Hempseed Cake as a Protein Feed for Ruminants

Linda Karlsson
Faculty of Natural Resources and Agricultural Sciences
Department of Agricultural Research for Northern Sweden
Umeå

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Cover: Hemp (*Cannabis sativa* L.) field in Umeå
(photo: Michael Finell)

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Abstract

Increasing the use of locally produced protein feeds in ruminant production systems in northern Europe would be a valuable development. Hemp (*Cannabis sativa* L.) can be cultivated at high latitudes and cold-pressing the seeds to extract the oil produces a residue, hempseed cake (HC), with a high protein content. The overall aim of the studies presented in this thesis was to investigate the possibilities of using HC as a protein feed for ruminants.

The *in vitro* gas production technique was developed further in order to estimate rumen protein degradation; the effective protein degradation (EPD) of HC determined by this method was low (0.33). When calculated from *in situ* crude protein (CP) disappearance, HC had a high EPD value (0.71-0.84) and a low *in vitro* intestinal digestibility (0.18-0.31) for the rumen undegradable protein (RUP). Moist heat treatment could be used to shift the site of CP digestion from the rumen to the small intestine. The highest temperature considered, 130°C, was associated with the largest amount of RUP and rumen undegraded amino acids with the highest intestinal digestibility. A high proportion of the neutral detergent fibre in HC was indigestible (0.85), which contributed to a low calculated metabolisable energy value (9.5 MJ/kg dry matter).

Increasing the proportion of HC (0, 143, 233 or 318 g/kg dry matter) in the diet of dairy cows produced effects on the yields of milk, energy corrected milk and milk protein, fat and lactose that could be described by quadratic functions; the highest production was achieved by cows fed the diet containing 143 g HC/kg dry matter. There were linear decreases in the concentration of milk fat and protein and CP efficiency (milk protein/CP intake), and a linear increase in milk urea, with increasing proportions of HC.

Supplementing barley-based diets with peas or rapeseed cake improved the growth performance of lambs, but inclusion of HC resulted in an average daily gain similar to that achieved with the control diet containing no protein supplement.

The results from the studies underlying this thesis indicate that the nutritional value of HC will limit its use as a protein feed for ruminants.

Keywords: Amino acids, *Cannabis sativa* L., Crude protein efficiency, Growth performance, Heat treatment, Intestinal digestion, Milk production, Rumen degradation

Author's address: Linda Karlsson, SLU, Department of Agricultural Research for Northern Sweden, 901 83 Umeå, Sweden
E-mail: Linda.Karlsson@njv.slu.se

Dedication

In memory of my grandfather, Harald Karlsson (1921-2009),
a great farmer and friend

Hon är eldfast och stark, hon är sårbar och öm
Lars Winnerbäck

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I L. Karlsson, M. Hetta, P. Udén and K. Martinsson (2009). New methodology for estimating rumen protein degradation using the *in vitro* gas production technique. *Animal Feed Science and Technology* 153, 193-202.

II L. Karlsson, M. Finell and K. Martinsson (2010). Effects of increasing amounts of hempseed cake in the diet of dairy cows on the production and composition of milk. *Animal* 4, 1854-1860.

III L. Karlsson and K. Martinsson. Growth performance of lambs fed different protein supplements in barley-based diets (submitted).

IV L. Karlsson, M.D. Stern, M. Ruiz Moreno and K. Martinsson. Effects of temperature during moist heat treatment on ruminal degradability and intestinal digestibility of protein and amino acids in hempseed cake (manuscript).

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The contribution of Linda Karlsson to the papers included in this thesis was as follows:

I Planning the research jointly with the co-authors, performing the experiments, processing the data and writing the manuscript.

II Planning the research jointly with the co-authors, carrying out the experiment, processing the data and writing the manuscript.

III Planning the research jointly with the co-author, carrying out the experiment, processing the data and writing the manuscript.

IV Planning the research jointly with the co-authors, performing the experiments, processing the data and writing the manuscript.

Abbreviations

AA	Amino acid(s)
ADG	Average daily gain
CP	Crude protein
DM	Dry matter
EAA	Essential amino acids
EPD	Effective protein degradation
GP	Gas production
HC	Hempseed cake
iNDF	Indigestible neutral detergent fibre
IVDP	*In vitro* degradable crude protein
ME	Metabolisable energy
MUN	Milk urea nitrogen
NDF	Neutral detergent fibre
NPN	Non-protein nitrogen
TCA	Trichloroacetic acid
THC	Tetrahydrocannabinol
PUFA	Polyunsaturated fatty acids
RDP	Rumen degraded protein
RUP	Rumen undegraded protein

1 Introduction

1.1 Use of protein feeds in animal production systems

In northern Europe, feed rations for ruminants are often based on grass silage. Animals such as high yielding dairy cows and fast growing lambs, with high energy and protein requirements, usually receive supplements of concentrates to fulfil this need. While silage is almost exclusively cultivated on the same farm as the animals, it is less common for protein supplements to be locally produced.

Annually in the EU, 25 million tons of protein feeds are produced. Around 27 million tons/year are imported, accounting for approximately 40% of the total world trade, which makes the EU the biggest importer of protein feeds in the world (Björklund *et al.*, 2010). Protein feed consumption in Sweden amounted to 633 000 tons during 2008, of which 286 000 tons was in the form of seed meals of rape, turnip rape, or mustard and 255 000 tons was soybean meal (Björklund *et al.*, 2010). Of the protein feeds commonly used in Sweden, soybeans are imported in largest amounts. Importing soybeans, which mainly originate from Brazil, has been questioned in recent decades. The cultivation of soybeans has expanded greatly during the last 15 years and is one of the major reasons for destruction of the rainforests in Brazil, with huge consequences for humans and the environment, both locally and globally (Bartholdson *et al.*, 2010).

