PROJECT REPORT No. OS45

EVALUATION OF HEAT-TREATED LUPINS, BEANS AND RAPESEED MEAL AS PROTEIN SOURCES FOR DAIRY COWS

AUGUST 2000

Price: £4.50

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by

A MOSS
ADAS FENS, Alcester Road, Stratford upon Avon, Warwickshire CV37 9RQ

R ALLISON, A STROUD AND C COLLINS
ADAS Bridgets, Martyr Worthy, Winchester, Hampshire SO21 1AP

This is the final report of a one-year research project, which started in March 1999. The work was jointly funded by the Home-Grown Cereals Authority (project 2156 - £24,573), the Ministry of Agriculture, Fisheries and Food (project AR0132 - £20,000), the Milk Development Council (£38,817) and the Processors and Growers Research Organisation (£24,500).

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ABSTRACT

The UK ruminant feed industry is heavily reliant on fishmeal and soyabean meal as sources of high quality protein within rations. However, there is increasing concern over the sustainability of fish stocks and supplies of imported soyabean meal. There are several oil and protein crops which will grow under UK conditions including oilseed rape, sweet white lupins and beans. However, their protein tends to be more degradable in the rumen of dairy cows (i.e. lower quality protein). Previous work funded by the Milk Development Council and MAFF conducted at ADAS Bridgets has identified several issues limiting higher inclusion rates of home-grown proteins within the ration of dairy cows. For UK grown proteins to be attractive alternatives to fishmeal or soyabean meal, processing strategies such as heating which improve protein quality (by reducing degradability in the rumen) must be developed.

Within this project, the optimum duration and temperature of the heat treatment method to optimise rumen degradability of the protein for sweet white lupins, beans and rapeseed meal was determined. This treatment method was then scaled up to produce 3 tonnes of lupins and 6 tonnes of both beans and rapeseed meal. In a 10 week feeding study, 60 high yielding Holstein cows in early lactation, yielding in excess of 30 kg/day were fed complete diets based on high quality grass silage which contained one of five different combinations of protein sources. These included fishmeal + soyabean meal (Control), heat treated rapeseed meal (2.7 kg/cow/day), heat treated sweet white lupins (3.0 kg/cow/day), heat treated beans (4.0 kg/cow/day) or a combination of the heat treated home-grown proteins.

The optimum heat treatment was found to be the same for rapeseed meal, sweet white lupins and beans; the protein being heated at 120ºC for 35 minutes.

The results of this study demonstrate that fish meal and soyabean meal can be replaced with either heat treated rapeseed meal, heat treated beans or a combination of heat treated proteins without any reduction in milk yield or quality. The results also demonstrate that heat treated rapeseed meal and beans can be successfully included in the ration at up 32% and 34% respectively of the concentrate, without any adverse effect on milk quality. There was no evidence that tannins, known to be present in beans, had any adverse effect on protein digestibility. Additionally, feeding heat treated lupins had no adverse effect on milk yield, milk fat content or milk lactose content when replacing soyabean meal/fishmeal in a ration, although, both milk protein and casein N content were reduced by 5%.

Margin over purchased feed cost was similar for cows fed rations based on fish and soyabean meal or heat treated rapeseed meal when soyabean meal, fish meal and heat treated rapeseed meal were valued at £125, £381 and £143/tonne respectively. Replacing fish and soyabean meal with heat treated lupins (£180/tonne), beans (£134/tonne) or a combination of heat treated home-grown proteins (£148/tonne) resulted in lower margins due to the higher feed costs and reduced milk sales for lupins.

In order to exploit the considerable potential for home grown proteins, it is essential that less expensive techniques which are equally effective in terms of protein protection are developed. This would ensure far greater use of home grown proteins. An increase of 1% in the average inclusion rate in UK ruminant compound feeds of home-grown proteins would demand an extra 40,000 tonnes/year, equivalent to 23,000 ha of rapeseed or 15,000 ha of beans.

SUMMARY

1. INTRODUCTION

The UK ruminant industry is currently heavily reliant on fishmeal and soya bean meal as sources of high quality, digestible undegraded protein (DUP). However, there is increasing concern over the sustainability of world fish stocks and the recent BSE crisis has increased public awareness regarding the feeding of fishmeal to herbivores. Additionally, over 27% of the protein required for livestock feed is imported, of which 75% is soyabean meal (over 1.3 million tonnes in 1998). This imported protein is subject to fluctuations in world market price and the issue of genetically modified organisms.

There are a number of protein crops which will grow under UK conditions including oilseed rape, linseed, peas and beans. In addition, there have been recent advances in the development of lupin varieties to better suited to the UK climate. All these home-grown proteins have lower protein contents than fishmeal or soyabean meal and it tends to be more degradable in the rumen of dairy cows (Benchaar *et al* 1994, Corbett *et al* 1995, Guillaume *et al* 1987, Moss and Givens 1994). However, they may be other benefits, for example rapeseed protein has a higher methionine and lysine content than soyabean meal (Emanuelson 1994). If protein from UK sources was treated to reduce protein rumen degradability, their use as alternatives to fishmeal and soybean meal in the diets of high yielding dairy cows might increase.

In a series of studies funded by the MDC and MAFF, conducted at ADAS Bridgets (Mansbridge 1997a, Mansbridge 1997b), it was demonstrated that yields in excess of 9,000 litres could be achieved by dairy cows using diets based on linseed, lupins or high levels of rapeseed meal. However, these studies identified several areas of concern, including a significant reduction in milk yield (by up to 5.2 kg/cow/day) when cows were fed high levels of rapeseed meal. The importance of this will depend on which milk buyer as there was no significant change in milk fat/protein yield. In addition, feeding high levels of rapeseed meal and lupins increased urea and non-protein nitrogen levels in milk. High levels of these nitrogen fractions lower true protein levels and may reduce cheese yield, with implications for cheese makers.

There are various techniques to protect dietary proteins from rumen degradation. Heat treatments usually denature the protein and lead to reduced solubility in the rumen. Overprotection using heat treatment can occur. This is due to a number of chemical reactions involving reducing sugars, tannins and lignins and can, in extreme cases, lead to a burnt material which is undegradable in the rumen, but also completely indigestible. Carefully controlled conditions of heat and moisture have been shown to reduce degradability without adversely affecting protein digestibility (Herland 1996). Other methods, such as the use of xylose and heat in novel protection processes have also been shown to protect proteins from rumen degradation (Nakamura *et al* 1992, Windschitl and Stern 1988).

For UK grown protein sources to be attractive alternatives to fishmeal or soyabean meal, processing and management strategies which reduce their degradability in the rumen must be developed. The obvious benefits from increased use of UK grown protein sources are highlighted by the fact that an increase of 1% in the average inclusion rate in UK ruminant compound feeds of home-grown proteins would require an extra 40,000 tonnes/year, equivalent to 23,000 ha of rapeseed or 15,000 ha of beans. This study funded by a consortium of funding bodies (HGCA, PGRO, MDC and MAFF) was undertaken with the objective of identifying the optimum processing method to protect the protein in UK grown rapeseed meal, lupins and beans, and evaluating these feeds in high yielding dairy cow rations.

2. OBJECTIVES

The objectives of this project were:-

1. To reduce the milk yield depression reported in cows fed high levels of rapeseed meal by effectively protecting the protein through heat treatment.

2. To reduce the adverse reported effects of lupins on milk protein quality by effectively protecting the protein through heat treatment.

3. To evaluate the use of beans as a home-grown protein source, the protein in beans being effectively protected by heat treatment.

4. To evaluate a combination of the heat treated home-grown protein sources (lupins, rape or beans).

3. WORK PROGRAMME

To achieve these objectives, the work was split into 5 phases:-

Phase 1
Determination of the optimum temperature and pressure to achieve maximum rumen protection of the protein in lupins, rape and beans.

Phase 2
Determination of the optimum conditions for the protection of protein in lupins, rape and beans using a novel protection process.

Phase 3
Comparison of each product for the degree of protection achieved by either heat treatment or the novel processing technique, using an enzyme based test method (Ficin test).

Phase 4
Determination of protein degradability using the *in situ* dacron bag technique on samples of the most effectively protected lupins, rape and beans identified in *Phase 3* of the study, together with samples of fishmeal, soyabean meal and grass silage to be used in *Phase* 5. In addition, amino acid profiles of the dacron bag residue were determined.

Phase 5
Comparison of the protected lupins, rape, beans and a combination of the proteins on milk production and milk quality of high yielding dairy cows when fed within a grass silage based diet.

4. MATERIAL AND METHODS

Phases 1-3: Determination of optimum protein protection method

Samples of sweet white lupin seed (*Lupinus albus*) (ex farm Herefordshire), beans (*Vicia faba*) (ex farm Dorset) and solvent extracted rapeseed meal were collected. Identical samples were supplied to Borregaard UK Limited to be treated using their novel patented process for rumen protecting feed.

Lupin seeds and beans were milled through an 8 mm screen, rapeseed meal required no further milling. Sub-samples were heat treated in an autoclave according to the following schedule to provide nine treatments per protein source.

Time (minutes)	Temperature (°C)		
	108	**120**	**132**
20	√	√	√
35	√	√	√
50	√	√	√

The established method within the current UK metabolisable protein (MP) system (AFRC 1993) for estimating the degradability of protein in feeds is the *in situ* technique (Orskov and McDonald 1979), however this method is costly and requires the use of surgically modified animals. Therefore, in this study an *in vitro* method was used for initial screening of heating combinations. The two most promising heating combinations together with the samples treated using a novel patented rumen protection process were then incubated *in situ* for 12 hours and the residue subjected to a pepsin/pancreatin digestion in order to determine nitrogen digestibility in the small intestine. This would allow a more accurate estimate of protein degradability in the rumen and digestible undegradable protein (DUP) content.

Phase 4: Protein evaluation of processed proteins and feeds used in the animal feeding study

Sweet white lupin seeds (*Lupinus albus*) and beans (*Vicia faba*) were sourced on farm having been harvested in the previous season (1998/1999). The solvent extracted rapeseed meal was obtained from Unitrition Ltd. Samples of other feedstuffs to be fed in the animal study included fish meal, solvent extracted soyabean meal, untreated solvent extracted rapeseed meal and first cut Italian Ryegrass silage.

Lupins and beans were milled using a mobile cyclone hammer mill using an 8 mm sieve, while the rapeseed meal received no further milling. The heat treatment chosen for each protein (identified in *Phases 1-3*) was scaled to 3 tonne batches by Unitrition Ltd. resulting in 3 tonnes of processed lupins and 6 tonnes each of processed beans and rapeseed meal.

The measurement of *in situ* rumen degradability for dry matter and nitrogen, using the polyester fibre bag technique, was undertaken for each of the main feedstuffs (heat treated beans, lupins and rapeseed meal, rapeseed treated using the novel protection process, fish meal, extracted soyabean meal, untreated extracted rapeseed meal and grass silage) for the dairy feeding study (*Phase 5*). The amino acid content of the feedstuffs and the resultant residue after a 12 hour incubation in the rumen were determined by Aspland and James Ltd.

