminary *in sacco* study with fistulated cattle conducted in 1998 in collaboration with Dr Colin White, Principal Research Scientist, CSIRO Division of Animal Production showed that protein in Chalus was 93% degradable, compared to 94% in chickpea, faba bean and field pea and 90% in lupins. Protein had a quickly soluble fraction of 53% compared to lupins at 35%.

- A preliminary standard broiler chicken assay conducted in 1998 by Mr Bob Hughes, Pig and Poultry Production Institute, SARDI indicated that apparent metabolisable energy of Chalus was 12.3 MJ/kg comparing favourably with field peas (12.0 MJ/kg) and lupin (10.0 MJ/kg). No detrimental effects on birds were recorded.

- A full inclusion rate trial with pigs grown from 20-110 kg body weight (97 days of feeding) was completed in February 1999 in collaboration with Dr Bruce Mullan, Senior Research Officer, Dept of Agriculture WA (Table 2). All measured characters showed no difference between performance on Chalus relative to soybean meal, this applied to both the initial period (0-40 day) and the total (0-97 day) feeding period. Average daily gains were above the industry average (750 g) and feed conversion rates were substantially lower than the industry average (2.60). Post-slaughter examinations showed no indications of detrimental effects of ODAP on animal health and internal organs. Preliminary results have been published (Mullan *et al*., 1999). ODAP in animal tissue was not investigated.

Table 2: Summary of *Lathyrus cicera* cv Chalus inclusion rate trial for pigs fed from 20-110 kg live weight (soybean used as standard diet).

Character	Chalus inclusion rate (%)				LSD (P<0.05)
	0	10	20	30	
Average daily gain (g)	899	896	895	912	69.7
Voluntary feed intake (kg/d)	1.96	1.76	1.77	1.81	0.290
Feed conversion rate (feed consumed/live weight increase)	2.18	1.97	2.05	1.99	0.279

- A sheep feeding study completed in December 1999 in collaboration with Dr Colin White, Principal Research Scientist, CSIRO Division of Animal Production compared Chalus with lupins fed *ad libitum* to immature Merino wethers over a 10 week period (White *et al*., 2002). Chalus and lupin were fed at two rates, 35% and 70% of the diet, the remainder being hay. There were 20 sheep per diet group, fed individually. The sheep fed Chalus showed greater liveweight gains, dressed carcass weights, voluntary feed intakes and efficiencies of feed conversion than those fed lupins (Table 3). There was no behavioural indication of animal ill health, nor were there any apparent problems with the animal carcasses. Biochemical analyses indicated good animal health throughout the experiment. Quality testing of meat showed equal or better results for Chalus compared to lupins. Overall, the results indicated that sheep fed the Chalus diets performed better than those fed the lupin diets. Testing of sheep loin muscle for ODAP showed no detectable traces (ie below 0.1 ppm).

Table 3: Summary of *Lathyrus cicera* cv Chalus inclusion rate trial for sheep fed for 10 weeks. Compared to hay only and lupin (*Lupinus angustifolius*).

Character	Hay	Lupin (%)		Chalus (%)		LSD (P<0.05)
		35	70	35	70	
Average liveweight gain (g/d)	84	136	119	164	187	26
Voluntary feed intake (kg/d)	1.17	1.26	1.02	1.42	1.40	0.17
Feed conversion efficiency (% live weight increase/feed consumed)	5.5	8.0	6.6	9.7	12.8	1.1

It is highly likely that the level of ODAP in Chalus (and in future *Lathyrus* cultivars) is sufficiently low not to pose a problem for monogastrics, as shown in the large pig feeding trial and to a limited extent by the preliminary broiler assay. This is consistent also with the pleasing performance of ruminants on Chalus to date. Chalus is seen as a low production cost (and hence low priced) grain legume for animal feed purposes, the egg industry in southern Australia is an industry to which it is suited. The suitability and levels of inclusion, however, need to be established experimentally as no studies have been performed.

1.3 Economics

The potential economic value of *L. cicera* cv Chalus to the egg industry was assessed by parametric cost analysis of Chalus included in typical layer diets with commercially relevant nutrient specifications and ingredient costs current in SA in February 2000 by Mr Fil Ciancio, Ridley Agriproducts. Nutrient specifications for Chalus were based on the compositional analyses (minerals, amino acids, energy) performed by CLIMA.

The inclusion rate of Chalus in a high specification layer diet suitable for high performance breeds of hens from 18-30 weeks of age rose quickly from 8.8 to 12.3% when the cost of Chalus fell below $136/tonne. Similarly, the inclusion rate jumped from 6.6% to 11.1% when the price fell below $126/tonne for Chalus in a layer diet with lower specifications for older hens.

The least cost diet formulations were very sensitive to small changes in protein content of Chalus (and presumably amino acids). A relatively small increase in protein content (from 25 to 26%) could result in significantly higher inclusion rates of Chalus in diets with similar nutrient specifications provided other raw ingredients did not change in nutrient levels or prices.

Estimates of cost of production of Chalus by grain producers is $90-$100 per ha. With on-farm yields of the order of 1-1.5 t/ha it is anticipated that $140 per tonne would be a first estimate of price. However, yields are expected to improve with grower experience with what is still a new crop, consequently price per tonne would be expected to drop.

On the basis of these preliminary cost assessments, we conclude that Chalus is a potentially useful ingredient for inclusion in layer diets provided it can be shown to support a cost effective level of performance in hens without detriment to the quality of eggs, or excreta condition.

2. Objectives

To establish *Lathyrus cicera* cv Chalus as a low cost grain legume of sufficient quality to be included in layer hen rations. To be achieved by evaluation of layer performance, visual observations, egg quality and incidence of soiled eggs in long and shorter term layer feeding trials at inclusion rates up to 30% Chalus.

3. Methodology

In order to assess the performance and safety aspects of layers using Chalus it was necessary to do three things:

- To establish that ODAP does not present a health issue to consumers of eggs or bird tissue.
- To show that the presence of low levels of ODAP in Chalus does not have a deleterious affect on layer performance.
- To demonstrate that inclusion of Chalus in the diet shows similar performance in terms of egg production, egg quality and soiling of eggs to current industry accepted diets.

Accordingly three layer hen experiments were carried out in 2000/2001 as follows.

3.1 Long term feeding trial No. 1.

Prior to commencement of a costly feeding study, it was essential to assess the risk of accumulation of ODAP in layers and subsequent transfer to eggs for human consumption. Thus the purpose of a long term feeding study commenced at the start of the project period was to provide information on ODAP in eggs and tissues of hens. A small flock of hens were given a wheat-based diet containing 0, 15 or 30% *L. cicera* cv Chalus (for composition of diets see Table 9, for chemical composition of L. cicera cv Chalus see Table 10, both tables in Appendix). Each diet was given to six hens for a period of 15 weeks commencing when hens were 18 weeks of age. Eggs were sampled for analysis of ODAP at the beginning and at weeks 4, 8 and 15. Breast meat, liver and brain samples were analysed at week 15. All of the eggs and tissues not required for analysis were destroyed. This experiment was commenced on 31 August 2000.

