Effects of heat treatment on protein feeds evaluated in vitro utilizing the method utilizable protein

M. Vaga, M. Hetta & P. Huhtanen
Dept.of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden.
Correspondence: merko.vaga@slu.se

Introduction

There is an increasing interest in using domestic feeds in diets for dairy cattle in Sweden. Due to increasing energy prices as well as ethical considerations and growing demand for certified organic feeds it is not rational anymore to depend on imported protein supplements for dairy cattle. Seeds of field beans (*Vicia faba*), and lupines (*L. angustifolius)* have high crude protein (CP) and starch content (NRC, 2001) and therefore have potential to replace imported soybean meal(May et al. 1993; Tufarelli et a. 2012).

Meeting dietary protein requirements of high producing dairy cows rely largely on the proportion of ruminally undegraded protein which also has high total digestibility. Heat treating protein feeds have been suggested to decrease protein degradability and increase nitrogen efficiency in dairy production (Broderic & Graig, 1980; Cros et al. 1991; Canbolat et al. 2005). One of the challenges has been to evaluate the effects of heat treatments with relevant laboratory methods as production trials are expensive. Several new methods for evaluation of forage protein for ruminants have been presented, e.g. Karlsson *et al*. (2009). The technique described by Karlsson *et al*. (2009) originates from the method developed by Raab *et al*. (1983), which utilises the production of ammonia nitrogen (NH_3-N) for the estimation of protein degradation *in vitro*. The method has also been modified into another promising method for estimation of protein supply from forages by using utilisable crude protein (uCP) (Edmunds *et al*., 2012). It quantifies simultaneously the availability of protein as the sum of the microbial *de novo* synthesis of protein in combination with estimations of the degradation of feed protein resulting in a total estimate of supply of protein from the rumen to the small intestine. Using *in vitro* methods for protein evaluation remove the problems like escape of soluble non-ammonia-N and particle losses, which occur in *in situ* methods (Ørskov and McDonald, 1979). The aim of this experiment was to evaluate the effects of heat treatment on protein feeds utilising the *in vitro* method utilisable protein.

Materials and Methods

Feeds and Experimental design

The experiment had 2x2x4 factorial design plus one control, including two protein feeds, two heat treatment methods and three temperature levels + untreated and one control diet, resulting in a total of 17 treatments. The two protein feeds used in this experiment were grains of field beans and lupines. Each protein feed was mixed together with standard silage and barley in isonitrogenous total mixed rations (TMR) in order to simulate real diets and take into account effects on microbial protein synthesis which provides about two thirds of the of the protein supply to the ruminant. The TMR diets consisted of 500 mg of standard grass silage, barley and protein feed, combined to give a CP concentration of 180 g/kg DM (Table 1). The control diet consisted of only standard silage and barley (1:1, fresh weight) with total CP concentration of

132 g/kg DM. Each diet was randomly distributed within and between *in vitro* series (run) and replicated twice within two of total eight runs, resulting in four observations per sample. In each run, a blank (buffered rumen fluid without a sample) and the control diet were incubated in duplicates.

Sample preparation and heat treatments

The feeds were dried at 60°C in force air oven for 48h and milled through a 1mm screen (cutter mill; SM300, Retsch GmbH, HAAN, Germany). Thereafter the samples of protein feeds, silage and barley were analysed for dry matter (DM) (105^{o}C for 16 h), ash (AOAC 1984) and CP (AOAC 1984). The chemical composition of the feeds and diets are presented in Table 1.

Table 1 Chemical composition of experimental feeds and diets

			Protein feeds		Diets		
	Silage	Barley	FB	LP	FBD	LPD	Control
DM, g/kg	939	866	905	882	914	907	903
OM, g/kg DM	941	969	960	959	956	956	960
CP, g/kg DM	143	120	299	348	180	180	132

FB – field beans; LP – lupines; FBD – field beans diet (field beans meal+silage+barley); LPD – lupines diet (lupines meal+silage+barley); Control - silage+barley diet.

The two protein feeds were heat-treated for 60 minutes at 120, 140 and 160°C in a ventilated oven (ULE 400, Memmert, Germany) with dry air or at 105, 120 and 135°C in an autoclave (PACS 50, Getinge Biofoe, Sweden) with steam pressure. The milled feed samples were placed on aluminium trays and evenly spread (2 cm deep layer). After the heat treatments, the samples were allowed to cool in open air for 24 h to regain original moisture content (avg. 92% DM) before packing and storing. A sample of each unheated feed was retained for use in the control treatments.