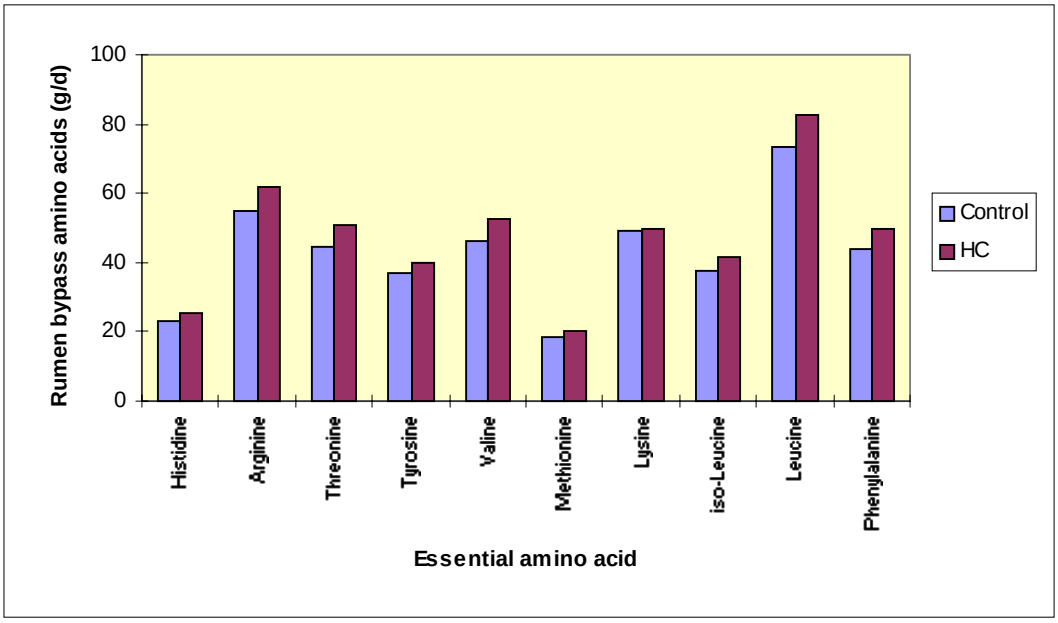
Phase 5: Animal feeding study

A total of 60 second and subsequent parity Holstein-Friesian dairy cows were used in the study. They were divided into 12 blocks of five cows, on the basis of parity and days in milk. Within each block cows were allocated at random to one of the five treatments (Control, HR, HL, HB and HC) to give a total of 12 cows per treatment. Each treatment diet differed in the combination of protein sources used to meet protein requirements as follows:-

Diet	Protein source (s)
Control	Fishmeal + soyabean meal
HR Rapeseed meal	Heat treated rapeseed meal
HL Lupins	Heat treated lupins
HB Beans	Heat treated beans
HC Combination	Heat treated beans, lupins and rapeseed meal

The five treatment diets were formulated using feed data generated from *phase 4* and analysis of raw materials prior to the study, to meet the energy and protein requirements for maintenance + 38 kg milk/day with no live weight loss, using the current UK metabolisable energy (ME) and metabolisable protein (MP) (+15%) systems (AFRC 1993). In addition, the Control and HC rations were formulated using the amino acid data to achieve similar supplies of the 10 essential amino acids (as defined by Fleet and Mepham (1985)) (Figure 1).

Figure 1: Formulated rumen bypass essential amino acid supply for Control and HC rations.



Dry matter intake, milk yield and milk quality were measured in the covariate week (week -2) when all cows were fed a commercial ration, and these values were used as the covariate in the statistical analysis. During the changeover week (week -1) cows were changed onto the treatment diets. The experimental period ran for 8 weeks (weeks 1 - 8). The data obtained during the animal study (dry matter intake, milk yield, milk quality, milk N fractions, live weight and body condition score) were

subjected to analysis of variance (ANOVA), and where there were significant differences between treatments, statistical comparisons were made against Control.

5. DISCUSSION

5.1 Optimum protection of feed protein

There are various methods of protecting protein, with the most common being combinations of temperature and time. In this study, the optimum heat treatment process was found to be heating rapeseed meal, lupins and beans at 120ºC for 35 minutes based on cost and maximising DUP supply.

Bencharr *et al* (1994) reported that the optimum temperature of processing beans was 195ºC, and likewise, Kung *et al* (1991) used a process involving heating whole lupins at 175ºC. However, in these examples, although the extrusion/roasting methods used higher temperatures, the residence time was seconds not minutes. McKinnon *et al* (1995) examined the effect of various temperatures and durations on rapeseed meal, and found heating to 125ºC reduced rumen degradability, whereas heating at 145ºC led to a significant reduction in digestibility. This over protection may be associated with Maillard type reactions where proteins bind to sugars rendering the protein indigestible. In this current study, there was no evidence of reduced digestibility in beans when the processing temperature increased from 120 to 132ºC.

Acid detergent insoluble nitrogen (ADIN) has been used as a measure of protein damage as it includes Maillard reaction products and tannin protein complexes (Van Soest *et al* 1987). Goering *et al* (1972) found that nitrogen bound to acid detergent fibre (ADF) was indigestible and Schroeder *et al* (1996) demonstrated that ADIN content was a good indicator of heat damage to the protein in sunflower cake. To reflect these observations, ADIN is used in the UK MP system (AFRC 1993) as a measurement of indigestible protein. In this study, ADIN was 2.7, 6.6 and 1.6 g/kg DM for heat-treated lupins, rapeseed meal and beans respectively, higher than published values of 1-2, 0.5 and 3.6-4.8 g/kg for untreated lupins, rapeseed meal and beans respectively (ADAS 1995, AFRC 1993). Values were however considerably lower than the upper limit of 120 - 150 g/kg suggested by Schroeder *et al* (1996), who speculated that exceeding this limit may lead to a reduction in the supply of amino acids from the undegraded protein of sunflower cake.

Similar heat treatments to those employed in this study are extensively used in Sweden and Finland where they have been demonstrated to reduce effective protein degradability by up to 20% (Tuori 1992) while having a minimal effect on digestibility. Overall, the results achieved in this study are comparable with values obtained by other research workers (Table 1).

The calculated DUP content was 157, 165 and 115 g/kg DM for heat-treated rapeseed meal, lupins and beans respectively. For beans and lupins, these values were much higher than published values for untreated proteins (59 and 51 g/kg DM respectively) demonstrating the potential value of heat treatment in improving protein quality. However, the difference between the DUP contents of untreated and treated rapeseed meal was small because the level of DUP in the untreated rapeseed (147 g/kg DM) was much higher than anticipated (78 g/kg DM, AFRC 1993). Comparison of the protein degradability of untreated rapeseed meal with published figures (Table 2) suggests that the standard rapeseed meal used in this study had a lower than expected degradability.

Table 1: Comparison of rumen bypass protein content of heat treated rapeseed meal, lupins and beans with published values.

Protein	Treatment process	Rumen bypass protein (% CP)	Reference
Rapeseed meal	**Moist heat (120 °C for 35 min)**	**59**	**This study**
	Moist heat (2-3 atm < 30s)	54	Herland 1996a
	Moist heat (2-3 atm < 30s)	48	Bertilsson *et al* 1994
	Moist heat (2-3 atm < 30s)	60 and 61	Herland 1996
	Moist heat (2-3 atm < 30s)	34	Huhtanen and Heikkila 1996
	Moist heat 130ºC	51	Dakowski *et al* 1996
	140ºC	77	
	150ºC	80	
Lupins	**Moist heat (120 ºC for 25 min)**	**60**	**This study**
	Heat (300 ºC for 1-4 min)	21	Zaman *et al* 1995
	Heat 130ºC	55**	Kung *et al* 1991
	175ºC	61**	
	Roasted	33	Robinson and McNiven 1993
	Roasted	45	Singh *et al* 1995
	Pressure toasted	47 and 51	Goelema *et al* 1998
	Extruded	61	Benchaar *et al* 1991
Beans	**Moist heat (120ºC for 25 min)**	**49**	**This study**
	Extrusion (195ºC)	58*	Benchaar *et al* 1992
	Pressure toasting	48 and 57	Goelema *et al* 1998

* = PDIA (≡ DUP)
** = Based on N disappearance after 12 hour incubation in rumen fluid

Table 2: Comparison of the determined effective degradability of untreated rapeseed meal with published values.

Source	Effective protein degradability (% CP)
This study	**0.44**
Allison 1999	0.46
Bertilsson *et al* 1994	0.72
Huhtanen and Heikkila 1996	0.78
MAFF 1990	0.59 - 0.79
AFRC 1993	0.69 and 0.73
ADAS 1989	0.59 - 0.70

The low degradability of the untreated rapeseed meal in this study could be due to differences in variety or agronomy, or more likely to differences in the processing of rapeseed during commercial oil extraction, as any heating or drying of the extracted meal may further protect the protein. Kendall *et al* (1991) stated that variation in the protein quality of rapeseed meal can be related to methods used at the

processing plant during oil extraction, and the results from this study highlight the variability in the UK of rapeseed meal protein quality and its potential consequence on ration formulation.

The use of the novel treatment process on rapeseed meal increased the amount of protein which bypassed the rumen, but reduced DUP content compared with the heat treated rapeseed meal. This may be due to a reduced digestibility of the protein as evidenced by a higher ADIN level and a lower pepsin/pancreatin digestibility. However, the 12 hour rumen residue study indicated that novel treated rapeseed meal tended to have lower amino acid degradabilities, and that tyrosine and methionine were particularly slowly degraded. Methionine is generally regarded as the first limiting amino acid for milk protein synthesis (Rulquin and Verite 1993, Schwab *et al* 1976), and the potential rumen bypass methionine supplied by the novel treated rapeseed meal may be beneficial to high producing dairy cows.

5.2 Animal performance

Objective 1 - Rapeseed meal

Standard rapeseed meal has a relatively high effective rumen degradable protein (ERDP) and low DUP content. Research at ADAS Bridgets (Mansbridge 1997a) found that feeding 5.8 kg (DM basis) of rapeseed meal to meet MP (and hence DUP) requirement led to a 5.2 kg/day depression in milk yield. The reduction could not be explained by dry matter intake as this was unaffected. However, the supply of rumen degradable protein was high relative to rumen fermentable metabolisable energy (FME) (ERDP:FME = 12.7) probably leading to the excess nitrogen being excreted in the urine, with the possible consequence of increased energy requirement. For example, Twigge and van Gils (1988) estimated that the energy cost associated with a daily surplus of 500 - 1000 g of rumen degradable protein would be 1.3 to 2.6 kg of fat corrected milk production. Reducing the degradability of protein in rapeseed meal might reduce the adverse effects of feeding rapeseed meal as the sole DUP source in dairy rations.

In this study, rapeseed meal was included at 31% of the concentrate component to maintain DUP supply when replacing fish meal and soyabean meal i.e. at a level which is twice of inclusion in UK dairy compounds (15% - MAFF Statistics (MAFF Statistical Service)). At this level, feeding a combination of untreated and heat treated rapeseed meal as the major protein sources produced the same performance (milk yield, milk quality and live weight) as a diet based on fish meal and soyabean meal. This agrees with the results of Garnsworthy (1997) who replaced fish meal with rumen protected rapeseed meal and showed no adverse effect on production. In addition, milk urea content remained at Control levels in this study suggesting that urea excretion was not elevated to the levels found in the previous study.

Production responses to heat treated rapeseed meal can be variable (Tuori 1992), and the benefits of heat treating rapeseed meal are not always evident. In studies where heat treating rapeseed meal had no effect on performance, the difference in protein degradability between untreated and treated rapeseed meal was small (0.06 - 0.17 as a proportion of total CP), suggesting that if protein degradability of the standard rapeseed meal is low (as in this study), heat treatment may not be required. However, the use of heat treatment when applied to “standard” rapeseed meal (i.e. with a protein degradability over 0.65), gives significant responses in dairy cow performance (Bertilsson *et al* 1994, Herland 1996).

Overall, it was demonstrated that high levels of rapeseed meal with a low protein degradabilities can be formulated in grass silage based dairy rations using the current UK ME and MP systems, instead of fish meal and soyabean meal, without any reduction in milk yield and quality, or increase in milk urea content.

Objective 2 - Lupins

Raw lupins are extensively degraded in the rumen (over 70% of CP, AFRC 1993) which can lead to increased milk urea content (Mansbridge 1997a and 1997b). Additionally, feeding lupins to dairy cows has lead to reductions in milk protein content (Guillaume *et al* 1987, Robinson and McNiven 1993, Singh *et al* 1995). These effects suggest a reduced efficiency of utilisation of feed protein, possibly due to a reduction in N utilisation for microbial protein synthesis. Benchaar *et al* (1994) reported a protein solubility of 30% and effective degradability of 64%, while UK sources (AFRC 1993; Mansbridge 1997a; Mansbridge 1997b) have reported higher degradabilities (71 and 69%). This study investigated whether reducing the protein degradability of lupins can reduce the adverse effect on milk protein content.