3.2 Intense short term feeding study.

There were 6 dietary treatments of wheat-based diets with Chalus included at 0, 5, 10, 15, 25 and 30% Chalus (for composition of diets see Table 9, Appendix). Each diet was fed to 16 replicates of 8 birds each (2 cages of 4 birds) for a period of 8 weeks. Bird weights were recorded at the beginning and end of the experiment. Bird behaviour was observed daily for neurotoxic symptoms. The experimental diets were commenced on 4th May 2001 when the birds were 26 weeks old. Feed consumption was monitored weekly.

In the seventh week excreta from each cage was collected for moisture determination. In the final week egg weight of all eggs laid in a 3 day period was recorded. Shell characteristics and yolk colour of approximately 600 eggs (100 eggs per treatment) were measured. The proportion of dirty eggs was visually assessed. On the final day four samples (pooled) of eggs, breast meat, whole brain and liver per treatment were sampled for analysis of ODAP.

3.3 Long term feeding trial No. 2.

An additional long term feeding study was conducted on some birds retained after the 8 week feeding study described above. Each of the six dietary treatments was given to three birds for a further period of 24 weeks. Eggs were analysed for ODAP after a further 12 weeks (on 19th September 2001), eggs and tissues were analysed for ODAP at the end of the study (on 5th December 2001) as done previously. All of the eggs and tissues not required for analysis were destroyed

All of the ODAP analyses were performed at the Chemistry Centre WA under supervision of Dr David Harris. Samples were extracted with water and analysed using HPLC with fluorescence detection enabling detection down to 0.1 ppm. There were no signs of neurotoxic effects during any of the three experiments, consequently there was no need to euthanase any birds apart from those included in the scheduled tissue analysis as previously described.

4. Results

4.1 Production and quality

4.1.1 Long term feeding trial No. 1

The first long term feeding trial showed no detrimental effects of Chalus on hen performance, egg production and feed intake. These were all typical for the breed and age (data not shown). However, this experiment was not designed to rigorously test production or quality as the number of birds on each treatment was small but adequate for ODAP assessment.

4.1.2 Intense short term feeding study.

There was no significant effect of diet on egg production (Fig. 1) in either the first four weeks, the last four weeks or over the total time of the experiment. Egg production was typical for these birds at this age (26-34 weeks; Fig. 2). Bird weights at the end of the 8 week period were not significantly affected by inclusion of Chalus in diets, similarly weight gains were also unaffected (Table 4).

Table 4: The effect of Chalus content in the diet on hen body weights and weight gain over the 8 week intense experimental period. Mean weights with standard errors (se).

Chalus (%)	Start weight mean (kg)	se	End weight mean (kg)	se	Weight gain (kg)	se
0	1.905	0.023	2.020	0.022	0.116	0.017
5	1.864	0.019	1.999	0.022	0.135	0.028
10	1.914	0.028	2.050	0.023	0.135	0.018
15	1.902	0.009	2.016	0.016	0.113	0.013
25	1.875	0.027	2.027	0.019	0.152	0.033
30	1.883	0.026	2.035	0.021	0.151	0.023

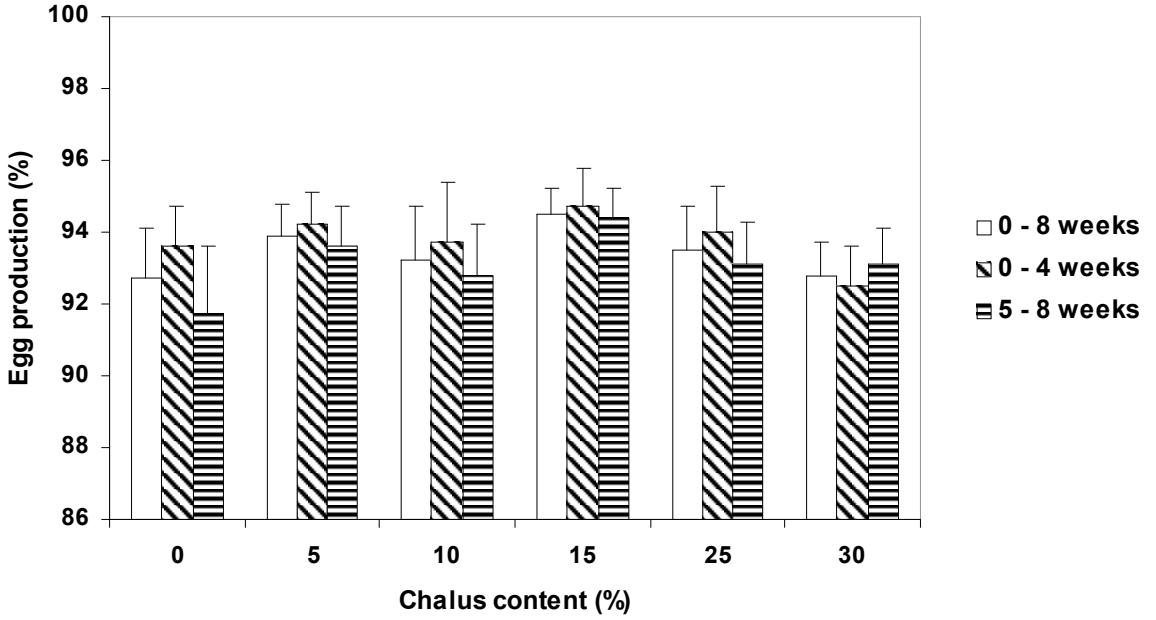

Fig. 1: The effect of Chalus content in the diet on hen-day egg production. Means with standard error bars.

Fig. 2: Hen-housed egg production of flock used for *Lathyrus cicera* cv Chalus experiment. Arrow indicates start of the 8 week intense experimental period.

The effect of inclusion of Chalus in the diet had a significant effect ($P<0.05$) on feed intake only in the first four weeks of the experiment (Fig. 3), the 25% and 30% diet birds consumed typically 3-4% more than the controls, by the last four weeks of the experiment this effect was not apparent. At all other times and diets there was no significant effect of inclusion rate on feed intake. There was no significant effect of Chalus inclusion on the feed conversion rate (Fig. 4).