Growing concerns about the environmental impact of farming systems have increased interest in finding protein crops that can be produced in the northern regions of Europe. Wallman *et al.* (2010) investigated the environmental consequences of five feed ration choices in dairy production, using a life cycle assessment. Their results showed that the feed ration comprising only locally produced protein feeds (rapeseed and peas) and silage from nitrogen fixing clover-mixed leys was associated with the lowest

energy use and climate impact. Even though more land was used and there was greater eutrophication, the authors concluded that this feed ration was the best option considered in the study. Of the protein feeds commonly used in Sweden, peas, field beans and rapeseeds are mainly locally produced (Björklund *et al.*, 2010). There are possibilities for utilising whole-crop silages from these protein crops in ruminants' diets; for example, pea-oat silage has been shown to produce concentrate-sparing effects in dairy cow diets (Rondahl *et al.*, 2007). However, the short growing season limits the production of mature pods or seeds from legumes and oilseed crops at high latitudes. Hence, crops adapted to the Nordic climate that are suitable as protein sources for ruminants are required.

1.2 Hemp (*Cannabis sativa* L.)

1.2.1 History

Hemp was one of the first plants to be cultivated and, for centuries, was considered one of the most important agricultural crops. From 1000 B.C. until the late 19th century, it provided necessities such as cordage, cloth, food, lighting oil and medicine (Clarke, 1999). The exact origin of hemp is unclear, but it is believed to have spread from Central Asia to East Asia, South Asia and Europe in the pre-Christian era. Later, during the 15th and 16th centuries it reached Africa and the Americas (Clarke, 1999). The use of hemp varied between cultures. In Europe, it was mainly utilised as a source of fibre and seeds, while in other parts of the world it was primarily used as a source of a psychoactive drug (Clarke, 1999). European hemp breeders have focused on developing cultivars with high fibre production and low levels of the psychoactive substance *delta*-9-tetrahydrocannabinol (THC). Since 1970, the cultivation of the low-THC varieties, commonly called industrial hemp, has been strictly regulated and in some countries even banned (Bócsa & Karus, 1998). The rediscovery of hemp as a multi-functional crop occurred in the early 1990s and has resulted in the development of new varieties with higher quality fibre, increased seed and oil yields, and modified fatty acid profiles (Bócsa & Karus, 1998).

1.2.2 Botany, cultivation and agronomic performance

Cannabis belongs to the Cannabaceae family and taxonomists classify cannabis into a single species (*C. sativa*), two species (*C. sativa* and *C. indica*) or even three species including *C. ruderalis* (Clarke, 1999). However, in all three systems of nomenclature, *C. sativa* is the most diverse taxon and

contains the majority of varieties used for fibre, seeds and drugs. Hence, in this thesis, the word hemp refers to *C. sativa*.

Hemp is a medium to tall, annual herb. It is a short-day plant, meaning that shorter days accelerate the beginning of flowering. Naturally, it is dioecious, with male and female flowers on separate plants. The seeds develop from the female flowers and reach maturity after about four to six weeks (Bócsa & Karus, 1998). The cultivation of oil hemp for seed production differs from the cultivation of fibre hemp with respect to varieties, growing areas and techniques, but it is possible to cultivate the crop to produce both usable seed and fibre.

According to the Food and Agriculture Organization of the United Nations (2010), globally, the area used for hempseed production was 26 949 ha in 2009. The respective areas reported for cultivation in Asia, Europe and America were 13 556, 12 083 and 1 300 ha, while no areas were reported for Africa or Oceania. There were large variations between the average reported yields: 3 538, 598 and 1 000 kg/ha for Asia, Europe and America, respectively.

In Sweden, the cultivation of industrial hemp varieties with guaranteed low THC content has been legal since 2003 (Official Journal of the European Union, 2003) and hemp is mainly grown as a fibre or energy crop. In 2010, there were 56 farmers registered to cultivate hemp on a total area of 260 ha (Rolandsson, personal communication[1]). There are special rules for hemp cultivation in Sweden (Swedish Board of Agriculture, 2010). In 2010, there were 45 approved varieties, which all have to contain less than 0.2% THC. The seeds have to be certified and must be sown in pure stands. The hemp must be harvested after the end of seed development or later than 10 days after the end of flowering.

The early-blooming oil hemp variety Finola (breeder code FIN-314) was developed in Finland to suit cultivation in boreal regions (>50° latitude), where the length of daylight during the growing season is longer compared to regions with temperate or equatorial climates. The variety reaches approximately 1.5 meters tall, matures in less than 110 days and has produced seed yields of around 1 700 kg/ha at latitudes greater than 60° N (Callaway, 2002). In trials at SLU in Umeå, the estimated seed yield from Finola varied between 1 000 and 1 400 kg/ha when seeds were harvested by hand from small plots, covered by net cages, within a larger field (Finell *et al.*, 2006). However, actual harvested yields were much smaller and varied

[1] Rolandsson, Hans. Swedish Board of Agriculture, Jönköping, Sweden. Personal communication, June 2010.

between 110 and 670 kg/ha, because of seed consumption by birds and losses during harvesting.

1.2.3 The hemp industry in Europe

The state of the European hemp industry has been documented by Karus & Dominik (2004), mainly based on data from market surveys by the European Industrial Hemp Association between 2001 and 2003. The data show that hemp shives (the core of the stem) accounted for the largest production from hemp cultivation, followed by fibre and to a smaller extent seed. The hemp shives were almost exclusively used as animal bedding, mainly for horses, while a small amount was used as building material in the construction sector. The most important product line for the fibre was specialty pulp for cigarette papers and technical applications, accounting for 70-80% of the hemp fibre market, while the automotive industry accounted for around 15% of the market. Out of the 5 300 tons of hempseed produced by the primary hemp processors in the EU during 2002, more than 95% was used as animal feed, mainly bird feed. Only a small part was used as human food, which included oil and grains.