Feeding lupins which had been protected using heat treatment had no adverse effect on milk yield, milk protein yield, milk fat content and yield or milk lactose content and yield compared with Control diet containing fish meal and soyabean meal. However, consistent with other published findings (Bayourthe *et al*, 1998, Robinson and McNiven 1993), there was a significant reduction in milk crude protein and casein content. Several reasons for this reduction have been suggested. Firstly, the sulphur containing amino acids (methionine and cystine) content of the lupins were low compared with either rapeseed meal, soyabean meal or fish meal. Methionine is generally regarded as first limiting amino acid for milk protein synthesis (Rulquin and Verite 1993), and responses in milk protein output (largely due to increased casein synthesis) have been observed when methionine supply is increased (Sloan 1997).

Secondly, lupins contain around 10% oil (Moss *et al* 1996), and it is generally accepted that feeding oilseeds to dairy cows can reduce milk protein content (DePeters and Cant 1992, Garnsworthy 1999, Wu and Huber 1994). The oil content of the diet based on lupins was higher than any other diet which may explain the reduction in milk protein content.

A third explanation is that dry matter intake was significantly lower for cows fed heat treated lupins in weeks 5, 6 and 8 which would reduce nutrient supply for milk production in the mammary gland. It was however interesting to note that the effects of feeding heat treated lupins on dry matter intake was not evident until 5 weeks after its introduction in the diet.

In summary, heat treated lupins can be fed instead of soyabean meal and fish meal in grass silage based rations without any adverse effect on milk yield or milk fat content, however there was a milk protein depression.

Objective 3 - Beans

Beans are a traditional crop grown in the UK and recently have seen important breeding improvements in seed yield and harvesting index by producing determinate types. Their protein however is readily degraded in the rumen resulting in a low DUP content (AFRC 1993). Therefore, beans are not ideal supplements when fed with grass silages (Wilkins and Jones 2000), but protection of the protein could increase their value as a ruminant protein source. Beans are currently not used extensively in standard dairy feed compounds (MAFF Statistics) and have a low national average inclusion rate (1-2%). Additionally, there is virtually no published data regarding the feeding of heat-treated beans to dairy cows. This study provided valuable data on feeding heat treated beans at high levels (34% of ration supplement) in dairy cow rations.

Beans are a valuable source of protein, starch (339 g/kg DM), and have an excellent ME content (13.1 MJ/kg DM). In this study, replacing fish meal and soyabean meal with heat treated beans as a protein

source had no adverse effect on milk yield or quality, indicating that heat treated beans can be fed at levels higher than recommended for raw beans (16% of concentrates, Chamberlain and Wilkinson 1996). Peas, which are similar to beans, have been fed to high yielding dairy cows without any detrimental effect during early lactation (Corbett *et al* 1995).

Beans contain tannins which can have an adverse effect on protein digestibility (Chamberlain and Wilkinson 1996), but in this study, overall N *in vitro* digestibility was 891 g/kg DM, and comparable with other estimates of apparent digestibility (820 - 840 g/kg DM - ADAS Tech Bull. 90/2). It is interesting to note that beans, similar to lupins, contain low levels of the sulphur amino acids, but unlike lupins, have no effect on milk protein content. In summary, heat treated beans can be fed at high levels to replace fish meal/soyabean meal in dairy cow rations without affecting dairy cow performance.

Objective 4 - Combination of proteins

Rations to high yielding dairy cows traditionally contain more than one protein source, partly due to inclusion limits for certain individual protein sources (rapeseed meal, beans, etc.) and partly as a consequence of least cost rationing where proteins with different degradabilities are used to provide the 'perfect' balance. Some authors (Oldham 1994), have suggested that there may also be benefits in amino acid supply, as different protein sources can be deficient in one or more amino acids, for example, maize gluten which is low in lysine, and lupins which are low in methionine. Conversely, some protein supplements are high in specific amino acids e.g. fish meal which is high in lysine and methionine, and therefore blending proteins can lead to a supplement that has an "ideal" balance of amino acids for milk production. Schingoethe (1996) examined amino acid supply from mixtures of feed proteins, and found, for example, that a blend of soybean meal, maize gluten and meat/bone meal, had an amino acid balance that was closer to assumed requirements than any one of the individual ingredients. In this study, heat treated beans, lupins and rapeseed meal were formulated using a simple amino acid supply model to provide the same mixture of amino acids as the Control ration containing soyabean meal and fishmeal.

Results indicated no differences in any measure of performance between the Control and the blend of heat treated beans, lupins and rapeseed meal. This is consistent with work by Allison (1999), who demonstrated that a mixture of rumen protected vegetable proteins can replace fishmeal on a crude protein basis. The inclusion of a low level of lupins (4 versus 26% of supplement for HL and HC respectively) prevented any reduction in milk protein content. It is unclear from this work whether the effect of lupins on milk protein production is a consequence of its poor amino acid balance or some other factor. Further work is necessary to determine the maximum inclusion level of lupins in high yielding dairy cow rations when fed in combination with other protein (i.e. amino acid) sources.

5.3 Margin over purchased feed costs

Using standard farm measures of economic performance (i.e. margin over feed costs), cows fed the Control and HR diets had the highest margins over all feeds both per cow and per litre (Table 3). Replacing fish meal/soyabean meal with heat treated lupins, beans or a combination of proteins led to a reduction in margin over feed costs. This was due to a an increase in feed costs, although for lupins and the combination of proteins, there was also a reduction in the milk value. However, it must be noted that changes in market conditions circumstances such as increases in soyabean meal price (increased by £25 in the period between the conclusion of work and the preparation of this report) and reductions in bean/lupin prices, will change the margin over feed costs of heat treated UK grown proteins in dairy cow rations.

Table 3: Calculation of margins over feed costs for the dietary treatments.

Financial performance	Treatment				
	Control	HR	HB	HL	HC
Margin over all feed costs (p/l)	12.0	12.1	11.6	11.6	11.6
Margin over all feed costs (£/cow/day)	3.97	3.99	3.94	3.87	3.83
Milk value (p/litre)	17.6	17.5	17.3	17.1	17.4
Milk volume (litres)	33.0	32.9	33.9	33.4	33.2
Milk value (£/cow/day)	5.81	5.77	5.85	5.72	5.77
Feed cost (£/cow/day)*	1.84	1.78	1.91	1.85	1.92

* Calculated assuming feed costs: fish meal = £381/tonne, soyabean meal = £125/tonne, untreated rapeseed meal = £108/tonne, heat treated rapeseed meal = £143/tonne, heat treated beans = £134/tonne and heat treated lupins = £180/tonne. Heat treatment costs = £45/tonne (commercial rate).

From a financial perspective, feeding high levels of heat treated rapeseed meal in a total mixed ration based system was the most cost effective alternative to soyabean meal/fish meal. This would however be different for organic milk producers who cannot feed either fish meal or solvent extracted soyabean meal/rapeseed meal and also receive premiums for organic milk (over 25 p/litre). It must also be noted that there could be longer term effects of feeding high concentrations of heat treated rapeseed meal in rations on cattle health, this is being currently addressed in a separate HGCA funded project at ADAS Bridgets (Project: 2324).

Cheaper treatment technologies which retain the improvements in protein quality or alternatively using plant selection for varieties of beans, lupins and rapeseed meal containing protein which is less degradable in the rumen, would further encourage their use in dairy cow rations.

APPENDIX 1

Determination of the optimum heat treatment process and evaluation of protein quality

1. OBJECTIVE

The objective of this component of the work was two-fold. Firstly, to determine the optimum heating conditions for UK grown rapeseed meal, lupins and beans. Secondly, to evaluate the protein quality of feeds to be fed in the animal feeding phase. This part of the work was split into four phases:-

Phase 1
Determination of the optimum temperature and pressure to achieve maximum protection of the protein in lupins, rape and beans.

Phase 2
Determination of the optimum conditions for the protection of protein in lupins, rape and beans using a novel protection process.

Phase 3
Comparison of each product for the degree of protection achieved by either heat treatment or the novel processing technique using an enzyme based test method (Ficin test).

Phase 4
Determination of protein degradability using the *in situ* dacron bag technique on samples of the most effectively protected lupins, rape and beans, identified in *Phase 3* of the study, together with samples of fishmeal, soyabean meal and grass silage to be used in the animal feeding study. In addition, amino acid profiles of the dacron bag residue were determined.

2. MATERIAL AND METHODS

2.1 Phases 1-3: Determination of optimum protein protection method

2.1.1 Protein sources

Samples of sweet white lupin seed (*Lupinus albus*) (ex farm Herefordshire), beans (*Vicia faba*) (ex farm Dorset) and solvent extracted rapeseed meal were collected. Identical samples were supplied to Borregaard UK Limited to be treated using their novel patented process for rumen protecting feed. Samples were delivered to ADAS Nutritional Sciences Research Unit (NSRU) and stored at 4 ºC.

2.1.2 Sample preparation

Lupin seeds and beans were milled (Christy Norris, UK) through an 8 mm screen. Rapeseed meal required no further milling. Sub-samples (150g of each) had 50 g/kg water added and were heat treated in an autoclave according to the following schedule to provide nine treatments per protein source.

Time (minutes)	Temperature (°C)		
	108	**120**	**132**
20	√	√	√
35	√	√	√
50	√	√	√

Each autoclave run at the specified heat treatment contained all three protein sources. Samples were put into the autoclave when the temperature reached 60°C, and the cooking time began when the autoclave reached the required temperature for that treatment. After the appropriate cooking time, the autoclave was switched off and cooled to a temperature which allowed the autoclave to be opened. Samples were removed, cooled to room temperature and stored at 4°C.

2.1.3 Measurement of ficin degradability

The established method within the current UK metabolisable protein system (AFRC 1993) for estimating the degradability of protein in feeds is the *in situ* technique (Orskov and McDonald 1979), however this method is costly and requires surgically modified animals. *In vitro* methods are being developed, e.g. the plant enzyme ficin assay, which can be used for initial screening of protein

sources. Kosmala *et al* (1996) found that values of ficin degradability agreed closely ($R^2 = 0.92$) with values measured using the *in situ* method.

A 20g sub-sample of each of the treated feeds was milled through a 1 mm screen using the Cyclotec mill (Tecator, Sweden). Dry matter (DM) content was determined in duplicate after 18 hours at 100°C and the nitrogen content of the untreated samples used to calculate the weight of sample for the ficin test.

2.1.3.1 Protein degradation assays

Samples were accurately weighed to provide an equivalent of 0.1 g of protein into labelled glass 100 ml vials. The samples were incubated singularly on two separate occasions with 10 ml phosphate buffer (0.1 M, pH 7.0) for 30 min at 39°C. After this pre-incubation period 10 ml of Ficin (from fig tree latex, EC 3.4.22.3, activity 0.28 U/mg, Sigma Chemicals, 3.214 mg/ml in phosphate buffer) was added and incubated for a further 2 hours in a shaking water bath (50 rpm) at 39°C. At the end of the incubation time the fermentation was terminated and the contents filtered through Whatman No. 4 filter paper and the solid residues washed three times with 250 ml distilled water (95°C). Total nitrogen was determined on the residue by the Kjeldahl method (MAFF, 1986). Protein degradability was calculated by the difference between the protein in the original sample and the protein in the residue.