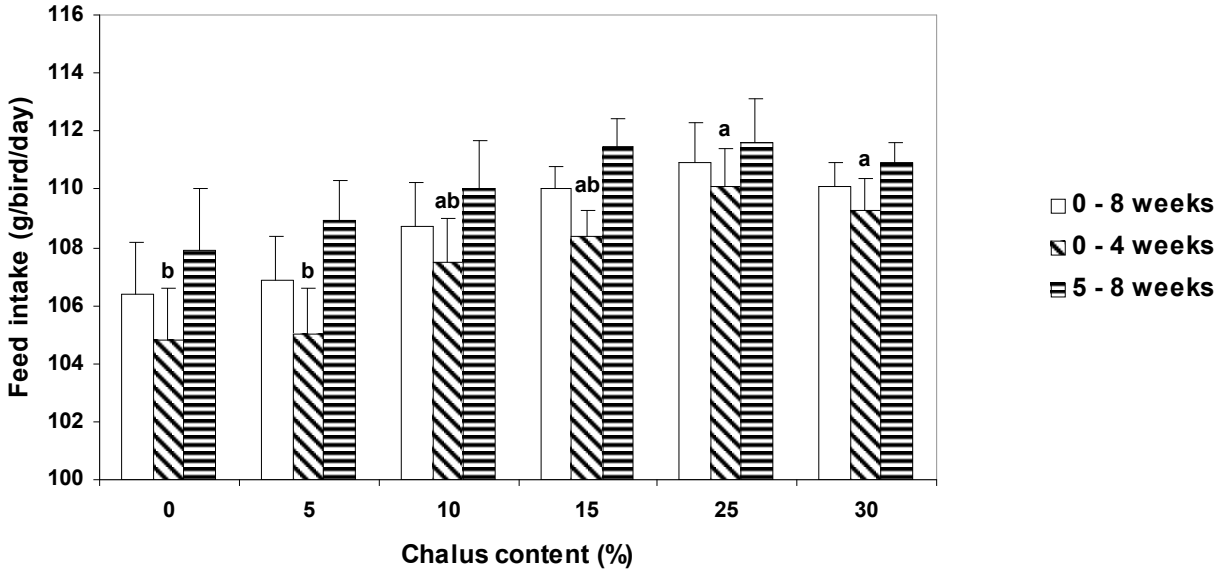

Fig. 3: The effect of Chalus content in the diet on feed intake. Means with standard error bars. Significant differences only in 0-4 week period where the means with a common letter are not significantly different ($P<0.05$).

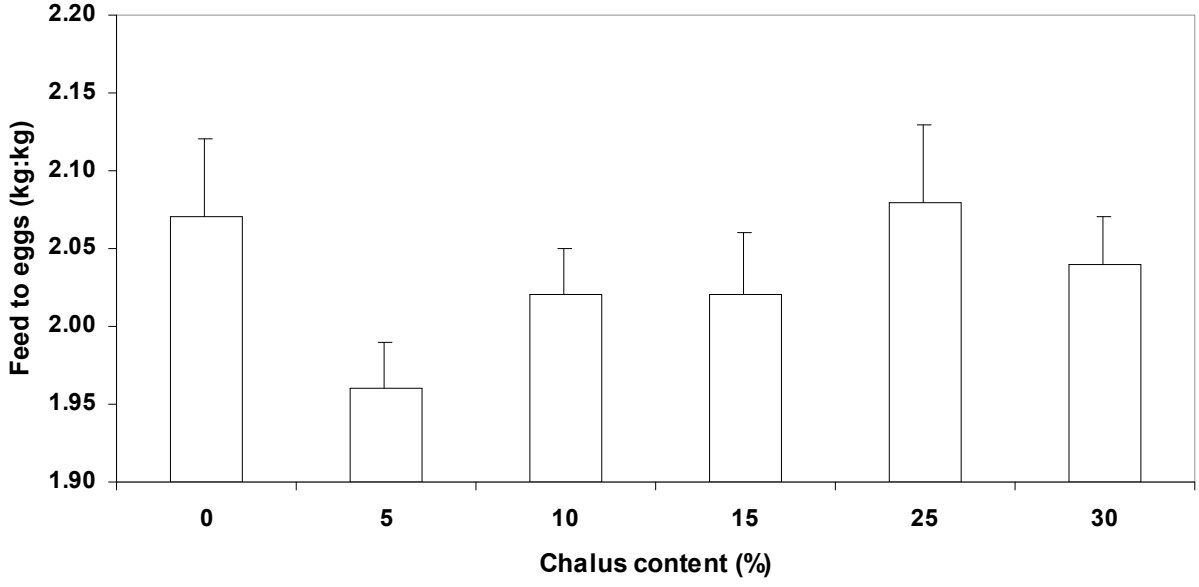

Fig. 4: Effect of Chalus content of diet on conversion rate of feed to eggs at 34 weeks of age (means ± standard errors).

There were no significant effects of Chalus inclusion on the quality characteristics of egg weight (Fig. 5), shell proportion (Fig. 6) and shell thickness (Fig. 7). However, there was a significant increase in the Roche score (≈ 0.5 units) for yolk colour, higher in all Chalus inclusions except for 15% (Fig. 8).

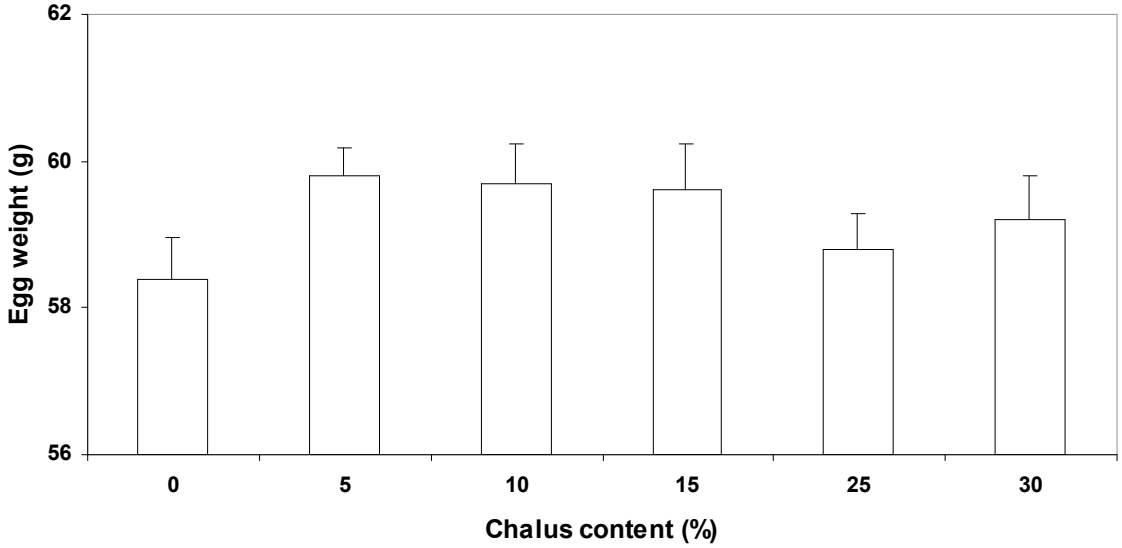

Fig. 5: Effect of Chalus content of diet on egg weight at 34 weeks of age (means ± standard errors).