In vitro procedures

Rumen fluid was collected about two hours after morning feeding from two dry and ruminally cannulated Swedish red cows fed on a diet containing grass silage (600 g/kg DM) and commercial concentrates (400 g/kg DM). The rumen fluid was filtered through four layers of cheese cloth into a buffered mineral solution (Menke and Steingass, 1988), with a 1:4 volume ratio of rumen fluid to buffer. About 1 g of each TMR diet was incubated in 60 ml of buffered rumen fluid in 250-ml serum bottles (Schott, Mainz, Germany). The bottles were placed in water baths at 39°C for 48 h and continuously agitated. The *in vitro* gas production (GP) was recorded as described by Hetta et al. (2003). The *in vitro* procedures were performed with a fully automated system (Cone et al., 1996), recording GP every 12 minutes. Simultaneously with gas recordings, determinations of NH_3-N concentrations in the liquid phase were made at 8 h, 24 h and 48 h. The concentration of NH_3-N of the buffered rumen fluid in the incubation bottles was determined by sampling 0.4 ml of fluid with plastic syringes as described by Karlsson et al. (2009). The fluid samples were transferred into Eppendorf tubes kept on ice and thereafter, 0.016 ml of 96% H_2SO_4 was added for preservation. The samples were stored frozen (-20°C) until

analysis. The sample tubes were thawed, centrifuged (12500 x g, 10 min) and 0.1 ml of supernatant was transferred to test tubes and diluted 1:20 with distilled water. The concentrations of NH_3-N was analysed using a continuous flow analyser (AutoAnalyzer 3 HR, SEAL Analytical Ltd).

Data handling and statistical analysis

For each treatment, we calculated the concentrations of uCP as described by Edmunds et al. (2012) (eqn. 1) as:

$$\text{uCP (g/kg DM)} = \frac{NH_3N_{blank} + N_{sample} - NH_3N_{sample}}{\text{weight (mg DM)}} \text{ x } 6.25 \text{ x } 1000 \text{ (eqn. 1)},$$

where NH_3N_{blank} is the average amount (g) NH_3N in the two blanks at the time of interest, N_{sample} is the amount (g) of N in the sample at the start of the incubation and NH_3N_{sample} is amount (g) of NH_3N in the incubation bottles at the time of interest for the treatment evaluated.

The effect of heat treatments on uCP and NH_3-N were analysed using the GLM procedure in SAS (SAS Institute 2008). The model included feed, run, treatment temperature and method. Treatment effects were considered to be significant at $P \leq 0.05$.

Results and discussion

The use of heat treatments effectively increased the uCP concentrations for both the field bean and lupine diets. The lowest temperature for both the oven- and autoclave-treatments actually reduced the uCP concentrations for the field bean diets after 8 h. This reduction was expected as it is generally known that modest heat treatments can improve protein degradability (Fennema, 1976). Increasing the temperatures for oven-treated field beans caused linear increase of uCP based on the 24 h incubations (Table 2). However for lupines, only the medium and high temperatures had positive effects, but low temperature treatments instead decreased the concentration of uCP.

The lupines were less affected by the heat treatments than the field beans. The high temperature oven-treatment increased uCP levels from 120 to 166 g/kg DM for the field bean diets and from 167 to 187 for the lupine diets at 24 h. For the autoclaved feeds the increases were from 120 to 173 g/kg DM for field beans and from 167 to 178 g/kg DM for lupines. Similar effects were reported by Martinussen et al. (2013) who found that Lupines required higher temperatures than field beans to achieve the same reducing effect on soluble protein. Despite the different temperatures, both oven and autoclave treatments seemed to have similar effect on uCP and NH_3-N concentrations in this study.

Changes in concentrations of NH_3-N 0, 8 and 24 h incubations are shown in Figure 1. The autoclave treatments seemed to decrease NH_3-N concentrations already after 8 h while for oven treated feeds, the effect of heat treatment was significant after 24 h of incubation. These results are supported by Tagari et al. (1986) who noted that autoclave treatment of feeds increased the NH_3-N concentrations at lower temperatures compared to dry heat treatments. Heat treatment with both oven and autoclave significantly ($P < .001$) decreased the NH_3-N concentrations as shown in Table 2.