1.2.4 Nutritional value of hempseed

Botanically, the fruit of hemp is a small nut, although it is customary to call them seeds. They are light-grey to green and their diameter ranges from 2-3.5 mm (Bócsa & Karus, 1998). The seeds typically contain over 30% oil, of which more than 80% is polyunsaturated fatty acids (PUFA) (Callaway, 2004). Hempseed oil is especially rich in two essential fatty acids – linoleic acid (18:2 *omega*-6) and *alpha*-linolenic acid (18:3 *omega*-3) – and the ratio of *omega*-6 to *omega*-3 normally ranges between 2:1 and 3:1 (Callaway, 2004). In a review, Matthäus & Brühl (2008) concluded that hempseed oil is interesting for human nutrition due to its unique fatty acid composition, especially the amounts of *gamma*-linoleic acid and stearidonic acid, which are rare in domestic plants. Hempseed oil is very susceptible to oxidative deterioration and is much less stable than rapeseed or sunflower oil, even though it contains relatively high levels of the antioxidant *gamma*-tocopherol (Matthäus & Brühl, 2008).

The protein content of hempseed is around 25% and the two main proteins, albumin and edestin, contain significant amounts of essential amino acids (EAA) (Callaway, 2004). In a study by Wang *et al.* (2008), isolates of hempseed protein had a higher proportion of EAA compared to soya protein and they concluded that it is a good source of protein for humans.

Hempseed has been reported to contain 1.7 g/kg dry matter (DM) of calcium and 6.0 g/kg DM of phosphorous (Spörndly, 2003).

Hempseed oil is commonly removed mechanically using a low heat technique, so called cold-pressing. This method results in a by-product known as hempseed cake (HC, Figure 1), which contains various amounts of remaining fat. Other methods of oil extraction are the application of heat or the use of solvents; these usually result in an expeller or a meal with lower fat content than cold-pressed feed cakes. Although hempseed seems to have a high nutritional value, there are only a few published studies describing investigations of the potential use of the seeds or the by-products of oil production in animal feeding.

Figure 1. Hempseed (left) and cold-pressed hempseed cake (right) (photo: Linda Karlsson).

Varying results have been reported with respect to the *in situ* rumen degradation of hempseed protein. Mustafa *et al.* (1999b) found hempseed meal to have a low effective protein degradation (EPD), comparable to that of heat treated canola (rapeseed) meal. In contrast, Sehu *et al.* (2010) reported the highest EPD value for hempseed among a total of six oilseeds and meals. Mustafa *et al.* (1999b) also found that hempseed meal could replace rapeseed meal as a protein supplement for lambs, up to 200 g/kg diet DM, without detrimental effects on DM intake or nutrient utilisation. Feeding cold-pressed HC or a mix of soybean meal and barley to growing cattle resulted in similar weight gains and carcass traits (Hessle *et al.*, 2008). Studies including steers have shown that it is possible to alter the fatty acid profile of the meat by supplementing the animals' diet with either full-fat hempseed (Gibb *et al.*, 2005) or cold-pressed HC (Turner *et al.*, 2008). Cold-pressed HC has also been fed to laying hens, resulting in increased

concentrations of linoleic acid and *alpha*-linolenic acid in the eggs (Silversides & Lefrancois, 2005).

Oilseed may contain anti-nutritional factors, which reduce the nutritional value. Matthäus (2004) determined the amount of four anti-nutritional factors in different oilseeds and neither glocosinolates nor sinapine were found in hempseed. The amount of inositol phosphate was similar and the tannin content was significantly lower, compared to that in rapeseed.

1.3 Protein in ruminant nutrition

1.3.1 Characterisation of feed protein

Proteins are complex organic compounds of high molecular weight, built up of numerous amino acids (AA). The AA are linked together to peptides by peptide bonds, which form when the amino group (-NH_2) of one AA reacts with the carboxyl group (-COOH) of a second AA to release a water molecule and form a covalent bond (Horton *et al.*, 2002). The structure of a protein is divided into four different levels: the primary structure is the AA sequence of the polypeptide chain, the secondary structure refers to the conformation of the AA chain, the tertiary structure refers to the overall, three-dimensional shape of a single protein molecule and the quaternary structure describes a protein consisting of more than one polypeptide chain (Horton *et al.*, 2002).

Dietary protein fed to ruminants generally refers to crude protein (CP), defined as the N content × 6.25. This definition is based on the assumptions that all N in the feed is present as protein and that the average feed protein contains 16% N. The N content of feeds is usually determined by a modified version of the Kjeldahl technique (e.g. Nordic Committee on Food Analysis, 1976; AOAC, 1984) developed by Johan Kjeldahl in 1883. An alternative technique for determining total N is the Dumas method (AOAC official method 990.03). Since these techniques quantify N both from protein and other N sources, calculated values for CP include non-protein N (NPN) such as peptides, free AA, nucleic acid, amides, amines and ammonia. About 60-80% of total plant N is true protein, while soluble NPN and small amounts of lignified N account for most of the remainder (Van Soest, 1994).

1.3.2 Protein metabolism in ruminants

It is the AA, and not the protein *per se*, that are the required nutrient for the host animal and that are used as building blocks for the synthesis of protein required for maintenance, growth, reproduction and lactation. In ruminant

CP feeding, consideration must be taken of both the N requirements of rumen microbes and the AA requirements of the host animal. Lack of supply to either of these could adversely affect animal performance. A schematic representation of the fate of dietary CP is shown in Figure 2.