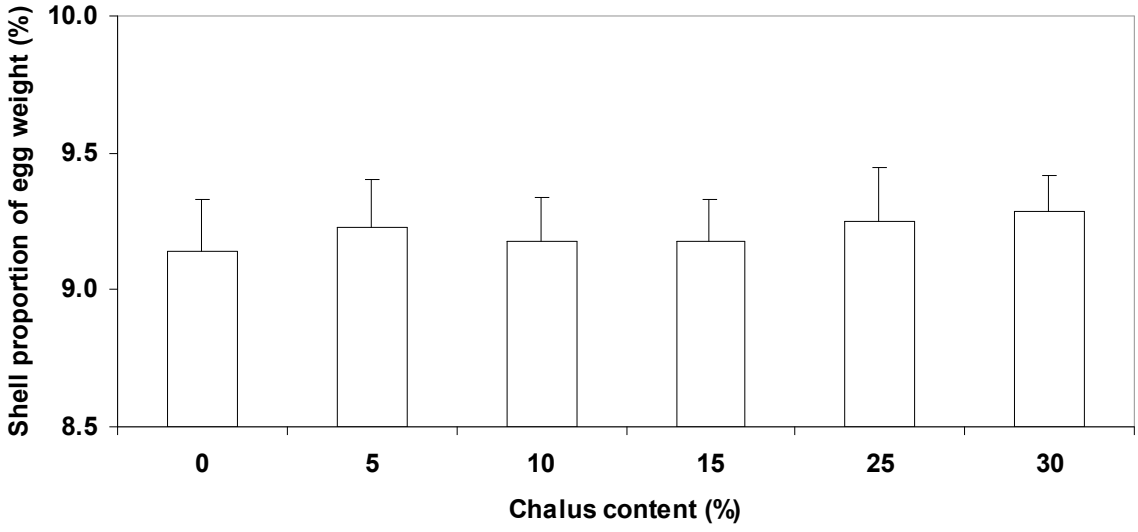

Fig. 6: Effect of Chalus content of diet on shell proportion at 34 weeks of age (means ± standard errors)

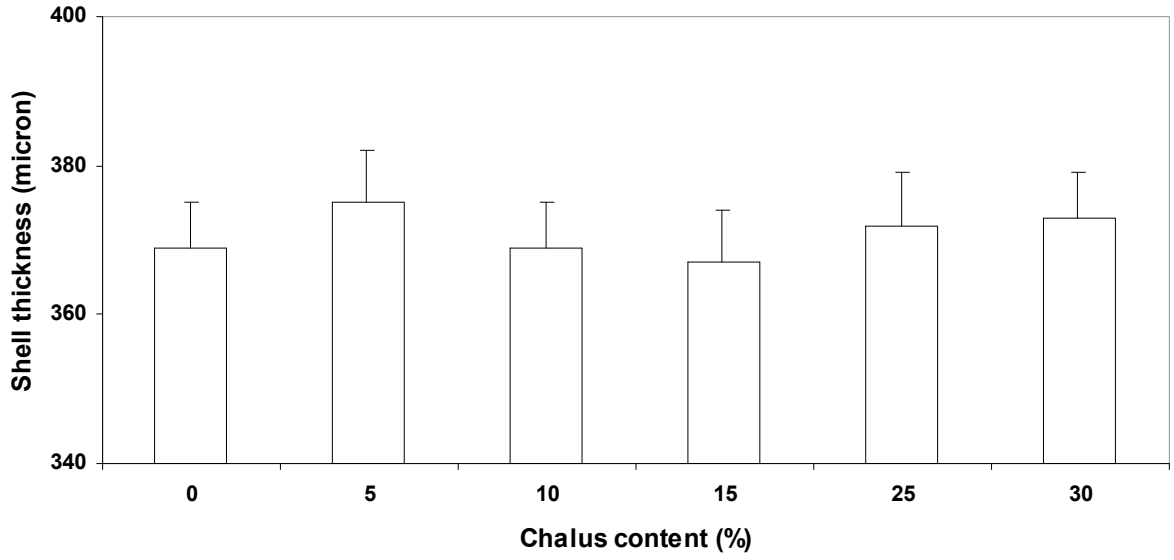

Fig. 7: Effect of Chalus content of diet on shell thickness at 34 weeks of age (means ± standard errors)

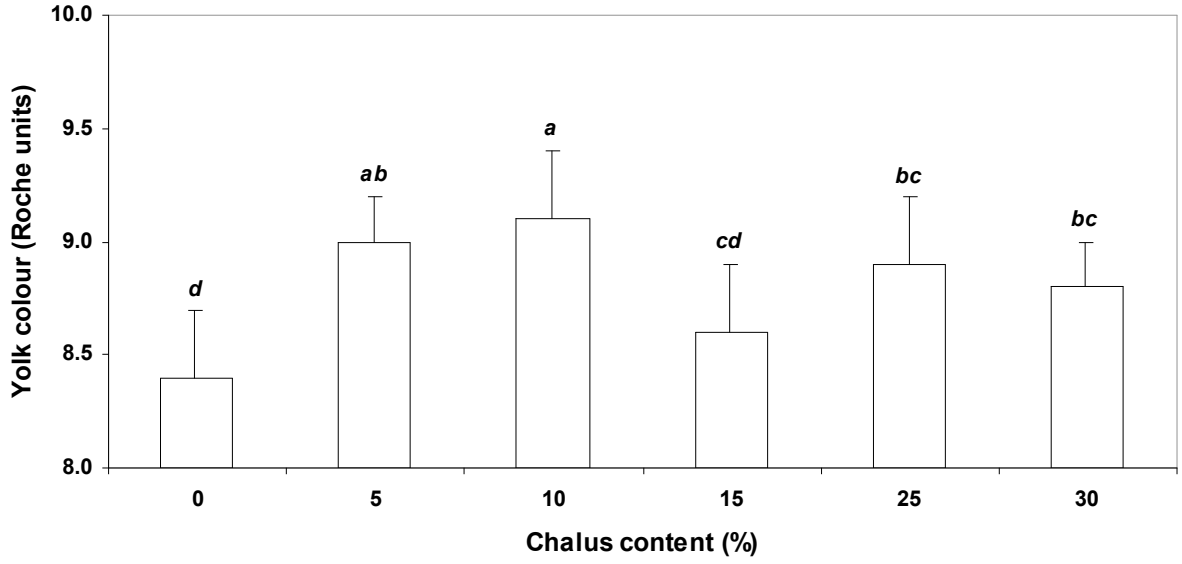

Fig. 8: Effect of Chalus content of diet on yolk colour at 34 weeks of age (means ± standard errors). Significant effect (P < 0.05) of diet, means with a common letter are not significantly different.