2.1.4 Measurement of nitrogen disappearance from the rumen (10 hours) and pepsin/pancreatin digestion of residue

The fresh material was accurately weighed (to provide approximately 5 g fresh weight) into pre-dried and weighed polyester fibre bags (43 μm pore size, 200 x 90 mm internal diameter). All samples (each in duplicate) were incubated together for 10 hours in one cow with samples introduced into the rumen at 08.30h, prior to the morning feed. The animal was maintained on a mixed diet comprising on a DM basis of, 0.8 grass silage and 0.2 rolled mineralised barley. The ration was offered in two discreet meals at 08.30 and 16.30 h. Fresh water was freely available at all times.

After incubation, residues were mechanically washed, dried at 60ºC for 48 hours and the proportional loss of DM during incubation calculated. Nitrogen (N) content was determined using the Kjeldahl method for each bag and an equivalent to 15 mg of N was accurately weighed into labelled 50 ml centrifuge. The residue was incubated for 1 hour in a shaking water bath at 38°C with 10 ml of a pH 1.9, 0.1 M HCl solution containing 6 g per l of pepsin (1:10,000 i.u.). After incubation, 0.5 ml of a 1 M NaOH solution and 13.5 ml of a pancreatin (Sigma p-7545) solution (0.5 M KH_2PO_4 buffer standardised at pH 7.8 containing 50 ppm of thymol and 3 g of pancreatin) was added and vortexed. The samples were incubated for a further 24 hours at 38ºC in a shaking water bath, with samples vortexed every 8 hours. After incubation, the solution and residue was quantitatively transferred to labelled and pre-weighed filter papers and thoroughly washed with 200 ml of distilled water (at 38ºC) to remove enzyme, soluble nitrogen and soluble oil. The residues were dried for 16 hours at 60ºC, weighed and analysed for N.

2.1.5 Statistical analysis

The ficin degradability results were analysed using analysis of variance (ANOVA) with sample, time of treatment and temperature as factors.

2.2 Phase 4: Protein evaluation of processed proteins and feeds used in the animal feeding study

The measurement of *in situ* rumen degradability for DM and N, using the polyester fibre bag technique, was undertaken for each of the main feedstuffs (heat treated beans, lupins and rapeseed meal, rapeseed treated using the novel protection process, fish meal, extracted soyabean meal, untreated extracted rapeseed meal and grass silage) for the dairy feeding study (*Phase 5*).

2.2.1 Samples, preparation and processing

Sweet white lupin seeds (*Lupinus albus*) and beans (*Vicia faba*) were sourced on farm being harvested in the previous season (1998/1999). The solvent extracted rapeseed meal was obtained from Unitrition Ltd. Samples of other feedstuffs to be fed in the animal study included fish meal (Provimi 66, United Fish Industries UK Ltd.), solvent extracted soyabean meal, untreated extracted rapeseed meal and first cut Italian Ryegrass silage.

Lupins and beans were milled using a mobile cyclone hammer mill using an 8 mm sieve (B and W Mobile Milling Ltd.), while the rapeseed meal received no further milling. The heat treatment chosen for each protein (identified in *Phases 1-3*) was scaled to 3 tonne batches by Unitrition Ltd. resulting in 3 tonnes of processed lupins and 6 tonnes each of processed beans and rapeseed meal.

2.2.2 *In situ* nitrogen degradability and digestibility by pepsin/pancreatin

The fresh weight equivalent to provide 5g DM was incubated within polyester fibre bags (pore size 43 μm) in the rumens of non-lactating Friesian cows for 2, 5, 8, 12, 24, 48 and 72 hours. Each time period was incubated in duplicate in three separate animals. All bags (per time period) were inserted into the rumens initially just prior to the morning feed and bags were removed after the appropriate time had lapsed. The three animals were maintained on a mixed diet comprising on a DM basis of, 0.8 grass silage and 0.2 dairy compound. The ration was offered in two discreet meals at 08.30 and 16.30h. Fresh water was freely available at all times. In addition, duplicate samples for the 12 hour time period were prepared and incubated for amino acid analysis (see section 3.2.5).

After incubation the residues were mechanically washed, dried at 60ºC for 48 hours and the proportional loss of DM during incubation calculated. The incubation residues from all samples were analysed for N by the Leco method and the extra 12 hour residues were combined and analysed for pepsin/pancreatin digestibility (as described in section 2.1.4) and amino acids. In addition, the original samples of feed were analysed for Leco N, acid detergent insoluble N (Goering and Van Soest, 1970) and amino acids. An estimate of the initial loss of the DM and N was made by mechanically washing non-incubated material in the polyester fibre bags in cold water.

Curves describing the disappearance of DM and N were fitted to the mean data using the exponential model of Ørskov and McDonald (1979):

$$P=a+b(1-e^{-ct})$$

where P=% degradation at time t, a=the immediately soluble fraction, b=the insoluble but potentially degradable fraction and c=the fractional rate of degradation of the b fraction.

Where necessary the data were fitted to the amended model of McDonald (1981) in order to calculate the lag phase and adjusted a and b values as follows:

$$\text{lag phase (h)}=1/c(\log_e(b'/(a'+b'-a))$$

where a', b', and c are described above and a=the actual amount of soluble material.

$$b=(a'+b')-a$$

where b=b' corrected for the difference between a' and a.

Effective degradability's were calculated according to McDonald (1981) and include where necessary the effects of lag phase. The ERDP (effective rumen degradable protein) and digestible undegradable protein (DUP) contents were calculated from the polyester fibre bag measurements as follows:

$$ERDP\ (g/kg\ DM) = CP(0.8a+(bc/(c+r)))$$
$$DUP\ (g/kg\ DM) = 0.9\{CP(1-a-(bc/(c+r)))-6.25\ ADIN\}$$

where CP=crude protein content of the feed (g/kg DM), ADIN=acid detergent insoluble nitrogen (g/kg DM), a, b and c are the degradability characteristics and r=the assumed ruminal outflow rate.

2.2.3 Dry matter and nitrogen solubility

The solubility of DM and N was measured in the laboratory by saturating approximately a 1g sample, in triplicate, of the eight final feeds in 40 ml of de-ionised water for approximately 1 hour with regular agitation. Each sample was then filtered under vacuum through a Whatman grade 541 filter paper, washed with three portions of 40 ml of de-ionised water and the filter paper and residue was dried at 100ºC. The filter paper plus residue was weighed to estimate DM solubility and the insoluble N present was measured by the Kjeldahl method.

2.2.4 Chemical analysis

Representative sub-samples of each sample of lupin were analysed for DM content, crude protein (CP) by the methods of MAFF (1986). Acid detergent insoluble nitrogen (ADIN) was determined essentially by the methods of Goering and Van Soest (1970).

2.2.5 Amino acid content of feeds and undegraded residue

The amino acid content of the feedstuffs and the resultant residue after a 12 hour incubation in the rumen (see section 2.2.2) were determined by Aspland and James Ltd. Amino acids were determined after hydrolysis of the sample with 6 M HCl containing phenol followed by separation of the amino acids by ion exchange chromatography on a Biochron 20 analyser (Pharmia Biochron Ltd., St Albans, UK) using post-column reaction with ninhydrin. For the analysis of methionine and cystine, an initial oxidation to methionine sulphone and cysteic acid respectively was carried out with a performic acid/phenol mixture before hydrolysis.

3. RESULTS

3.1 Phases 1-3: Determination of optimum protein protection method

3.1.1 Ficin degradability

The results of the ficin degradability of the samples of lupin seed, bean and rapeseed meal treated by the nine combinations of temperature and time are shown in Table 1. Ficin degradability significantly decreased with both time and temperature and there were significant ($P<0.001$) time x temperature and sample x temperature interactions. From these results two treatments were selected from each protein source on the basis of the lowest degradability obtained using the least invasive treatments. The treatments selected are shaded in Table 1.

Table 1: Ficin nitrogen degradability (%N) for samples of lupin seed, beans and rapeseed meal heat treated at 3 temperatures and 3 cooking times.

Temperature	Time (min)		
	20	35	50
Lupin seed			
108°C	86.9	84.1	81.5
120°C	76.1	75.4	74.7
132°C	73.0	77.0	75.3
Beans			
108°C	77.3	72.7	70.9
120°C	63.4	60.1	60.0
132°C	61.5	57.1	60.5
Rapeseed meal			
108°C	27.5	19.3	20.1
120°C	15.8	16.2	17.9
132°C	23.9	26.6	25.6
SED Sample x temperature	1.093***		
Time x temperature	1.093***		
Sample x time x temperature	1.893^{NS}		

3.1.2 Nitrogen disappearance from the rumen over 10 hours and pepsin/pancreatin digestion of residue

The results of the measurement of nitrogen disappearance from the rumen (10 hours), pepsin/pancreatin digestion of the resultant residue of the selected samples of lupin seed, bean and rapeseed meal and the samples treated with the novel protection method (Lupin 1 and 2, Bean 1 and 2, Rapeseed mea1) are shown in Table 2.

Table 2: Rumen nitrogen disappearance (%) and digestibility for the selected samples of lupin seed, beans and rapeseed meal heat and the samples treated with the novel protection method.

Feedstuff	Rumen nitrogen disappearance (%) after 10 hour incubation	Pepsin/pancreatin digestibility of the rumen residue (%)	Whole tract nitrogen digestibility (%)	Calculated DUP content (g/kg DM)
Lupin				
20 min, 132°C	48.8	78.9	89.2	164
35 min, 120°C	**40.5**	**72.7**	**83.8**	**178**
Lupin 1	58.8	88.1	95.1	138
Lupin 2	50.6	84.8	92.5	148
Beans				
35 min, 120°C	**44.9**	**87.4**	**93.1**	**140**
35 min, 132°C	43.4	87.9	93.1	148
Bean 1	57.2	71.0	87.6	88
Bean 2	33.9	73.3	82.4	137
Rapeseed meal				
20 min, 120°C	29.4	63.0	73.9	179
35 min, 120°C	**34.6**	**69.4**	**80.0**	**183**
Rapeseed meal 1	30.3	65.8	76.2	169

The optimum treatment process was chosen for each protein (highlighted in bold, Table 2) for scaling up to a commercial run on the basis of maximising DUP content calculated using the rumen N disappearance after 10 hours and pepsin/pancreatin digestibility of the residue and economics. For example, the small increase in DUP content was not justified considering the extra cost of treating proteins at 132°C compared with 125°C.

3.2 Phase 4: Protein evaluation of processed proteins and feeds used for the animal feeding study

3.2.1 Chemical analyses

The results of the chemical analysis of the grass silage, soyabean meal, fish meal, rapeseed meal, commercially treated rapeseed meal using a novel process, heat treated lupin seed, heat treated bean and heat treated rapeseed meal studied are shown in Table 3. The treated rapeseed meals compared with the untreated rapeseed meal had a slightly lower DM and N content, but considerably higher ADIN content (11.7 and 6.6 v 5.5 g/kg DM for novel treated, heat treated and untreated rapeseed meals respectively). The ADIN content of the treated rapeseed meals accounted for a substantially higher proportion of the total N (200, 113 and 92 g/kg N for novel, heat treated and untreated rapeseed meals respectively).

Table 3: Chemical composition of grass silage and a range of feedstuffs (g/kg DM or as stated).

	Grass silage	Fish meal	Soya bean meal	Rapeseed meal - novel treated	Rapeseed meal heat treated	Lupins heat treated	Beans heat treated	Rapeseed meal untreated
Oven dry matter (g/kg as fed)	282	940	890	889	907	885	906	919
Nitrogen	24.7	111.0	83.9	58.5	58.2	53.0	45.4	59.8
Crude protein	154	694	524	366	364	331	284	374
ADIN	0	<1	2.1	11.7	6.6	2.7	1.6	5.5

Table 4: Water solubility of silage and a range of feedstuffs.

	Grass silage	Fish meal	Soya bean meal	Rapeseed meal - novel treated	Rape seed meal heat treated	Lupins heat treated	Beans heat treated	Rape seed meal untreated
DM solubility (%DM)	28.1	24.8	22.6	21.1	16.9	15.9	12.6	17.5
N solubility (%N)	55.2	36.7	9.6	9.8	2.6	3.4	12.0	6.8

3.2.2 *In situ* nitrogen degradability and digestibility by pepsin/pancreatin

The DM and N water solubility determined for the eight samples of feedstuff are shown in Table 4. The nitrogen solubility was high for both the grass silage and the fish meal. Of the heat treated products, beans had the highest nitrogen solubility and the rapeseed meal the lowest.