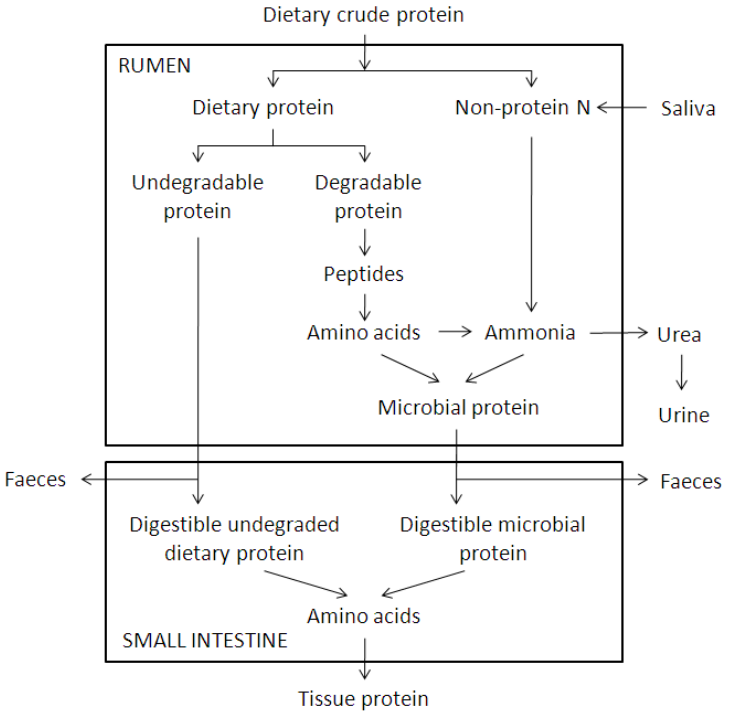

Figure 2. A schematic representation of dietary crude protein metabolism in ruminants.

Protein metabolism in the rumen can be divided into two separate actions: protein degradation, which provides N for the bacteria; and microbial protein synthesis. Bacteria, the most abundant microorganisms in the rumen, are the major organisms involved in protein degradation (Schwab *et al.*, 2005). Protein is hydrolyzed extracellularly by proteolytic bacteria and transported as AA and small peptides into the bacterial cells, where peptides can be hydrolyzed further to AA. The AA are used either for microbial protein synthesis or deaminated to volatile fatty acids, ammonia, carbon dioxide and methane (Tamminga, 1979). Protozoa and anaerobic fungi are also, but to a lesser extent, involved in protein degradation. The N requirements of rumen microorganisms are met by ammonia, AA and peptides, which are the end-products of microbial protein degradation, plus recycled urea (Schwab *et al.*, 2005).

The amount of protein that is degraded in the rumen depends on several factors, of which the chemistry of the feed CP is the single most important (NRC, 2001). The key aspects of feed CP chemistry are the proportions of NPN and true protein as well as the physical and chemical characteristics of the true protein. Differences in the three-dimensional structure and chemical bonding within and between proteins and between proteins and carbohydrates affect microbial access to the proteins, which in turn affects the rate and extent of ruminal protein degradation (NRC, 2001). Another important factor is the predominant microbial population, which in turn depends on the type of ration, ruminal passage rate and pH (Bach *et al.*, 2005).

Ruminal microbial protein synthesis is dependent on carbohydrates as an energy source. Increasing amounts of readily fermentable carbohydrates decrease the concentration of ammonia in the rumen as a result of enhanced N uptake by the microbes. Microbes that ferment structural carbohydrates (cellulose and hemicellulose) grow slowly and use ammonia as their N source, while the microbes that ferment non-structural carbohydrates like starch, pectin and sugars, grow more rapidly and use either ammonia or peptides and AA as N sources (Russell *et al.*, 1992).

The AA absorbed by the animal originate from protein synthesised by the rumen microbes, rumen undegraded protein (RUP) and to a small extent endogenous protein. In most feeding situations, the major proportion of the absorbed AA will come from microbial protein (Schwab *et al.*, 2005). Ten of the twenty primary AA in proteins are usually classified as essential (or indispensable) and can either not be synthesised by animal tissues or are synthesised at insufficient rates to meet requirements (NRC, 2001).

1.3.3 Efficiency of protein utilisation by ruminants

Emission of N is a major environmental issue and N output from ruminant production systems should be minimised. Efforts are being made to store and handle manure appropriately and to increase the conversion of dietary CP into meat and milk protein. The efficiency of CP utilisation in ruminants is generally low but is very variable. A large data set from North European feeding trials with dairy cows showed an average CP efficiency (milk N/N intake) of 27.7%, ranging from 16.4 to 40.2% (Huhtanen & Hristov, 2009).

Rumen microbes are able to utilise NPN compounds for protein synthesis and supply the animal with large amounts of the required protein. Hence, ruminants can be productive on diets in which the CP consists of NPN. However, rumen degraded protein (RDP) from NPN sources may

not be as effective source of nutrition as true protein. Replacing RDP from soybean with that of urea resulted in decreased microbial protein synthesis in the rumen and reduced yields of milk and milk protein (Broderick & Reynal, 2009). Several studies indicate that it is possible to reduce dietary CP levels and decrease N excretion without decreasing growth or milk production. A number of different studies found no further improvement in milk yield when dietary CP was increased from 167 to 184 g/kg CP (Broderick, 2003), from 157 to 192 g/kg DM (Groff & Wu, 2005) or from 165 to 194 g/kg DM (Olmos Colmenero & Broderick, 2006). In addition, an increase in urinary N and milk urea N (MUN) reflecting the linear decrease in CP efficiency (milk N/N intake) with increasing CP in the diet has been found (Olmos Colmenero & Broderick, 2006).