Table 2 uCP and NH_3-N concentrations of untreated and treated field beans and lupines diets after 24 hour incubations

	Untreated	Oven treated			SE	P	Autoclave treated			SE	P
Temperature, °C		120°C	140°C	160°C			105°C	120°C	135°C		
	uCP 24h (g/kg DM)										
Field beans	121	141	158	167	18.7	***	168	158	173	18.7	***
Lupines	167	153	179	187	17.2	***	171	190	179	18.8	***
Control	166										
	NH_3-N 24h (mg/L)										
Field beans	451	405	357	340	42.6	***	317	363	320	42.6	***
Lupines	341	373	313	290	40.6	***	331	323	309	47.7	***
Control	226										
Blank	309										

*** P<0.001

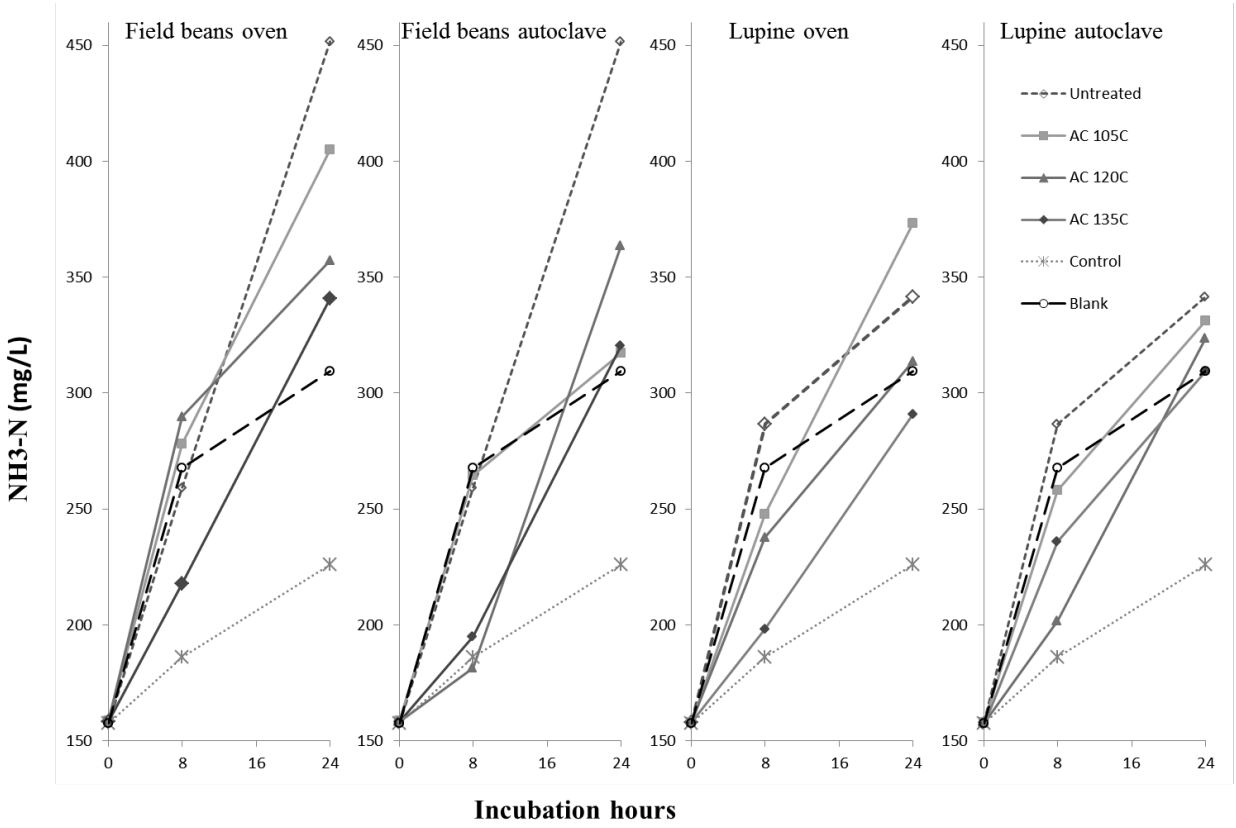

Figure 1 Effect of heat treatments of field beans and lupines on NH3-N concentrations *in vitro*.

There were higher correlations (r = -0.97 and r = -0.80) between temperature and NH3-N concentrations for oven-treated and autoclave-treated lupines than for field beans (r = -0.66 and r = -0.70).