The DM and N degradability data for all the samples of feedstuff are shown in Table 5. For DM, all the samples of rapeseed meal regardless of treatment had similar *a* and *b* fractions and varied mainly in the rate of degradation (*c*) of the *b* fraction, with novel treated rapeseed meal having the lowest rate followed by the heat treated rapeseed meal and finally untreated rapeseed meal. The heat treated lupins and beans had similar *a* and *b* fractions to soyabean meal with similar rate of degradation for lupins, but a slower rate for beans. Fish meal had slowest rate of degradation (*c*) of the *b* fraction but a high *a* fraction.

For N, the water solubilities were lower than *a* fractions (Tables 4 and 5) for all the samples with the exception of soyabean meal and novel treated rapeseed. The rate of degradation of N in the *b* fraction was similar for soyabean meal, heat treated beans and the untreated rapeseed meal. Both the treated rapeseed meal products and the heat treated lupins had similar rates of degradation and these were lower than the soyabean meal. The fish meal had the slowest rate of degradation and the grass silage the fastest. The effectively degradable N fractions (at 0.08 h^{-1}, adjusted for water solubility) was lowest for the heat treated rapeseed meal. The novel treated rapeseed and heat treated lupins had similar effective degradability's, while the untreated rapeseed meal had a slightly higher value. The soyabean meal, fish meal and heat treated beans had higher effective degradability's and all were similar. The grass silage had the highest effective nitrogen degradability.

Table 5: Degradation characteristics and effective degradability of silage and seven feedstuffs.

	Grass silage	Fish meal	Soya bean meal	Rapeseed meal novel treated	Rape seed meal heat treated	Lupins heat treated	Beans heat treated	Rape seed meal untreated
Dry matter degradability								
a (%)	39.5	43.1	24.1	22.7	18.7	21.4	25.6	21.2
b (%)	47.6	57.0	76.0	68.5	68.1	77.5	71.9	62.6
c (h^{-1})	0.061	0.008	0.069	0.034	0.044	0.067	0.053	0.057
lag (hours)	1.6	0.7	1.9	2.2	2.5	1.4	1.1	1.7
Effective DM degradability at outflow: 0.08 (h^{-1})	57.6	48.2	54.3	39.8	38.4	52.9	51.7	43.9
Nitrogen degradability								
a (%)	65.0	54.9	5.6	8.8	13.6	10.5	16.4	14.3
b (%)	25.4	54.2	94.5	91.3	86.5	93.7	83.7	82.9
c (h^{-1})	0.077	0.020	0.059	0.035	0.037	0.036	0.058	0.052
lag (hours)	2.6	1.2	0.05	2.3	4.4	-0.6	0.1	1.2
Effective N degradability at outflow: 0.08 (h^{-1})	75.2	55.9	45.4	32.1	32.7	41.0	51.1	44.0
Effective N degradability at outflow: 0.08 (h^{-1}) adjusted for water solubility	68.1	48.4	47.6	32.9	24.1	36.3	48.6	39.1

The results of the measurement of nitrogen disappearance from the rumen 12 hour/pepsin-pancreatin digestion residue and the calculation of effective degradable protein and undegradable protein of the eight samples of feedstuff are shown in Table 6. Fish meal and soyabean meal had the highest ERDP, while all the treated protein sources had similar ERDP values. Fish meal and soyabean meal had the highest DUP. The two treated rapeseed meals and the untreated rapeseed meal had similar DUP values, while there were lower amounts in heat treated lupins and beans.

Table 6: ERDP and DUP contents calculated at a rumen outflow of 0.08 h^{-1}.

	ERDP (g/kg DM)	DUP (Calculated AFRC 1993) (g/kg DM)	N digestibility of 12 hour residue (%)	Overall N digestibility of 12 hour residue (%)
Soya bean meal	233	245	90.1	95.5
Fish meal	331	263	72.8	87.1
Grass silage	100	31	39.2	86.6
Rapeseed meal - novel treated	128	142	68.5	75.7
Rapeseed meal - heat treated	139	157	71.5	77.8
Lupins - heat treated	124	165	67.1	82.3
Beans - heat treated	137	115	71.7	89.1
Rapeseed meal - untreated	165	147	79.2	88.5

3.2.3 Amino acid degradation in the 12 hour residues

The amino acid profile of the eight feedstuffs studied are shown in Table 7. Fish meal had the highest content of lysine and methionine plus cystine. Treatment of the rapeseed meal did not alter its' amino acid profile. Lupin seed was low in the sulphur amino acids methionine and cystine compared with the other feedstuffs.

The amino acid degradability of the 12 hour rumen residues for the eight samples of feedstuffs studied are shown in Table 8. For soyabean meal all the amino acids had disappeared to a similar extent (50-60%) in the 12 hour residue, whereas for fish meal there was very little degradation of cystine, tyrosine, methionine, leucine and threonine.

All of the amino acids in grass silage were extensively degraded by 12 hours with the exception of arginine which had disappeared less. The amino acids in the treated rapeseed meal products were less degraded than the untreated rapeseed meal and this was particularly notable for novel treated rapeseed. Tyrosine and methionine were very slowly degraded for novel treated rapeseed compared with both the heat treated and untreated rapeseed meal and all the other feedstuffs.

Table 7: Amino acid profile of eight samples of feedstuffs studied (g/kg DM).

Amino acid	Soyabean meal	Fish meal	Grass silage	Rapeseed meal Novel treated	Rapeseed meal Heat treated	Lupin seed Heat treated	Beans Heat treated	Rapeseed meal untreated
Aspartic acid	51.9	53.7	10.9	22.4	25.4	32.5	29.9	27.1
Serine	25.4	30.1	5.3	13.3	14.7	15.3	13.9	16.4
Glutamic acid	83.5	79.0	11.0	53.2	59.0	62.2	47.8	64.3
Glycine	19.7	67.1	6.8	14.6	16.0	11.1	11.3	17.9
Histidine	11.7	11.6	2.0	7.2	8.1	6.3	6.7	9.2
Arginine	32.9	43.5	3.3	13.0	16.5	28.6	24.8	19.5
Threonine	18.4	22.9	5.4	13.3	14.7	11.0	10.2	16.0
Alanine	19.4	41.4	11.8	13.5	14.7	10.5	11.2	16.4
Proline	23.6	35.5	9.4	18.9	21.3	12.9	11.7	22.0
Cystine	6.4	3.9	1.0	5.2	6.3	3.8	3.2	7.5
Tyrosine	16.5	15.6	3.2	9.0	9.8	13.1	9.7	9.9
Valine	20.3	24.2	7.8	15.0	16.4	11.7	11.7	17.6
Methionine	7.4	13.7	1.7	5.2	5.8	1.9	2.1	6.4
Lysine	28.5	40.8	5.0	11.4	17.3	14.6	17.8	20.0
iso-Leucine	19.8	19.4	5.9	11.5	12.8	12.4	10.5	13.2
Leucine	34.4	36.2	10.0	21.5	23.6	22.4	19.7	24.4
Phenylalanine	24.7	19.8	5.7	12.0	15.2	14.0	11.3	13.7

Table 8: Amino acid degradability of the 12 hour rumen residues for the eight samples of feedstuffs studied (% amino acid degraded).

Amino acid	Soyabean meal	Fish meal	Grass silage	Rapeseed meal novel treated	Rapeseed meal Heat treated	Lupin seed Heat treated	Beans Heat treated	Rapeseed meal untreated
Aspartic acid	56.4	49.0	79.8	29.7	35.8	49.3	63.2	55.1
Serine	51.9	46.7	74.4	11.8	22.9	40.8	59.7	48.1
Glutamic acid	58.2	50.8	77.8	31.2	41.1	55.3	67.4	61.0
Glycine	53.3	73.1	76.7	19.3	30.6	45.4	60.1	53.2
Histidine	56.9	43.2	78.7	22.1	34.3	50.6	64.5	57.6
Arginine	57.7	58.1	57.8	17.6	28.1	58.7	68.8	50.6
Threonine	53.7	36.6	77.1	16.1	26.8	43.1	62.4	46.5
Alanine	54.5	59.8	86.9	23.2	32.7	47.7	62.9	56.3
Proline	54.3	64.2	83.5	22.9	36.7	49.6	62.1	54.7
Cystine	54.7	22.3	61.3	22.6	36.6	50.0	62.3	58.3
Tyrosine	50.4	21.3	71.0	3.0	21.5	44.8	62.7	39.2
Valine	56.8	44.0	83.6	28.6	35.4	49.2	63.9	56.0
Methionine	61.7	37.9	68.1	9.6	20.2	38.3	59.4	47.4
Lysine	61.9	49.1	76.8	33.4	45.2	51.8	66.5	62.9
iso-Leucine	57.6	41.7	82.9	28.6	36.3	50.1	64.5	54.1
Leucine	52.1	36.6	78.3	20.7	30.4	46.4	60.0	50.1
Phenylalanine	53.3	45.2	78.3	16.4	35.7	46.0	58.2	43.4

4. DISCUSSION

There are various methods of protecting protein, with the most common being combinations of temperature and time. In this study, the optimum heat treatment process was found to be heating rapeseed meal, lupins and beans at 120ºC for 35 minutes based on cost and maximising digestible undegradable protein supply. The fact that the same heating process led to optimum treatment in all three proteins was surprising as Beever and Thomson (1981) found that when using a protection method, 64% of casein protein was protected compared with only 3% protection of peanut protein.

Bencharr *et al* (1994) reported that the optimum temperature of processing beans was 195ºC, and likewise, Kung *et al* (1991) used a process involving heating whole lupins at 175ºC. However, in these examples, although the extrusion/roasting methods used higher temperatures, the residence time was seconds not minutes. McKinnon *et al* (1995) examined the effect of various temperatures and durations on rapeseed meal, and found heating to 125ºC reduced rumen degradability, whereas heating at 145ºC led to a significant reduction in digestibility. This over protection may be associated with Maillard type reactions where proteins bind to sugars rendering the protein indigestible. In this current study, there was no evidence of reduced digestibility in beans when the processing temperature increased from 120 to 132ºC.

ADIN has been used as a measure of protein damage as it includes Maillard reaction products and tannin protein complexes (Van Soest *et al* 1987). Goering *et al* (1972) found that nitrogen bound to acid detergent fibre (ADF) was indigestible and Schroeder *et al* (1996) demonstrated that ADIN content was a good indicator of heat damage to the protein in sunflower cake. To reflect these observations, ADIN is used in the UK metabolisable protein (MP) system (AFRC 1993) as a measurement of indigestible protein. In this study, ADIN was 2.7, 6.6 and 1.6 g/kg DM for heat-treated lupins, rapeseed meal and beans respectively, higher than published values of 1-2, 0.5 and 3.6-4.8 g/kg for untreated lupins, rapeseed meal and beans respectively (ADAS 1995, AFRC 1993). Values were however considerably lower than the upper limit of 120 - 150 g/kg suggested by Schroeder *et al* (1996), who speculated that exceeding this limit may lead to a reduction in the supply of amino acids from the undegraded protein of sunflower cake.

Similar heat treatments to those employed in this study are extensively used in Sweden and Finland where they have been demonstrated to reduce effective protein degradability by up to 20% (Tuori 1992) while having a minimal effect on digestibility. Overall, the results achieved in this study are comparable with values obtained by other research workers (Table 8).