Several strategies can be used to increase the conversion of feed CP into milk and meat protein and to reduce N wastage (Schwab *et al.*, 2005):

i) Feeding to increase the synthesis of microbial protein. This will increase the chances of utilising recycled N and end-products from protein degradation in the rumen. It may also increase the efficiency of use of absorbed AA, since microbial protein is considered to have an AA profile that is closer to the profile required by the animal compared to practically all feed proteins (NRC, 2001).

ii) Balance the supply of RDP and RUP so that the requirements for both are met but not in excess.

iii) Fine-tune and balance the diet for EAA. For high yielding dairy cows, it is preferable that the diet is supplemented with digestible RUP that improves the AA profile of the metabolisable protein and meets the requirements of the cow (NRC, 2001). Various physical and chemical treatments can be used to protect dietary CP from microbial degradation in the rumen and thereby increase the proportion of RUP. Both dry heat treatment and moist heat treatment can effectively reduce rumen protein degradation. Commercially, toasting is probably the most commonly used method for heat treatment of oilseeds. Other common methods are expeller processing, extruding, expanding, roasting, pressurised toasting and micronising (Van der Poel *et al.*, 2005). Heat treatment reduces the accessibility of the substrate and forms linkages resistant to enzyme attack (Van Soest, 1994). The Maillard, or non-enzymatic browning, reaction involves condensation of amino groups with sugar residues and the overall reaction sequence results in permanently bound and indigestible N (Van Soest, 1994). Generally, moderate heat treatment does not have a negative impact on the intestinal digestibility of CP, but excessive heat may decrease it (Van der Poel *et al.*, 2005). Hence, it is important to balance the treatment

so that the rumen protein degradation is decreased without overprotecting the protein. Another approach to balance the diet for AA is by additions of the most limiting AA, as often used in monogastric feeding systems. Since free AA are degraded rapidly in the rumen, these supplements need to be fed in a rumen protected form to increase the supply of the specific AA to the small intestine (Kung & Rode, 1996).

1.3.4 Techniques to estimate protein degradation and digestion

There are several methods available to quantify the degradability and the digestibility of a feed, including *in vivo*, *in situ* and *in vitro* approaches. Since measurements made *in vivo* represent the actual animal response, this method should be used as a reference method when evaluating other methods. *In vivo* measurements require animals fitted with cannulae in the abomasum or small intestine, preferably with an additional cannula in the terminal ileum (Broderick *et al.*, 1991). Measurements of the amount and composition of digesta flow can then be made by using specific markers. However, *in vivo* experiments are expensive, laborious, time-consuming and not suitable for evaluating large numbers of feeds. Measurements of rumen degradation or intestinal digestion may exhibit large variation and errors associated with digesta flow markers, microbial markers and inherent animal variation (López, 2005). Therefore, *in vitro* and *in situ* techniques are used to estimate degradability and digestibility in feed evaluation systems such as those published by, for example, the National Research Council (NRC, 2001) and the Nordic Feed Evaluation System (Gustafsson *et al.*, 2005).

Ruminal degradation

The *in situ* technique (Ørskov & McDonald, 1979) is a standard method for determining ruminal degradation of protein. When using this method, small bags containing feedstuff are incubated in the rumen of a cannulated animal for a particular time interval. The disappearance of the substrate from the bag is assumed to represent actual degradation by ruminal microbes. This is however not completely true, since there may be losses of undegradable feed particles from the bag or microbial contamination of the feed residue in the bag (Broderick *et al.*, 1991; López, 2005). Furthermore, the bag can be considered as an independent compartment in the rumen and the conditions inside the bag might not be the same as in the surrounding rumen content (López, 2005).

Another approach used to measure protein degradation in the rumen is determining the production of ammonia-N, rather than the loss of feed N. One problem with this method is that degradation of feed protein, and thus

release of ammonia, is counterbalanced by a concomitant uptake of ammonia in the *de novo* synthesis of microbial protein (Tamminga, 1979). To overcome this problem, Broderick (1987) devised an inhibitor *in vitro* method, in which hydrazine sulphate and chloroamphenicol were used to inhibit microbial protein synthesis. The proportion of undegraded protein was calculated by subtracting the N recovered as ammonia or AA from the added N. Degradation rates were determined by linear regression between logarithms of the undegraded proportion and time. However, this method is only suitable for short term incubations since the microbial population will slowly die as a result of the inhibition of protein synthesis. The added inhibitors prevented microbial growth without reducing the degradation rate over 4 h, but longer incubations were not recommended (Broderick, 1987).

Raab *et al.* (1983) had previously presented another method for estimating protein degradation, dealing with the confounding protein degradation and synthesis in a different way. In this approach, protein degradability is determined by measurements of ammonia-N release and gas production (GP) when feed and graded amounts of carbohydrate are incubated in rumen fluid. Extrapolation of the linear regression between ammonia-N (y, mg) and GP (x, ml) gives a y-intercept that is assumed to represent the amount of ammonia-N that would be released if no fermentable carbohydrates were available and, hence, no bacterial protein synthesis could occur (Figure 3). The *in vitro* GP technique for determining protein degradation has only been used in a few published studies (Krishnamoorthy *et al.*, 1990; Getachew *et al.*, 1998; Mota *et al.*, 2005). The technique requires several incubation flasks for each feed and the rumen fluid has a considerable impact on the results (Krishnamoorthy *et al.*, 1990). In the original method, the degradability was generally estimated after a single incubation period of 24 h, which gave no information about the degradation pattern over time.

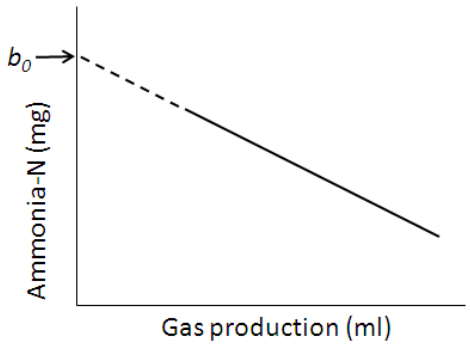

Figure 3. Extrapolation of the linear regression to zero protein synthesis (b_0) for predicting the amount of ammonia-N liberated from feed protein using the gas production technique.