The concentrations of NH_3-N (mg/L) had high variance among and within runs at 8 h (SE=24) and 24 h (SE=42), but very small variance at 0 h (SE=1). Small variance at the start of the incubations when no interactions had occurred yet, suggest a potential for good precision of the analytical method.

Conclusions

This study shows that heat treatment was effective in increasing the concentrations of uCP of in both field beans and lupines, but lupines needed higher temperatures to achieve the same effect as in field beans. The new analytical method provided by Edmunds et al. (2012) for estimating uCP seems to have good potential for evaluating protein quality *in vitro*. However more studies need to be carried out to evaluate the accuracy of this method. Especially, we need to evaluate the effects of heat treatments in production experiments with dairy cows in order to evaluate the relevance of the *in vitro* methods for potential use in feed evaluation systems.

References

AOAC, 1984. *Official methods of analysis of AOAC*. Washington DC, USA: Association of Official Analytical Chemists.

Broderick, G.A. & Craig W.M., 1980. Effect of heat treatment on ruminal degradation and escape, and intestinal digestibility of cottonseed meal protein. J. Nutr. 110, 2381.

Canbolat, O., Kamalak, A., Efe, E., Sahin, M. & Ozkan, C.O., 2005. Effect of heat treatment on in situ rumen degradability and in vitro gas production of full-fat soyabeans and soyabean meal. South African J. Anim. Sci. 3, 186-194.

Cone, J.W., van Gelder, A.H., Visscher, G.J.W. & Oudshoorn, L., 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. Anim. Feed Sci. Technol. 61, 113–128.

Cros, P., Vernay, M. & Moncoulon, R., 1991. In situ evaluation of the ruminal and intestinal degradability of extruded whole horse beans. Reproduction Nutrition and Development, 31, 249–255.

Edmunds, B., Südekum, K.-H., Spiekers, H., Schuster, M. & Schwarz, F.J., 2012. Estimating utilisable crude protein at the duodenum, a precursor to metabolisable protein for ruminants, from forages using a modified gas test. Anim. Feed Sci. Technol. 175, 106-113.

Fennema, O.R., 1976. Principles of Food Science. Part I. Food Chemistry. Marcel Dekker, Inc., New York, NY.

Hetta, M., Cone, J.W., Gustavsson, A.-M. & Martinsson, K., 2003. The effect of additives in silages of pure timothy and timothy mixed with red clover on chemical composition and in vitro rumen fermentation characteristics. Grass Forage Sci. 58, 249–257.

Karlsson, L., Hetta, M., Udén, P. & Martinsson, K., 2009. New methodology for estimating rumen protein degradation using the in vitro gas production technique. Anim. Feed Sci. Technol. 153, 193-202.

Martinussen, H., Jørgensen, K.F., Strudsholm, F. & Weisbjerg, M.R., 2013. Heat treatment increases the protein value in Faba beans and Lupins. Proceedings of the 4th Nordic Feed Science Conference, 287, 34-38.

May, M.G., Otterby, D.E., Linn, J.G., Hansen, W.P., Johnson, D.G. & Putnam, D.H., 1993. Lupins (Lupinus albus) as a protein supplement for lactating Holstein dairy cows. J. Dairy Sci. 76, 2682–2691.

Menke, K.H. & Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Animal Research and Development, 28, 7-25.

NRC, 2001. Nutrient Requirements of Dairy Cattle, 7th ed. National Academy Press, Washington, DC, USA.

Ørskov, E.R. & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. J. Agr. Sci**.** 92, 499–503.

Raab, L., Cafantaris, B., Jilg, T. & Menke, K.H.,1983. Rumen protein degradation and biosynthesis. 1. A new method for determination of protein degradation in rumen fluid in vitro. Brit. J. Nutr. 50, 569–582.

SAS, 2002. User's Guide: Statistics. SAS Institute, Inc., Cary, NC, USA.

Tagari, H., Pena, F. & Satter, L.D., 1986. Protein degradation by rumen microbes of heat-treated whole cottonseed. J. Anim. Sci. 62, 1732.

Tufarelli, V., Khan, R.U. & Laudadio, V., 2012. Evaluating the suitability of field beans as a substitute for soybean meal in early-lactating dairy cow: production and metabolic responses. J. Anim. Sci. 83(2), 136-40