The incidence of soiled eggs was in general lower with Chalus inclusion than in the controls but this was not significant (P<0.05). However, at 15% inclusion the incidence was significantly lower (Fig. 9). The results for soiled eggs were mirrored in the similarly small (and not significant, P<0.05) reductions in excreta moisture when Chalus was included in the diet (Fig. 10).

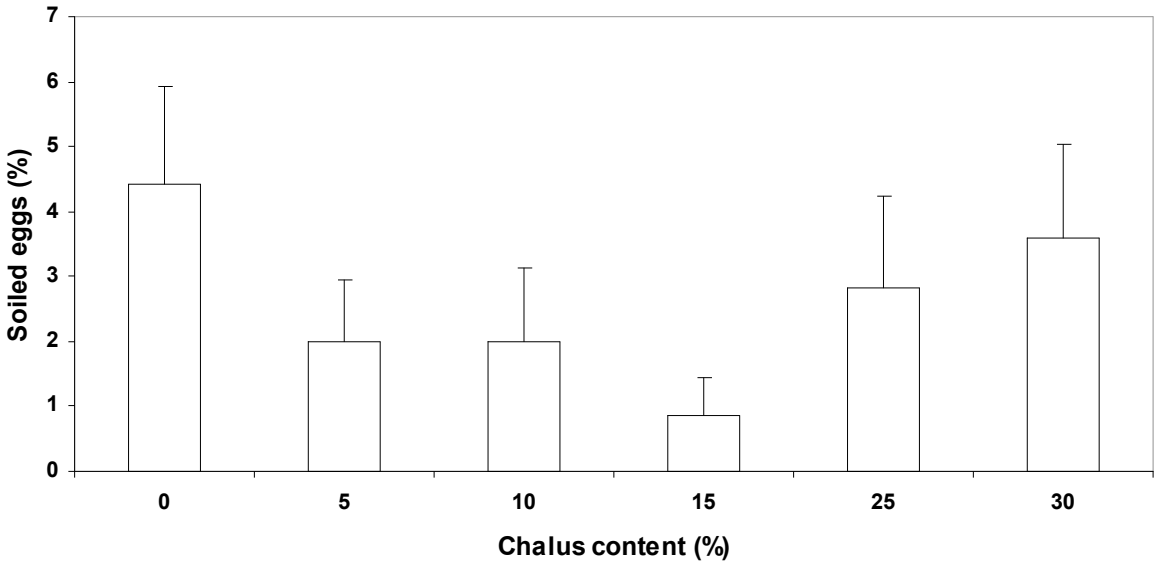

Fig. 9: Effect of Chalus content of diet on incidence of soiled eggs at 34 weeks of age (means ± standard errors).

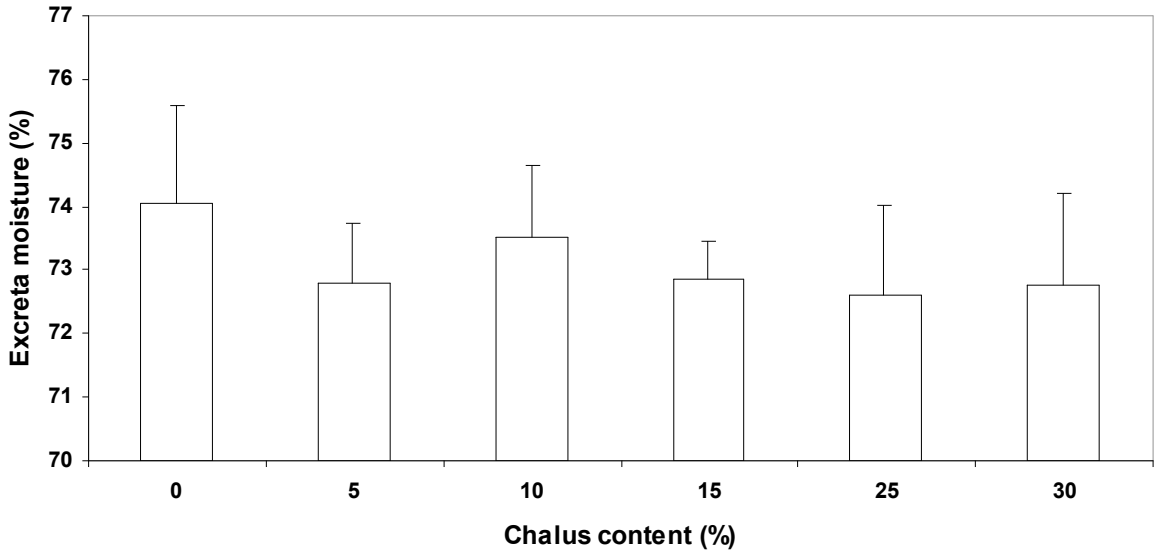

Fig. 10: Effect of Chalus content of diet on excreta moisture content at 33 weeks of age (means ± standard errors).

4.2 ODAP analyses

4.2.1 Long term feeding trial No. 1

There was little indication that ODAP accumulated in tissue and eggs of birds fed Chalus. There was no indication that ODAP occurred in egg white or yolk. Similarly at the end of the long term feeding there was no indication of ODAP in breast meat or brain tissue. However, in the liver a trace of ODAP was detected, this was consistent in both of the Chalus containing diets and was approximately proportional to the content of Chalus in the diet (Table 5).

Table 5: Effect of Chalus content of diet on concentration of ODAP in the liver following an extended feeding period of 15 weeks in long term feeding trial No. 1.

Chalus (%)	ODAP (ppm)
0	0.0
15	0.2
30	0.4

4.2.2 Long term feeding trial No. 2

Similarly to the first long term feeding experiment no ODAP was detected in any egg white. In contrast to the preliminary experiment there was no indication that ODAP was present in the liver. The egg yolk showed trace levels of ODAP in the 8 and 20 week samples, by the 32 week samples only one treatment (25% Chalus) showed the minimum detectable level (0.1 ppm; Table 6). Breast meat also showed the minimum detectable level (0.1 ppm) only in the diet containing the highest proportion of Chalus (30%) at week 32. The brain showed the most consistent detection of ODAP at week 32, occurring as traces in all Chalus containing diets (Table 7). There was, however, no indication of effects of neurotoxicity in any of the hens and mortality was minimal (approx. 1-5%) in hens fed all diets including the control (Table 8).

Table 6: Effect of Chalus content of diet on concentration of ODAP in egg yolk sample over an extended feeding period of 32 weeks in the second long term feeding experiment.