Other approaches to estimate protein degradation in the rumen utilise ^{15}N markers to differentiate microbial protein from undegraded feed protein (e.g. Hristov & Broderick, 1994). There are also techniques where the feed is incubated in buffer solutions containing commercial enzymes, e.g. proteases (Krishnamoorthy *et al.*, 1983), instead of rumen fluid. When utilising enzymes, the protein degradability can be affected by factors such as type of protease, variability between batches, incubation pH and enzyme:substrate ratio (López, 2005). Nitrogen solubility in buffers or other water solutions has also been used to estimate protein degradability. However, soluble proteins vary in their degradation rates and solubility cannot be considered to be synonymous with degradation (Hedqvist & Udén, 2006).

Intestinal digestion

The *in situ* mobile bag technique has been used to determine intestinal CP digestion (Hvelplund, 1985). Feed samples or residues after rumen incubation are weighed into small bags, introduced into the abomasum or proximal duodenum and collected either from the ileum or from the faeces. The sources of variation include the porosity of the bags, animal and diet effects, retention time, the site of bag collection and microbial contamination (Hvelplund, 1985).

There are also *in vitro* methods available for estimation of CP digestibility, where enzymes are utilised to simulate abomasal and intestinal digestion. The most commonly used technique is the three-step procedure, where the feed is pre-incubated in the rumen, then incubated in acid-pepsin, and finally in pancreatin-phosphate-buffer (Calsamiglia & Stern, 1995). The

technique was developed to simulate closely the physiological conditions found in ruminants and, as the original study indicated that the digestion of the RUP may be different from that of the original feed, all three steps (ruminal, pepsin and pancreatin incubation) were included in the procedure (Calsamiglia & Stern, 1995). Some modifications to the method were made by Gargallo et al. (2006) to reduce costs and labour. In the modified three-step procedure, the rumen pre-incubated feeds are incubated in Dacron bags in a Daisy[II] batch culture incubator (Ankom, Fairport, NY). The corrosive and toxic trichloroacetic acid (TCA) is not needed and the procedure leaves a feed residue after enzyme digestion that can be analysed for AA content, for example.

1.3.5 Protein evaluation

Several different protein evaluation systems have been developed that predict the rate of rumen CP degradation, microbial protein synthesis, the input of RUP into the intestine, and the amount of protein that is available to the animal. Ruminal protein degradation has most commonly been described by a first-order disappearance model (Ørskov & McDonald, 1979) dividing the CP into fractions A, B and C. The A fraction is assumed to be instantly degraded, fraction C is assumed to be completely undegraded and the rest of the CP is the potentially degradable protein (fraction B). The proportion of fraction B that is degraded is determined by the fractional rate of degradation in combination with an assumed fractional rate of passage. The RDP (equivalent to EPD) and RUP of feed CP can then be calculated using the equations:

$$RDP = A + B\ (kd/(kd + kp))$$

$$RUP = B\ (kp/(kd + kp) + C$$

where A, B and C are as defined above, kd is the rate of degradation of fraction B, and kp is the assumed rate of passage from the rumen equal to 0.08/h for concentrates. The sum of RDP and RUP equals 100%. Many evaluation systems, e.g. the AAT/PBV system (Madsen, 1985), the Nordic Feed Evaluation System NorFor (Gustafsson *et al.*, 2005) and the Nutrient Requirements of Dairy Cattle (NRC, 2001), use the model above in combination with data derived *in situ* to determine CP degradability. In addition, *in vitro* and enzymatic digestion data generally fit a model that divides the CP into these three fractions (Broderick *et al.*, 1991). However, the model has been questioned, given that fraction A is not completely degraded in the rumen and passage of feed particles from the rumen cannot

be described as a single first-order compartment (Huhtanen & Hristov, 2009).

The Cornell Net Carbohydrate and Protein System (Sniffen *et al.*, 1992) uses another approach, in which the pool sizes of feed CP are estimated by standard chemical analyses. This method divides CP into five fractions (A, B1, B2, B3 and C) according to their rates of ruminal degradation. Protein degradation rates are estimated by an *in vitro* procedure using protease, and ruminal passage rates are a function of DM intake and different feed characteristics. The intestinal absorption of the undegraded fractions is assumed to be 100% for fractions B1 and B2 and 80% for the B3 fraction. Metabolisable protein (defined as digested feed and bacterial protein minus bacterial nucleic acids) in the diet is calculated as the sum of metabolisable protein from each feed (Sniffen *et al.*, 1992).

In addition, the NRC (2001) approach takes account of some of the factors affecting the passage rates of undegraded feed and hence the amount of RUP. An estimated intestinal digestibility is used to calculate the contribution of the RUP of each feed to metabolisable protein (defined as the true protein that is digested post-ruminally and the component AA absorbed by the intestine). The intestinal digestibility values are derived from studies using the *in situ* mobile bag technique or the *in vitro* three-step procedure (NRC, 2001).

The AAT/PBV protein evaluation system (Madsen, 1985; Madsen *et al.*, 1995) expresses the protein value of feeds in grams of amino acids absorbed in the small intestine (AAT) and the protein balance in the rumen (PBV). The formulae developed for calculations of AAT and PBV include factors which are either constant or variables that can be linked to analysis of the feeds.

The Nordic Feed Evaluation System NorFor (Gustafsson et al., 2005) also expresses the protein value of feeds as AAT and PBV but differs from the AAT/PBV system by not being additive. The system includes interactions between feed and animal characteristics in a non-linear model. Instead of constant factors for ruminal passage rate, effectiveness of microbial protein synthesis, intestinal digestibility of RUP and proportion of AA in RUP, these values are variable. Hence, the feeds do not have fixed AAT and PBV values since they vary with diet composition and DM intake (Gustafsson *et al.*, 2005).