The calculated DUP content was 157, 165 and 115 g/kg DM for heat-treated rapeseed meal, lupins and beans respectively. For beans and lupins, these values were much higher than published values for untreated proteins (59 and 51 g/kg DM respectively) demonstrating the potential value of heat treatment in improving protein quality. However, the difference between the DUP contents of untreated and treated rapeseed meal was small because the level of DUP in the untreated rapeseed (147 g/kg DM) was much higher than anticipated (78 g/kg DM, AFRC 1993).

Table 8: Comparison of rumen bypass protein content of heat treated rapeseed meal, lupins and beans with published values.

Protein	Treatment process	Rumen bypass protein (% CP)	Reference
Rapeseed meal	**Moist heat (120 °C for 35 min)**	**59**	**This study**
	Moist heat (2-3 atm < 30s)	54	Herland 1996a
	Moist heat (2-3 atm < 30s)	48	Bertilsson *et al* 1994
	Moist heat (2-3 atm < 30s)	60 and 61	Herland 1996b
	Moist heat (2-3 atm < 30s)	34	Huhtanen and Heikkila 1996
	Moist heat 130 °C	51	Dakowski *et al* 1998
	140 °C	77	
	150 °C	80	
Lupins	**Moist heat (120 °C for 25 min)**	**60**	**This study**
	Heat (300 °C for 1-4 min)	21	Zaman *et al* 1995
	Heat 130 °C	55**	Kung *et al* 1991
	175 °C	61**	
	Roasted	33	Robinson and McNiven 1993
	Roasted	45	Singh *et al* 1995
	Pressure toasted	47 and 51	Goelema *et al* 1998
	Extruded	61	Benchaar *et al* 1991
Beans	**Moist heat (120 °C for 25 min)**	**49**	**This study**
	Extrusion (195 °C)	58*	Benchaar *et al* 1992
	Pressure toasting	48 and 57	Goelema *et al* 1998

* = PDIA (≡ DUP)
** = Based on N disappearance after 12 hour incubation in rumen fluid

Comparison of the protein degradability of untreated rapeseed meal with published figures (Table 9) suggests that the standard rapeseed meal used in this study had an lower than expected degradability.

Table 9: Comparison of the determined effective degradability of untreated rapeseed meal with published values.

Source	Effective protein degradability (% CP)
This study	**0.44**
Allison 1999	0.46
Bertilsson *et al* 1994	0.72
Hutanen and Heikkila 1996	0.78
MAFF 1990	0.59 - 0.79
AFRC 1993	0.69
ADAS 1989	0.59 - 0.70

This low degradability may be due to differences in the processing of rapeseed during commercial oil extraction, as any heating or drying of the extracted meal may further protect the protein. Kendall *et al* (1991) stated that variation in the protein quality of rapeseed meal can be related to methods used at the processing plant during oil extraction, and the results from this study highlight the variability in the UK of rapeseed meal protein quality and its potential consequence on ration formulation.

The use of the novel treatment process on rapeseed meal increased the amount of protein which bypassed the rumen, but reduced DUP content compared with the heat treated rape seed meal. This may be due to a reduced digestibility of the protein as evidenced by a higher ADIN level and a lower pepsin-pancreatin digestibility. However, the 12 hour rumen residue study indicated that novel treated rapeseed meal tended to have lower amino acid degradabilities, and that tyrosine and methionine were particularly slowly degraded. Methionine is generally regarded as the first limiting amino acid for milk protein synthesis (Rulquin and Verite 1993, Schwab *et al* 1976), and the potential rumen bypass methionine supplied by the novel treated rapeseed meal may be beneficial to high producing dairy cows.

APPENDIX 2

The effect of feeding heat treated rapeseed meal, lupins and beans on animal performance

1. OBJECTIVE

The main objective of this section of the work was to evaluate the heat treated rapeseed meal, lupins and beans in terms of dairy cow performance. The specific objectives of this project were:-

1. To reduce the milk yield depression reported in cows fed high levels of rapeseed meal by effectively protecting the protein through heat treatment.

2. To reduce the adverse reported effects of lupins on milk protein quality by effectively protecting the protein through heat treatment.

3. To evaluate the use of beans as a home-grown protein source, the protein in beans being effectively protected by heat treatment.

4. To evaluate a combination of the heat treated home-grown protein sources (lupins, rape or beans).

2. MATERIAL AND METHODS

2.1 Stock

A total of 60 second and subsequent parity Holstein-Friesian cows were used in the study. Cows were on average 65 days in lactation at the start of the study.

2.2 Housing

The cows were housed in cubicles, which were bedded daily with wood shavings and slurry removed at frequent intervals by automatic scrapers.

2.3 Treatments

Five diets were formulated using feed data generated from *phase 4* and analysis of raw materials prior to the study, to meet the energy and protein requirements for maintenance + 38 kg milk/day with no live weight loss, using the current UK metabolisable energy (ME) and metabolisable protein (MP) (+15%) systems (AFRC 1993). Each diet differed in the combination of protein sources used to meet protein requirements as follows:-

Diet		Protein source (s)
	Control	Fishmeal + soyabean meal
HR	**Rapeseed meal**	Heat treated rapeseed meal
HL	**Lupins**	Heat treated lupins
HB	**Beans**	Heat treated beans
HC	**Combination**	Heat treated beans, lupins and rapeseed meal

2.4 Experimental design and statistical analysis.

In a randomised block design, a total of 60 high yielding Holstein-Friesian cows in early lactation were formed into 12 blocks of five cows, on the basis of parity and days in milk. Within each block cows were allocated at random to one of the five treatments (Control, HR, HL, HB and HC) to give a total of 12 cows per treatment. Dry matter intake, milk yield and milk quality was measured in week -2 when all cows were fed a commercial ration, and these values were used as the covariate in the statistical analysis. During week -1 cows were changed onto the treatment diets. The experimental period ran for 8 weeks (weeks 1 - 8).

The data obtained during the animal study was subject to analysis of variance (ANOVA), and where there were significant differences between treatments, statistical comparisons were made against Control.

2.5 Feeding details

2.5.1 Diets

The formulation details of the five diets are given in Table 1 and the theoretical specification of each diet given in Table 2. The diets were formulated using measured N degradability data and amino acid content (*phase 4*) for each protein source, and the untreated rapeseed meal and grass silage used in the study (for data see Appendix 1). All diets were fed as total mixed rations (TMR's) to appetite on one occasion daily.

Table 1: Diet details (g/kg diet DM).

	Control	HR	HL	HB	HC
Grass silage	509	509	516	509	509
Wheat	199	199	202	119	199
Sugar beet feed (molassed)	165	115	100	136	107
Fish meal	21	-	-	-	-
Heat-treated					
Rapeseed meal	-	113	-	-	83
Beans	-	-	-	167	21
Lupins	-	-	124	-	20
Untreated rapeseed meal	42	42	33	45	38
Soya 48	41	-	-	-	-
Calcium salts of fatty acid*	13	13	14	13	13
Mineral/vitamin	10	9	11	11	10

* Megalac, Volac Ltd., Royston UK

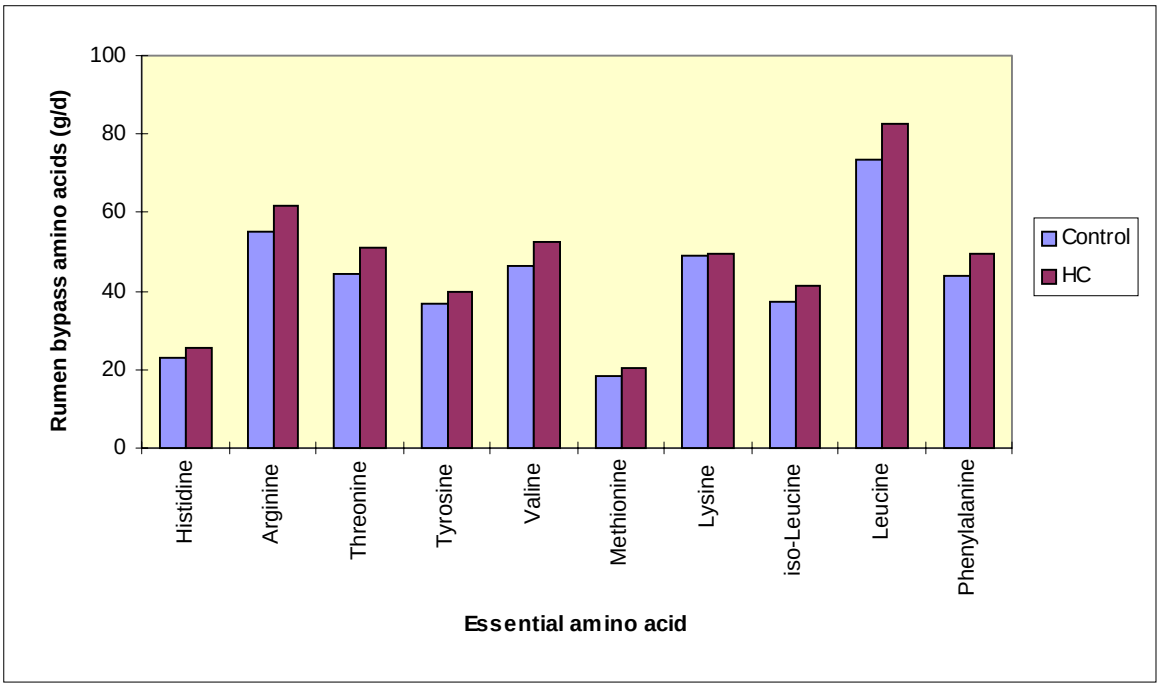
Table 2: Theoretical diet specification and nutrient supply from diets shown in Table 1.

	Control	HR	HL	HB	HC
Specification (g/kg diet DM)					
CP	190	190	189	191	190
Oil (acid hydrolysis)	41	42	51	41	44
Neutral detergent fibre (NDF)	346	369	351	350	362
Starch	142	146	148	153	153
Sugar	64	60	45	60	56
Supply (units/day)					
Dry matter (DM) (kg)	21.6	21.6	21.3	21.6	21.6
Metabolisable energy (ME) (MJ)	266	263	265	264	265
Fermentable ME (FME) (MJ)	218	214	209	216	214
ERDP:FME	10.9	10.7	10.7	10.6	10.7
MP (g)	2657	2628	2636	2697	2643
Digestible undegradable protein (DUP) (g)	1147	1177	1212	1236	1187

(ERDP = effective rumen degradable protein)

In addition, the Control and HC rations were formulated using the amino acid data (Appendix 1) to achieve similar supplies of the 10 essential amino acids (as defined by Fleet and Mepham (1985)) (Figure 1).

Figure 1: Formulated rumen bypass essential amino acid supply for Control and HC rations as outlined in Tables 1 and 2 using determined rumen bypass amino acid content of feedstuffs.



2.5.2 Silage

The grass silage was made from first cut Italian Ryegrass, ensiled on 1/05/1999 without the use of an additive.

2.5.3 Concentrates

Premixes containing the non-forage components of each diet were prepared in batches. Each diet was prepared daily by adding the forage and premix into a mixer wagon and mixing thoroughly immediately prior to feeding.

2.6 Feed analysis

2.6.1 Grass Silage

During the experiment, the grass silage was sampled in weeks 1, 4, 6, and 8. At the end of the study the accumulated frozen samples were bulked up and sent to the ADAS laboratory at Wolverhampton. The sample was analysed for the following:-

Dry matter, pH, ammonia N as % total N, crude protein, water soluble sugars, neutral detergent fibre and total ash. Organic matter digestibility (OMD) and metabolisable energy contents were determined by NIR.

2.6.2 Raw materials

Protein supplements were sampled in weeks 1, 4, 6 and 8, bulked up and sent to the ADAS laboratories at Wolverhampton for determination of :-

Dry matter, crude protein, water soluble nitrogen, neutral cellulase gamanase digestibility (NCGD), oil (acid hydrolysis), starch, neutral detergent fibre and total ash.