Time on diets (weeks)	0	8	20	32
Chalus (%)	**ODAP in yolk (ppm)**			
0	0.0	0.0	0.0	0.0
5	0.0	0.1	0.1	0.0
10	0.0	0.5	0.0	0.0
15	0.0	0.8	0.7	0.0
25	0.0	0.0	0.2	0.1
30	0.0	0.0	0.2	0.0

Table 7: Effect of Chalus content of diet on concentration of ODAP in brain sample after an extended feeding period of 32 weeks in the second long term feeding experiment.

Chalus (%)	ODAP (ppm)
0	0.0
5	0.5
10	0.8
15	0.4
25	0.7
30	0.7

Table 8: Mortality in hens fed for 8 weeks on diets containing a range of inclusion of Chalus.

Character	Proportion of Chalus in diet (%)					
	0	**5**	**10**	**15**	**25**	**30**
Deaths	5	4	5	1	6	5
Total no. hens fed	128	128	128	128	128	128

5. Discussion

Overall the presence of ODAP in the grain resulted in no appreciable residues in eggs (yolk or white), breast tissue, liver or brain. Traces occurred inconsistently in all samples, excepting egg white where none was ever detected. The longest term feeding (32 weeks, from age 26 weeks to 58 weeks) consistently showed traces of ODAP in the brain. This is consistent with the known ability of ODAP to cross the blood brain barrier. The low levels of ODAP did not affect the hen behaviour or production, indicating that the threshold of tolerance was not exceeded. Symptoms of lathyrism in young rats have been associated with brain ODAP levels of 19 ppm (Cheema *et al*., 1969), about 20 times what was recorded in hens in the current study. Since the brain tissue was analysed following 32 weeks of continuous feeding it is extremely unlikely that laying birds fed any longer than this will show anything more than a trace level of ODAP. The brain concentration of ODAP does not pose any threat to potential consumers, either human or other animal, of the brain tissue. For example in the current experiments the diet containing 30% Chalus (the highest level of consumption) would contain on average 0.027% (equivalent to 270 ppm) ODAP. This is about 300 times the highest level detected in the birds or their eggs in the present study. The consumption of this material by humans is extremely unlikely to pose any health risk. Consumption of *Lathyrus* grain (containing at least 3000 ppm ODAP in countries with significant production of *Lathyrus* spp., eg India, Bangladesh and Ethiopia) does not present a problem as a regular part of a human diet, however, in cases of extreme malnourishment and exclusive consumption of only *Lathyrus* grain symptoms of neurotoxicity have been observed (Lambein, 2000). The feeding of Chalus to other monogastrics has proved to be safe and to not reduce growth at up to 30% inclusion in the diet (Mullan *et al.*, 1999). Similarly sheep fed 70% Chalus showed excellent performance (White *et al.*, 2002).

The assessment of egg production and quality showed that Chalus had no negative effects and in some cases had small positive effects in comparison to the field pea based control diet. The production of eggs was unaffected by the inclusion of Chalus in the diet and was at all times typical of expectations. Feed intake was marginally increased by including Chalus and generally increased with increased inclusion rate. Feed conversion rate was not significantly affected by Chalus but was modestly improved in four of the five Chalus diets. The excreta moisture was not significantly affected by the inclusion of Chalus, but there were indications that soiling of eggs was slightly reduced in comparison to the control diet, although even the levels found in the controls are below what would be found in industry situations.

The characteristics of shell proportion and thickness were unaffected by Chalus inclusion. Egg weight showed non-significant increases with Chalus inclusion. The Roche yolk colour score was significantly improved in four of the five diets containing Chalus. The increases of 0.5 Roche units are significant and may present an opportunity for an industry saving not envisaged in the original experimental proposal. However, it is expected that most producers would opt for safety by not reducing dietary concentrations of yolk colorant, particularly if this could pose a threat to value of eggs or to market share.

Certainly in comparison to field pea (commonly accepted as a good ingredient for layer diets) Chalus has proved to perform as well in terms of egg production. This has certainly fulfilled the objective of establishing Chalus to be a grain legume of sufficient quality to be included in layer hen diets.

6. Implications

Since Chalus (and other *Lathyrus* cultivars) are likely to be marketed at prices significantly lower than field pea (currently $220 per tonne), there is a clear opportunity for egg producers to decrease their feed costs without any penalty in egg production, feed efficiency or product quality. It is highly likely that Chalus (and other cultivars) will be marketed at prices less than lupins (currently $190 per tonne), but Chalus has shown in the current experiments to not have any detrimental effect on hen performance. Production prices for *Lathyrus* cultivars are still difficult to estimate. Original estimates still stand at about $140 per tonne as a starting figure for grain price, this was arrived at from estimates of the costs of grain production (approximately $100 per tonne: see Introduction). Considering that the current study has shown that Chalus performs equally well to peas this demonstrates a significant advantage over peas in terms of cost per dozen eggs.

One of the problems to be overcome is the lack of an established market (such as animal feed) to encourage growers to produce the grain. The positive results of studies such as this one are expected to boost confidence of growers that there is a market for the grain. Currently farmers are being encouraged to produce the grain for on-farm consumption, principally by sheep. The interest shown by pig producers and hopefully now egg producers can only assist in convincing growers to shift their interest from on-farm consumption to selling for feed milling purposes.

Further production of new *Lathyrus* cultivars is planned by CLIMA. These cultivars are expected to considerably improve yield, ease of production and the range of suitable growing environments. This in turn will provide a potentially larger supply of grain for the feed industry. Additionally there is already another cultivar (Lath BC) released in South Australia (through SGB Australia Ltd.) which has shown considerable potential in regions of mild winter temperatures. The adoption of this crop, despite its obvious advantages, has been severely limited by the absence of established markets. Any significant interest in the feeds industry is likely to lead to a rapid increase in production acreage, which will then result in a fall in the price of production and future cheap feed options for egg producers. The potential problem of ODAP has been examined and found to be of no threat in the present study, therefore the future use by the egg industry of new *Lathyrus* cultivars with low levels of ODAP and better agronomic attributes is free of impediment. Since the compositions of *L. cicera* and *L. sativus* are very similar (Hanbury *et al.*, 2000), cultivars of both species are highly likely to show similar good quality attributes to Chalus.

7. Recommendations

It is recommended that the positive findings of this study be disseminated to the feeds industry widely together with contact details for the researchers and other interested parties, such as CLIMA and SGB Australia Ltd. who are involved in producing *Lathyrus* cultivars adapted to different agro-climatic zones across southern Australia. The grains researchers will provide linkage between the feeds industry and those growers involved in adoption of new legume production systems, but not already considering production of a new legume such as Chalus specifically for the feed industry. This will provide sufficient feedback to encourage those grain producers with ready access to feed millers to consider producing Chalus or other *Lathyrus* cultivars in the short term. In the longer term this will provide an incentive for growers in more distant locations who may consider growing Chalus for other benefits such as green manure or on-farm animal use to market their grain also. Further economic analysis is recommended now that some substantial production data has been generated. This will almost certainly provide a more encouraging result than the preliminary analysis and further lead to enthusiasm on the part of feed millers to seek grain supplies.