2 Objectives

The overall aim of the studies described in this thesis was to investigate the possibilities of using HC as a protein feed for ruminants. Hemp can be cultivated at high latitudes and farmers in northern Europe would be able to increase their use of locally produced feeds if HC could replace protein sources such as imported soybeans. More specific objectives in order to investigate the potential use of HC were:

- To develop further the *in vitro* GP technique to estimate rumen protein degradation of HC and commonly used protein feeds.
- To study the effects of increasing amounts of HC in the diet of dairy cows on the production and composition of milk.
- To compare the growth performance of lambs fed peas, rapeseed cake or HC as their protein supplement.
- To study the effects of temperature during moist heat treatment on the *in situ* ruminal degradability and *in vitro* intestinal digestibility of CP and AA in HC.

3 Materials and methods

The hempseed used in all studies was the variety Finola. That used in the study described in Paper I was cultivated in Umeå, Sweden (2006), while the hempseed used in the studies for Papers II-IV was cultivated in Germany (2007).

3.1 Paper I

The original *in vitro* GP technique described by Raab *et al.* (1983) was modified in order to improve its tractability and reliability, hereafter this is referred to as the new GP technique. Five protein feeds (cold-pressed HC, cold-pressed rapeseed cake, rapeseed expeller, heat treated rapeseed meal and soybean meal) were incubated in bottles with 90 ml of buffered rumen fluid and a mix of carbohydrates at four concentrations. Blank samples, containing buffered rumen fluid only, were incubated in duplicates. The GP was recorded in a fully automated system (Cone *et al.*, 1996) and the amounts of ammonia-N were measured after 4, 8, 12, 16, 24 and 30 h within the same incubation bottle. The rumen fluid was pre-incubated with carbohydrates to enhance microbial activity and reduce the amount of background ammonia-N. *In vitro* degradable CP (IVDP) was estimated for each feed at each incubation time via linear regression of ammonia-N *vs.* GP, as described by Raab *et al.* (1983). The EPD and CP kinetic parameters of the feeds were estimated after fitting the IVDP data to a non-linear equation (Ørskov and McDonald, 1979).

3.2 Paper II

Forty Swedish Red dairy cows were included in a 5-week dose-response feeding trial to study the effects of increasing amounts of HC in the diet on

milk production and composition. The cows were allocated to one of four experimental diets based on grass silage and concentrate (50:50 on DM basis). By replacing compound pellets with cold-pressed HC, the diets contained 0, 143, 233 or 318 g HC/kg DM and ranged from 126 to 195 g CP/kg DM. The diets were offered as total mixed rations and feed intake was automatically recorded. Individual milk production was recorded at every milking and samples for determination of fat, protein, lactose and urea were collected weekly during four consecutive milkings and pooled to create one morning and one evening sample per cow.

3.3 Paper III

Forty-eight crossbred ewe lambs, on average 87 days old (SD=9), were penned to form 16 experimental units allocated to one of four experimental diets. The diets were barley-based with the addition of 101 g hay/kg DM. The control diet (B-0) did not contain any protein supplement and had a CP concentration of 112 g/kg DM. The other diets were formulated to contain 160 g CP/kg DM by replacing a proportion of the barley with peas (B-P), rapeseed cake (B-RC) or HC (B-HC). The lambs were fed according to their metabolisable energy (ME) requirements for an expected average daily gain (ADG) of 250-300 g (assuming a ME concentration of 13 MJ/kg DM for all diets). The lambs were weighed and assigned a body condition score (1 to 5) once a week during the eight-week continuous trial. The ruminal CP degradability of the concentrates was estimated, using the *in situ* technique (Ørskov & McDonald, 1979). Intestinal CP digestibility was determined, using the three-step *in vitro* procedure (Calsamiglia & Stern, 1995), in which the 16 h *in situ* residue was incubated in pepsin followed by pancreatin solutions.

3.4 Paper IV

Samples of cold-pressed HC were autoclaved for 30 min at 110, 120 or 130°C, using a steam heated steriliser. One sample of untreated HC was kept as a control. The ruminal degradability of CP was estimated, using the *in situ* technique (Ørskov & McDonald, 1979). Intestinal CP digestibility was estimated for the 16 h *in situ* residue, using the three-step *in vitro* procedure modified by Gargallo *et al.* (2006). The HC samples were analysed for AA content in the intact feed, after 16 h *in situ* incubation and after the three-step procedure, in order to determine the AA profile of HC and to estimate the AA ruminal degradability and intestinal digestibility.

4 Results

4.1 Paper I

The new *in vitro* GP technique provided estimates of IVDP that allowed CP kinetic parameters and EPD of the five protein feeds to be estimated. Comparing the CP kinetic parameters, the immediately available fraction (*a*) did not differ between the feeds, but both the potentially degradable fraction (*b*) and its rate of degradation (*c*) did differ between the feeds ($P<0.001$ and $P=0.015$, respectively). The *b*-fraction amounted to 0.46 for HC, while it varied between 0.79 and 0.98 for the other feeds. The degradation rate of fraction *b* ranged from 0.08 to 0.26/h, and was found to be highest for HC. The calculated EPD values differed depending on feed ($P<0.001$) and were 0.33, 0.36, 0.46, 0.59 and 0.67 for HC, rapeseed meal, rapeseed expeller, rapeseed cake and soybean meal, respectively.