In addition, samples of wheat and molassed sugar beet feed were sampled in weeks 1, 4, 6 and 8 and bulked up. The bulk samples were sent to ADAS Wolverhampton for the following analyses:-

Dry matter, crude protein, neutral detergent fibre and total ash.

2.7 Measurements

2.7.1 Feed Intake

The quantity of complete diet offered to each cow was recorded daily. Any complete diet remaining at the beginning of the following day was weighed and discarded. Each complete diet was sampled on three occasions each week for determination of dry matter and the values used to calculate daily individual dry matter intake.

2.7.2 Milk yield

Individual daily milk yield was recorded to the nearest 0.1 kg.

2.7.3 Milk composition

Two milk samples were taken from each cow on two consecutive milkings in weeks week -2 (covariate week), 2, 4, 6 and 8. One sample was submitted to the National Milk Records (NMR) laboratory for determination of fat and protein contents and a second sample was sent to the ADAS Laboratory for determination of urea, non protein N and casein N content.

2.7.4 Live weight

The cows were weighed in weeks -2 (covariate), 1, 5 and 8 of the study.

2.7.5 Health

Routine daily health records were kept throughout the study.

3. RESULTS

3.1 Feed analysis

3.1.1 Silage quality

The analysis of the grass silage used throughout the study is given in Table 3.

Table 3: Analysis of grass silage (g/kg DM(C) unless stated otherwise).

Dry matter (corrected for volatiles)	285
pH	3.8
Ammonia N as % TN	10
Total crude protein (corrected for ammonia)	178
Total ash	99
Neutral detergent fibre	445
Digestibility (D value) %	74
Metabolisable Energy (MJ/kg DM(C))	11.9
Fermentable Metabolisable Energy (MJ/kg DM (C))	8.6
Total fermentation acids (TFA)	137
Lactic acid	102
Acetic acid	30
Butyric acid	< 1

Analysis confirmed that the grass silage fed throughout the study was well preserved. The high energy and protein levels are typical of young leafy grass cut in early May.

3.1.2 Raw materials

The protein supplements fed in the animal study were heat treated (120ºC for 35 mins) rapeseed meal, lupins and beans, untreated rapeseed meal, soyabean meal and fish meal. The novel treated rapeseed meal was not selected on the basis of a lower digestible undegradable protein (DUP) content.

The analysis of the raw materials used in the study are shown in Table 4 with most values within published database ranges (AFRC 1993, MAFF 1990) and in agreement with previous MAFF/MDC work at ADAS Bridgets (Mansbridge 1997a, 1997b).

Table 4: Chemical composition of raw materials fed.

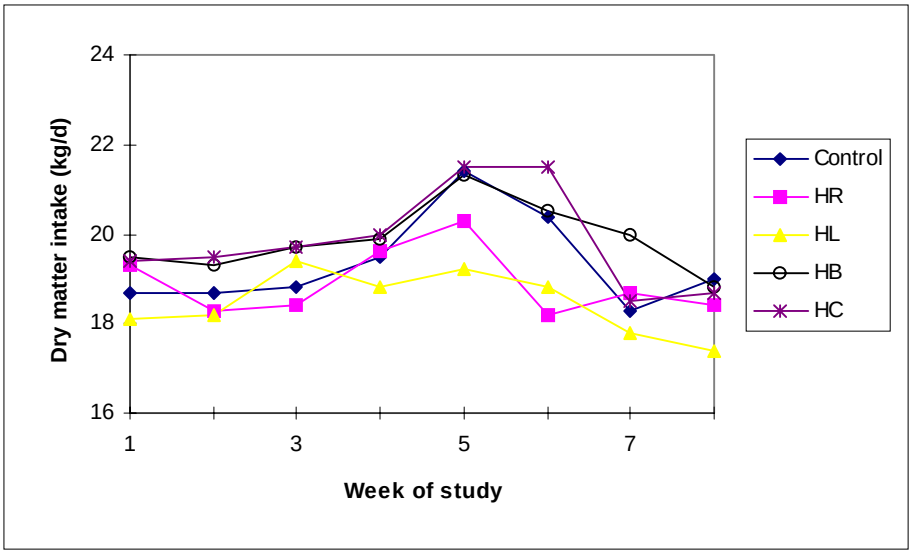
g/kg DM unless otherwise stated	Rapeseed meal		Heat treated beans	Heat treated lupins	Fish meal	Soyabean meal
	Untreated	Heat treated				
Dry matter	875	871	870	821	921	865
Ash	74	71	38	38	231	62
Crude protein	386	381	289	362	692	513
Water soluble nitrogen	8.2	3.4	5.3	6.1	47.3	4.1
Oil (Acid ether extract)	27	30	29	99	91	23
Neutral detergent fibre	321	413	166	226	194	123
Starch (Enzymatic)	7	12	339	22	< 1	20
Neutral cellulase gaminase digestibility (NCDG)	763	710	942	936	754	930
Metabolisable energy (ME) (MJ/kg DM)*	11.4	10.7	13.9	15.6	12.8	13.6

*Calculated using equation E3, (Thomas *et al* 1988).

3.2 Dry matter intake

There was a significant ($P < 0.01$) interaction between week and treatment (Table 5), weekly means are shown graphically in Figure 2. During weeks 1 - 4 there were no significant differences between Control and either HR, HL, HB or HC. However in weeks 5, 6 and 8, dry matter intake were significantly ($P < 0.01$) lower for cows in HL compared with Control. Additionally, in weeks 5 ($P = 0.07$) and 6 ($P < 0.001$), HR had a lower dry matter intake compared with Control. Dry matter intake was significantly ($P < 0.01$) higher for HB than Control in week 7.

Figure 2: Effect of week and treatment on dry matter intake (kg DM/day).



Treatment group means are presented in Table 5 below.

Table 5: Dry matter intake (kg/day) for weeks 1-8.

Treatment	DM intake (kg/day)	s.e.
Control	19.3	0.36
HR	18.9	0.34
HB	19.9	0.37
HL	18.5	0.36
HC	19.9	0.34
P values		
Treatment	*	
Week	> 0.2	
Treatment x week	**	

3.3 Milk yield

There was no significant interaction between treatment and week (Table 6). Additionally, there were no significant differences in milk yield between the five treatment groups. Mean milk yield values for cows in groups HR, HL, HB and HC were within 1 kg milk/day of cows in the Control (Table 6).

Table 6: Milk yield (kg/d) for weeks 1-8.

Treatment	Milk yield (kg/day)	s.e.
Control	33.0	0.71
HR	32.9	0.68
HB	33.4	0.71
HL	33.9	0.71
HC	33.2	0.67
P values		
Treatment	> 0.2	
Week	> 0.2	
Treatment x week	> 0.2	

3.4 Milk quality

3.4.1 Milk composition

No significant interactions between treatment and week were found for milk protein yield or content, there was however a significant ($P < 0.01$) difference between treatments for milk protein content (Table 7). Cows in the HL group had a significantly ($P < 0.01$) lower milk protein content than cows in the Control group. Cows in the other three treatment groups (HR, HB and HC) were not significantly different from Control cows. Milk protein yield was not significantly different between the five treatment groups of cows.

Table 7: Milk protein content (g/kg) and protein yield (kg/day).

Treatment	Milk protein content (g/kg)	s.e.	Milk protein yield (kg/day)	s.e.
Control	32.3	0.35	1.06	0.024
HR	32.1	0.35	1.04	0.022
HB	31.6	0.35	1.05	0.024
HL	30.6	0.35	1.00	0.024
HC	31.7	0.35	1.05	0.023
P values				
Treatment	**		> 0.20	
Week	*		> 0.20	
Treatment x week	0.09		0.15	

There were no significant interactions between treatment and week or main treatment effects on milk fat content or milk fat yield (Table 8).

Table 8: Milk fat content (g/kg) and fat yield (kg/day).

Treatment	Milk fat content (g/kg)	s.e.	Milk fat yield (kg/day)	s.e.
Control	45.5	1.35	1.48	0.068
HR	44.9	1.28	1.50	0.064
HB	42.2	1.35	1.38	0.068
HL	42.1	1.34	1.40	0.068
HC	43.5	1.34	1.43	0.068
P values				
Treatment	> 0.20		> 0.20	
Week	> 0.20		0.09	
Treatment x week	> 0.20		> 0.20	

Similarly, no significant interactions between treatment and week or main treatment effects were found for milk lactose yield or content (Table 9).

Table 9: Milk lactose content (g/kg) and lactose yield (kg/day).

Treatment	Milk lactose content (g/kg)	s.e.	Milk lactose yield (kg/day)	s.e.
Control	46.3	0.28	1.52	0.045
HR	45.6	0.26	1.49	0.042
HB	46.6	0.28	1.54	0.044
HL	46.4	0.28	1.54	0.044
HC	46.1	0.28	1.53	0.044
P values				
Treatment	0.12		> 0.20	
Week	> 0.20		> 0.20	
Treatment x week	> 0.20		> 0.20	

3.4.2 Milk protein fractions

There was no significant treatment x time interaction, but milk casein N content of milk was significantly ($P < 0.001$) different between the treatment groups (Table 10). Cows in group HL had a significantly lower milk casein N content than cows in the control group (0.38 and 0.40 g/100g respectively). Milk casein N contents for cows in groups HR, HB and HC were not significantly different to the Control group.

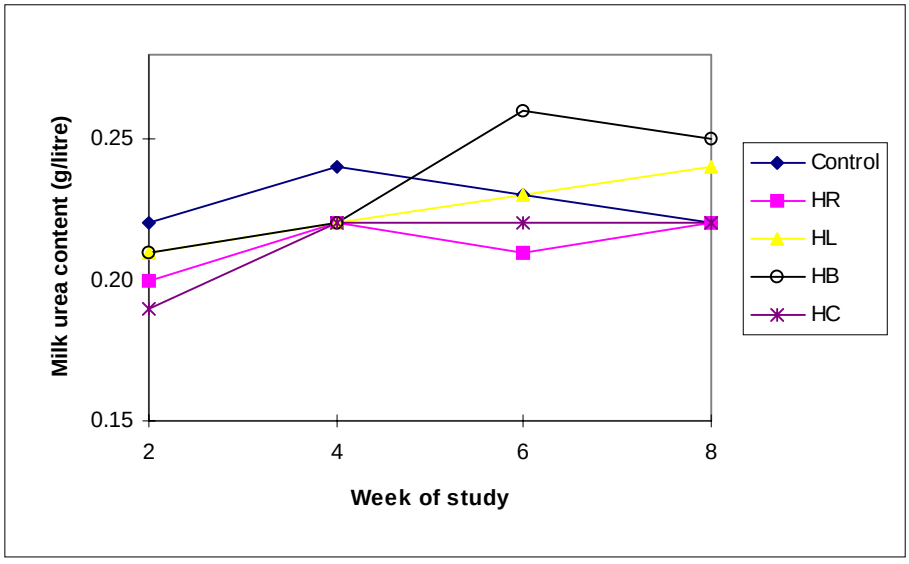
There was no significant interaction (week x treatment) or treatment effect on milk non-protein nitrogen (NPN) content across the five groups of cows.

Table 10: Milk casein N, non-protein N (NPN) and urea content.

Treatment	Milk casein N content (g/100g)	Milk NPN content (g/kg)	Milk urea content (g/l)
Control	0.40	0.29	0.23
HR	0.41	0.29	0.21
HB	0.39	0.29	0.24
HL	0.38	0.29	0.22
HC	0.40	0.29	0.21
s.e.	0.005	0.004	0.007
P values			
Treatment	***	>0.20	*
Week	0.13	*	*
Treatment x week	>0.20	0.13	*

There was a significant ($P < 0.05$) interaction of week and treatment for milk urea content. Cows in HC had a significantly ($P < 0.05$) lower milk urea content in week 2 but not weeks 4, 6 or 8. Cows fed HB had a higher milk urea content in weeks 4 ($P = 0.06$) and 6 ($P < 0.05$) compared with Control. Group HR tended to have a lower milk urea content in weeks 2 ($P = 0.09$) and 6 ($P = 0.08$) compared with Control (Figure 3).