Should future work encourage more interest from the pig industry and broilers this could only increase the availability of the grain and decrease the production price. This would provide the egg industry with the attractive prospect of further supplies of a cheap, good quality plant protein source.

The significant increases in yolk colour have been noted for Chalus in the present study and for lupins in recent unpublished work. Further research on the possible altered digestibility of fat when using these feed ingredients is warranted, the possible decreases in use of colorant may present a significant improvement in efficiency of production.

The future releases of *Lathyrus* spp. cultivars (both *L. cicera* and *L. sativus*) should be assessed for performance in feed diets. The present study has shown that the presence of ODAP at low concentrations has no negative effect on egg production. Newer cultivars will have similar or lower ODAP levels in the grain, accordingly the production value of other cultivars is the only factor that would need to be considered. In the present project a considerable amount of the budget was devoted to determining the safety issue of ODAP residues. Obviously this will not be necessary in any future cultivars, with similar ODAP concentrations to Chalus, that may be proposed as feed grains for the egg industry.

Part of the current application for an Australian Poultry Cooperative Research Centre involves development of in vitro techniques for assessing grain quality. Some recent work has been performed on the use of NIR techniques for the measurement of ODAP (Jaby El-Haramein *et al*., 1998) which have given results that correlated well with better established but more expensive techniques for measuring ODAP in grain (such as capillary zone electrophoresis and HPLC). This would be cheaply and readily adaptable to the requirements of the feeds industry to ensure that ODAP levels do not rise above a safe limit in grain deliveries. High ODAP levels are extremely unlikely to occur since ODAP concentration is genetically determined and influenced by growing environment to a much lesser degree (Hanbury *et al.*, 1999).

8. Appendices

Appendix 1

Table 9: Components of diets used in Chalus feeding trials at PPPI in 2000/2001.

	Proportion of Chalus in diet (%)					
	0	**5**	**10**	**15**	**25**	**30**
Ingredients (%)						
Wheat	51.50	51.70	51.80	52.00	52.20	52.30
Peas	30.00	25.00	20.00	15.00	5.00	0.00
Lathyrus cicera cv Chalus	0.00	5.00	10.00	15.00	25.00	30.00
Meat and bone meal	7.80	7.70	7.60	7.50	7.28	7.20
Marble powder	7.10	7.05	7.06	7.05	7.04	7.00
Marble chips	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	0.00	0.05	0.08	0.10	0.15	0.20
Tallow	1.20	1.13	1.10	1.00	1.00	1.00
Sunflower oil	0.50	0.50	0.50	0.50	0.50	0.50
Yolk colorant	0.20	0.20	0.20	0.20	0.20	0.20
Sodium bicarbonate	0.20	0.17	0.16	0.15	0.13	0.10
DL-methionine	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin/mineral premix	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride (60%)	0.04	0.04	0.04	0.04	0.04	0.04
Millrun premix diluent	0.16	0.16	0.16	0.16	0.16	0.16
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated composition (%)						
Fat	4.20	4.10	4.00	3.90	3.90	3.90
Ca	3.75	3.74	3.75	3.75	3.75	3.75
P total	0.58	0.58	0.58	0.58	0.58	0.58
P available	0.39	0.39	0.39	0.39	0.40	0.39
Meth	0.42	0.42	0.42	0.42	0.42	0.42
Lys	0.84	0.84	0.84	0.84	0.83	0.83
Linoleic acid	1.03	1.03	1.04	1.04	1.04	1.04
Na	0.16	0.15	0.15	0.15	0.15	0.15
K	0.53	0.53	0.53	0.52	0.52	0.52
Meth + cys	0.74	0.74	0.74	0.74	0.73	0.73
ME (MJ/kg)	11.49	11.50	11.51	11.51	11.54	11.54
Protein (N x 6.25 %)	17.47	17.50	17.50	17.53	17.54	17.55

Appendix 2

Table 10: Chemical composition of *Lathyrus cicera* cv Chalus as analysed by Chemistry Centre (WA) prior to the present study.

Nutrient	Units	Value
Moisture	**%**	10.6
Fat	%	0.7
Protein	%	27.6
Ash	%	3.1
NDF (enzyme modified)	%	24.5
ADF	%	10.7
Starch	%	42
Lignin	%	0.2
NFE (nitrogen free extractives)	%	61.8
In vitro digestibility (IVD)	%	80
Minerals		
P	%	0.33
K	%	0.91
Na	%	0.07
Ca	%	0.25
Mg	%	0.13
S	%	0.17
Fe	mg/kg	156
Mn	mg/kg	11
Zn	mg/kg	20
Cu	mg/kg	9
Essential amino acids		
Cystine	g/16g N	1.23
Methionine	g/16g N	0.82
Threonine	g/16g N	3.68
Valine	g/16g N	4.57
Isoleucine	g/16g N	4.01
Leucine	g/16g N	7.36
Phenylalanine	g/16g N	4.46
Lysine	g/16g N	7.13
Histidine	g/16g N	2.45
Arginine	g/16g N	8.81
Antinutritionals		
Tannins (total)	%	1.08
Tannin, catechin	%	0.51
Trypsin inhibitor activity	g/kg	2.07
Oligosaccharides	%	4.12
Chymotrypsin inhibitor activity	g/kg	3.46
Phytic acid	%	0.91
ODAP	%	0.09

Appendix 3

Publications related to this project

Proc. Aust. Poult. Sci. Symp. 2002, 14.

NUTRITIVE VALUE OF DWARF CHICKLING *LATHYRUS CICERA* CV CHALUS FOR LAYING HENS

R.J. HUGHES[1] and C.D. HANBURY[2]

Dwarf chickling *Lathyrus cicera*, a winter grain legume species new to Australia, has considerable potential as an alternative to field pea in rotation with cereals (Siddique *et al.*, 1996; Hanbury *et al.*, 1999). However, the presence in the seed of the neurotoxin 3-(-*N*-oxalyl)-L-2,3-diamino propionic acid (ODAP) restrains potential commercial releases to only lines having low ODAP levels. ODAP has been identified as the causal agent of lathyrism, a paralysis of the lower limbs, in humans and also in animals. Lathyrism occurs following consumption over an extended period of high levels of ODAP, and is most often associated with *L. sativus*. Recent studies have shown that ODAP concentrations in *L. cicera* (mean 1.8 g/kg) are typically lower than in *L. sativus* (mean 3.9 g/kg; Hanbury *et al.*, 1999). Chalus is a recently released cultivar of *L. cicera* with an ODAP concentration less than 0.1 g/kg.

This study examined the laying performance of Hyline Brown hens given diets containing *L. cicera* cv Chalus at 0, 50, 100, 150, 250 and 300 g/kg. *Lathyrus* replaced some of the wheat, peas and meat and bone meal in the control diet. Diets were formulated to contain (per kg) ME 11.5 MJ, protein 175g, calcium 37.5g, available phosphorus 4g, methionine 4.2 g and lysine 8.4 g. The hens were 26 weeks of age at the start of the 8-week experimental period. Hens were housed in groups of four in cages 50 cm wide and 54.5 cm deep. Two adjacent cages comprised an experimental plot. Each of the six diets was replicated 16 times. Eggs were collected daily, and feed intake was measured weekly. Eggs laid over three consecutive days in week 8 were used for measurements of egg weight, egg mass and shell thickness.

Lathyrus **content of diet (g/kg)**	**Egg production (eggs/100 hen-days)**	**Feed intake (g/bird/day)**	**Feed efficiency (g feed/g egg mass)**	**Egg weight (g/egg)**	**Shell thickness (µm)**
0	92.7	106.4	2.07	58.4	369
50	93.9	106.9	1.96	59.8	375
100	93.2	108.7	2.02	59.7	369
150	94.5	110.0	2.02	59.6	367
250	93.5	110.9	2.08	58.8	372
300	92.8	110.1	2.04	59.2	373
Pooled SEM	1.2	1.3	0.04	0.5	6

Dietary inclusion level of *Lathyrus* had no significant effects (P>0.05) on any of the measurements shown in the Table. The results indicate that dwarf chickling *L. cicera* cv Chalus is comparable in nutritive value to field peas for laying hens. Preliminary results from other studies at PPPI (data not shown here) suggest that there is no accumulation of ODAP in eggs, breast muscle or brain tissue from hens fed 300 g/kg *L. cicera* cv Chalus for 15 weeks. This will be examined in a 26-week feeding study due to finish in 2001.

Hanbury, C.D., Siddique, K.H.M., Galwey, N.W. and Cocks, P.S. (1999). *Euphytica,* **110**: 45.

Siddique, K.H.M., Loss, S.P., Herwig, S.P. and Wilson, J.M. (1996). *Aust. J. Exp. Agric*., **36**: 209.

A similar publication to this page will be prepared in July 2002 for the SA Pork and Poultry Fair for August 2002 and dealing with conclusions and outcomes of the present study:

RESEARCH HIGHLIGHTS 1999

Apparent metabolisable energy of Lathyrus species for broiler chickens

Bob Hughes[1], Colin Hanbury[2], Peter Zviedrans[1], and Shahajahan Miyan[3]

[1] SARDI, [2] CLIMA, [3] University of Adelaide

The Problem

As the global demand for grains increases for human consumption and feed for livestock, the poultry industry will be faced with ever increasing costs and competition for suitable feed ingredients. Other plant materials are potentially valuable sources of energy and protein but can contain anti-nutritive factors and toxic components.

Objective

To evaluate the nutritive value of two *Lathyrus* species selected for very low or negligible levels of neuro-toxic material such as ODAP. *L. cicera* cv Chalus, a flat pod pea vine developed at the Centre for Legumes in Mediterranean Agriculture (CLIMA) and *L. sativus*, a grass pea developed at the University of Adelaide were evaluated in an apparent metabolisable energy (AME) bioassay conducted over a 7-day period with commercial broiler chickens. *Lathyrus* was included at 30% in a sorghum and casein basal diet containing adequate levels of vitamins and minerals. *Lupinus angustifolius* was included for comparison.

Results

Dietary treatment	Feed conversion (g feed/g gain)	Growth (g/bird)	AME of ingredient (MJ/kg DM)	Excreta moisture (%)
Sorghum	2.21	323	15.1	63.7
L. cicera	2.30	297	12.3	66.4
L. sativus	2.22	334	11.3	69.3
Lupin	2.01	396	7.9	73.1

Implications for industry

- ⇨ *Lathyrus cicera* cv Chalus and *Lathyrus sativus* are potentially valuable sources of energy and protein for poultry.
- ⇨ There were no indications of any neuro-toxic effects in the 7-day study period.
- ⇨ Longer term feeding studies are warranted in broiler chickens and laying hens to determine whether there is any risk of toxic material in chicken meat, eggs and excreta.

Appendix 4

Other dissemination of results

- In May 2001 a workshop was organised by Dr C. Hanbury at CLIMA in Perth WA. The purpose of this workshop was to present outcomes of the GRDC funded project UWA 287 "On-farm use and industry development of *Lathyrus* in Australia" and related activities. Present were grain growers, feed manufacturers, nutritionists, animal scientists,grains researchers and rural/agricultural press. The results of animal feeding experiments concerning pigs, sheep and poultry (including the results of the present study as they were available at the time) were presented as part of the program. There was considerable interest from the feeds industry but this was qualified with seeing the final data of all feeds studies. The current report will be sent to all those concerned parties now that it is complete, it will also be circulated with all growers associated with the project in WA, SA, Vic and NSW. As with all other animal feeding work already completed Dr Hanbury will forward the present results to all grains researcher associates concerned with adoption of new grain legumes in farming systems of southern Australia.

- Dr C. Hanbury has delivered several farmer field day talks concerning his ongoing *Lathyrus* research in WA and Vic. The results of feeding studies have always been mentioned to foster interest from grain growers. Future such talks by Dr Hanbury will include mention of the successful outcomes of the present study.

- The current study will be written up as a scientific paper for publication in an internationally recognised journal. There are no detailed studies similar to this in the literature concerning *Lathyrus* spp. There is increased interest in Europe for cheap grain legumes that can be utilised in the feeds industry. *Lathyrus* spp. have a particular advantage in the EU in that they have been grown extensively up until relatively recent times (post WWII) and so have many available cultivars already adapted to regional environments. This study is certain to generate interest in these quarters. Additionally new cultivars of *L. sativus* are being promoted in North America, primarily for green manure, however the additional benefit of good quality animal feed grain is certain to increase the level of interest there also.

- Dr C. Hanbury edits an international newsletter for researchers (including a range of animal scientists) interested in *Lathyrus* spp. (the *Lathyrus* Lathyrism Newsletter available on-line at http://www.clima.uwa.edu.au/*Lathyrus*/). A summary of the present study is due to be published in the next edition of 2002 (Vol. 3(1)).

- Further research proposals for trials in the animal feed industry are to be submitted, including the WA dairy industry. The present results will be used to further foster confidence in the quality of *Lathyrus* grain and so increase the likelihood of adoption by grain growers and stabilise supply for the egg industry.

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