Figure 3: Effect of week and treatment on milk urea content (g/l).



3.5 Live weight and body condition score

There was no significant interaction or main effect of treatment on live weight or body condition score (Tables 11 and 12). Live weight change was not significantly different between treatments.

Table 11: Live weight (kg).

Treatment	Live weight (kg)	s.e.	Total liveweight change (kg)	s.e.
Control	652	5.4	-7.2	5.89
HR	646	5.1	+4.2	5.58
HB	650	5.1	+8.6	5.58
HL	650	5.4	-0.8	5.89
HC	641	5.1	-11.7	5.58
Treatment	> 0.20		0.09	
Week	0.18		-	
Treatment x week	> 0.20		-	

Table 12: Condition score (median for weeks 1, 2, 5 and 8).

Treatment	Condition score (week 0)	Condition score (week 2)	Condition score (week 5)	Condition score (week 8)
Control	2.0	2.0	2.0	2.0
HR	2.0	2.3	2.3	2.0
HB	2.0	2.3	2.0	2.0
HL	2.5	2.8	2.5	3.0
HC	2.5	2.4	2.0	2.0
P	> 0.20	> 0.20	> 0.20	> 0.20

4. DISCUSSION

4.1 Objective 1 - Rapeseed meal

Standard rapeseed meal has a relatively high effective rumen degradable protein (ERDP) and low DUP content. Research at ADAS Bridgets (Mansbridge 1997a) found that feeding 5.8 kg (DM basis) of rapeseed meal to meet MP (and hence DUP) requirement led to a 5.2 kg/day depression in milk yield. The reduction could not be explained by dry matter intake as this was unaffected. However, the supply of rumen degradable protein was high relative to rumen fermentable metabolisable energy (FME) (ERDP:FME = 12.7) probably leading to the excess nitrogen being excreted in the urine, with the possible consequence of increased energy requirement. For example, Twigge and van Gils (1988) estimated that the energy cost associated with a daily surplus of 500 - 1000 g of rumen degradable protein would be 1.3 to 2.6 kg of fat corrected milk production. Reducing the degradability of protein in rapeseed meal might reduce the adverse effects of feeding rapeseed meal as the sole DUP source in dairy rations.

In this study, rapeseed meal was included at 31% of the concentrate component to maintain DUP supply when replacing fish meal and soyabean meal i.e. at a level which is twice of inclusion in UK dairy compounds (15% - MAFF Statistics (MAFF Statistical Service)). At this level, feeding a combination of untreated and heat treated rapeseed meal as the major protein sources produced the same performance (milk yield, milk quality and live weight) as a diet based on fish meal and soyabean meal. This agrees with the results of Garnsworthy (1997) who replaced fish meal with rumen protected rapeseed meal and showed no adverse effect on production. In addition, milk urea content remained at Control levels in this study suggesting that urea excretion was not elevated to the levels found in the previous study.

Production responses to heat treated rapeseed meal can be variable (Tuori 1992), and the benefits of heat treating rapeseed meal are not always evident. In studies where heat treating rapeseed meal had no effect on performance, the difference in protein degradability between untreated and treated rapeseed meal was small (0.06 - 0.17 as a proportion of total CP), suggesting that if protein degradability of the standard rapeseed meal is low (as in this study), heat treatment may not be required. However, the use of heat treatment when applied to “standard” rapeseed meal (i.e. with a protein degradability over 0.65), gives significant responses in dairy cow performance (Bertilsson *et al* 1994, Herland 1996).

Overall, it was demonstrated that high levels of rapeseed meal with a low protein degradabilities can be formulated in grass silage based dairy rations using the current UK ME and MP systems, instead of fish meal and soyabean meal, without any reduction in milk yield and quality, or increase in milk urea content.

4.2 Objective 2 - Lupins

Raw lupins are extensively degraded in the rumen (over 70% of CP, AFRC 1993) which can lead to increased milk urea content (Mansbridge 1997a and 1997b). Additionally, feeding lupins to dairy cows has lead to reductions in milk protein content (Guillaume *et al* 1987, Robinson and McNiven 1993, Singh *et al* 1995). These effects suggests a reduced efficiency of utilisation of feed protein, possibly due to a reduction in N utilisation for microbial protein synthesis. Benchaar *et al* (1994) reported a protein solubility of 30% and effective degradability of 64%, while UK sources (AFRC 1993; Mansbridge 1997a, Mansbridge 1997b) have reported higher degradabilities (71 and 69%). This study investigated whether reducing the protein degradability of lupins can reduce the adverse effect on milk protein content.

Feeding lupins which had been protected using heat treatment had no adverse effect on milk yield, milk protein yield, milk fat content and yield or milk lactose content and yield compared with Control diet containing fish meal and soyabean meal. However, consistent with other published findings (Bayourthe

et al, 1998, Robinson and McNiven 1993), there was a significant reduction in milk crude protein and casein content. Several reasons for this reduction have been suggested. Firstly, the sulphur containing amino acids (methionine and cystine) content of the lupins were low compared with either rapeseed meal, soyabean meal or fish meal. Methionine is generally regarded as first limiting amino acid for milk protein synthesis (Rulquin and Verite 1993), and responses in milk protein output (largely due to increased casein synthesis) have been observed when methionine supply is increased (Sloan 1997).

Secondly, lupins contain around 10% oil (Moss *et al* 1996), and it is generally accepted that feeding oilseeds to dairy cows can reduce milk protein content (DePeters and Cant 1992, Garnsworthy 1999, Wu and Huber 1994). The oil content of the diet based on lupins was higher than any other diet which may explain the reduction in milk protein content.

A third explanation is that dry matter intake was significantly lower for cows fed heat treated lupins in weeks 5, 6 and 8 which would reduce nutrient supply for milk production in the mammary gland. It was however interesting to note that the effects of feeding heat treated lupins on dry matter intake was not evident until 5 weeks after its introduction in the diet.

In summary, heat treated lupins can be fed instead of soyabean meal and fish meal in grass silage based rations without any adverse effect on milk yield or milk fat content, however there was a milk protein depression.

4.3 Objective 3 - Beans

Beans are a traditional crop grown in the UK and recently have seen important breeding improvements in seed yield and harvesting index by producing determinate types. Their protein however is readily degraded in the rumen resulting in a low DUP content (AFRC 1993). Therefore, beans are not ideal supplements when fed with grass silages (Wilkins and Jones 2000), but protection of the protein could increase their value as a ruminant protein source. Beans are currently not used extensively in standard dairy feed compounds (MAFF Statistics) and have a low national average inclusion rate (1-2%). Additionally, there is virtually no published data regarding the feeding of heat-treated beans to dairy cows. This study provided valuable data on feeding heat treated beans at high levels (34% of ration supplement) in dairy cow rations.

Beans are a valuable source of protein, starch (339 g/kg DM), and have an excellent ME content (13.1 MJ/kg DM). In this study, replacing fish meal and soyabean meal with heat treated beans as a protein source had no adverse effect on milk yield or quality, indicating that heat treated beans can be fed at levels higher than recommended for raw beans (16% of concentrates, Chamberlain and Wilkinson 1996). Peas, which are similar to beans, have been fed to high yielding dairy cows without any detrimental effect during early lactation (Corbett *et al* 1995).

Beans contain tannins which can have an adverse effect on protein digestibility (Chamberlain and Wilkinson 1996), but in this study, overall N *in vitro* digestibility was 891 g/kg DM, and comparable with other estimates of apparent digestibility (820 - 840 g/kg DM - ADAS Tech Bull. 90/2). It is interesting to note that beans, similar to lupins, contain low levels of the sulphur amino acids, but unlike lupins, have no effect on milk protein content. In summary, heat treated beans can be fed at high levels to replace fish meal/soyabean meal in dairy cow rations without affecting dairy cow performance.

4.4 Objective 4 - Combination of proteins

Rations to high yielding dairy cows traditionally contain more than one protein source, partly due to inclusion limits for certain individual protein sources (rapeseed meal, beans, etc.) and partly as a consequence of least cost rationing where proteins with different degradabilities are used to provide the

'perfect' balance. Some authors (Oldham 1994), have suggested that there may also be benefits in amino acid supply, as different protein sources can be deficient in one or more amino acids, for example, maize gluten which is low in lysine, and lupins which are low in methionine. Conversely, some protein supplements are high in specific amino acids e.g. fish meal which is high in lysine and methionine, and therefore blending proteins can lead to a supplement that has an "ideal" balance of amino acids for milk production. Schingoethe (1996) examined amino acid supply from mixtures of feed proteins, and found, for example, that a blend of soybean meal, maize gluten and meat/bone meal, had an amino acid balance that was closer to assumed requirements than any one of the individual ingredients. In this study, heat treated beans, lupins and rapeseed meal were formulated using a simple amino acid supply model to provide the same mixture of amino acids as the Control ration containing soyabean meal and fishmeal.

Results indicated no differences in any measure of performance between the Control and the blend of heat treated beans, lupins and rapeseed meal. This is consistent with work by Allison (1999), who demonstrated that a mixture of rumen protected vegetable proteins can replace fishmeal on a crude protein basis. The inclusion of a low level of lupins (4 versus 26% of supplement for HL and HC respectively) prevented any reduction in milk protein content. It is unclear from this work whether the effect of lupins on milk protein production is a consequence of its poor amino acid balance or some other factor. Further work is necessary to determine the maximum inclusion level of lupins in high yielding dairy cow rations when fed in combination with other protein (i.e. amino acid) sources.

4.5 Margin over purchased feed costs

Using standard farm measures of economic performance (i.e. margin over feed costs), cows fed the Control and HR diets had the highest margins over all feeds both per cow and per litre (Table 3). Replacing fish meal/soyabean meal with heat treated lupins, beans or a combination of proteins led to a reduction in margin over feed costs. This was due to a an increase in feed costs, although for lupins and the combination of proteins, there was also a reduction in the milk value. However, it must be noted that changes in market conditions circumstances such as increases in soyabean meal price (increased by £25 in the period between the conclusion of work and the preparation of this report) and reductions in bean/lupin prices, will change the margin over feed costs of heat treated UK grown proteins in dairy cow rations.

Table 3: Calculation of margins over feed costs for the dietary treatments.

Financial performance	Treatment				
	Control	HR	HB	HL	HC
Margin over all feed costs (p/l)	12.0	12.1	11.6	11.6	11.6
Margin over all feed costs (£/cow/day)	3.97	3.99	3.94	3.87	3.83
Milk value (p/litre)	17.6	17.5	17.3	17.1	17.4
Milk volume (litres)	33.0	32.9	33.9	33.4	33.2
Milk value (£/cow/day)	5.81	5.77	5.85	5.72	5.77
Feed cost (£/cow/day)*	1.84	1.78	1.91	1.85	1.92

* Calculated assuming feed costs: fish meal = £381/tonne, soyabean meal = £125/tonne, untreated rapeseed meal = £108/tonne, heat treated rapeseed meal = £143/tonne, heat treated beans = £134/tonne and heat treated lupins = £180/tonne. Heat treatment costs = £45/tonne (commercial rate).

From a financial perspective, feeding high levels of heat treated rapeseed meal in a total mixed ration based system was the most cost effective alternative to soyabean meal/fish meal. This would however be different for organic milk producers who cannot feed either fish meal or solvent extracted soyabean meal/rapeseed meal and also receive premiums for organic milk (over 25 p/litre). It must also be noted that there could be longer term effects of feeding high concentrations of heat treated rapeseed meal in rations on cattle health, this is being currently addressed in a separate HGCA funded project at ADAS Bridgets (Project: 2324).

Cheaper treatment technologies which retain the improvements in protein quality or alternatively using plant breeding to select for varieties of beans, lupins and rapeseed meal containing protein which is less degradable in the rumen, would further encourage their use in dairy cow rations.

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