4.2 Paper II

Increasing the proportion of HC (0, 143, 233 or 318 g HC/kg DM) in the diet of dairy cows resulted in higher concentrations of CP, fat and neutral detergent fibre (NDF) and lower concentrations of starch. It also resulted in linear increases in CP, fat and NDF intake ($P<0.001$) and a linear decrease in starch intake ($P<0.001$), without either linear or quadratic effects on DM intake. A large proportion of the NDF in the HC was indigestible neutral detergent fibre (iNDF; 845 g/kg NDF), which contributed to a low calculated ME value of 9.5 MJ/kg DM. The proportion of HC in the diet produced effects on the yields of milk, energy corrected milk and milk protein, fat and lactose that could be described by quadratic functions ($P<0.05$). The highest production values for these parameters were recorded for cows fed the diet containing 143 g HC/kg DM. Increasing the

proportion of HC in the diet resulted in linear decreases in the concentration of milk fat and protein ($P<0.05$), a linear increase in milk urea ($P<0.001$) and a linear decrease in the CP efficiency (milk protein/CP intake, $P<0.001$).

4.3 Paper III

The RUP of the concentrates of barley, peas, rapeseed cake and HC was 231, 99, 298 and 291 g/kg CP, respectively, with intestinal digestibilities of 605, 707, 528 and 307 g/kg RUP. The diets containing protein supplement had similar CP concentrations (160-162 g/kg DM) but the inclusion of HC resulted in a lower calculated ME content than in the other diets. There were no significant differences in DM intake among the four diets. However, the CP intake was higher for the lambs fed B-P and B-HC ($P<0.001$) and the calculated ME intake was higher for the lambs fed B-P than for the lambs fed the other diets ($P=0.037$). The ADG was highest for the B-P treatment, followed by the B-RC treatment ($P<0.001$) and the feed conversion (DM/gain) was more efficient for lambs on these treatments ($P<0.001$). No significant differences in growth performance or feed conversion were found between the B-0 and the B-HC diets.

4.4 Paper IV

In situ ruminal degradability of CP decreased ($P=0.006$ and $P=0.030$ for linear and quadratic effects, respectively) and *in vitro* intestinal digestibility of RUP increased linearly ($P<0.001$) with temperature during moist heat treatment. The highest temperature used in this study, 130°C, resulted in the largest amount of RUP with the highest intestinal digestibility and, hence, the largest amount of intestinally available dietary CP. *In situ* ruminal degradability of total AA and individual AA decreased linearly with temperature ($P<0.001$ and $P<0.05$, respectively). *In vitro* intestinal digestibility of rumen undegraded AA increased with temperature and could be explained both by a linear and a quadratic model for total AA ($P<0.01$) and for most individual AA ($P<0.05$). The 130°C treatment decreased ruminal degradability of total AA from 822 to 264 g/kg, while the intestinal digestibility was increased from 269 to 820 g/kg ruminal undegradable AA, relative to the control.

5 Discussion

Since hemp is a crop that can be cultivated in the northern regions of Europe, the legalisation of cultivating industrial hemp in 2003 was welcomed by many Swedish farmers, researchers and other stakeholders. The potential of this multifunctional crop has been evaluated in field studies and the Finola variety gave reasonable seed yields at high latitudes (Callaway, 2002; Finell *et al.*, 2006); this is why hempseed from Finola was selected as the material used in the studies underlying this thesis. However, during this project, the Finola variety was removed from the EU list of approved varieties because THC levels above 0.2% had been detected. This has definitely decreased the chances of cultivating hemp for seed production at high latitudes. To the author's knowledge, there is no variety available on the market giving comparable seed yields in a Nordic climate. Breeding of new varieties or for decreased THC levels in the Finola variety is necessary to allow seed production in these regions. The current EU sampling and testing procedures (Annex I of EU Regulation No. 796/2004) have been questioned by Callaway (2008), who suggested a more reliable method for evaluating hemp THC levels. In addition to varieties that give acceptable yields, it will probably be necessary to make a profit from the PUFA-rich hempseed oil in order for the press residue to become an economic alternative animal feed. Even though the cultivation of hemp has increased in Europe during recent decades, production is still relatively limited and yields relatively low compared to other oilseed and legume crops. Table 1 shows the cultivated area and the hectare yields of protein crops in Sweden. Seed yields within the same range as those reported for rape cannot be expected for hemp. Under good agronomic conditions yields might be comparable with those of spring turnip rape or with yields of soybeans obtained in recent field experiments in the south of Sweden (Lagerberg Fogelberg & Fogelberg, 2009). The future of the cultivation of hemp for

seed production will depend not only on the agronomic performance of the crop, but also on political decisions and consumer interest, which is outside the scope of this thesis. It was the increased interest in finding protein feeds that can be locally produced that was the driving force for evaluating the potential use of hempseed for this purpose. The studies underlying this thesis focused on the nutritional value of hempseed for ruminants, with special emphasis on estimating its protein quality.

Table 1. *Cultivated areas and hectare yields of protein crops in Sweden.*

Crop	Cultivated area (ha)	Yield (kg/ha)
Autumn rape[1]	67 841	3 540
Spring rape[1]	29 245	1 880
Spring turnip rape[1]	282	1 320
Flax[1]	9 954	1 900
Peas[1]	24 705 (field beans and vetch included)	3 000
Field beans[1]		3 280
Soya[2]	Field experiments	1 600

[1]Statistics Sweden (2010). Yields during 2009, given for a moisture content of 15% for peas and field beans and 9% for oilseeds.

[2] Lagerberg Fogelberg & Fogelberg (2009).

5.1 Chemical composition of hempseed cake

The CP content of HC varied between 319 and 344 g/kg DM (Papers I-IV), which is comparable to the CP content of rapeseed cake or meal. The CP in HC contained less NPN and soluble CP, while the acid detergent insoluble fraction was similar or somewhat higher than in rapeseed cake (Paper I and III).

When evaluating the potential use of a protein feed, it is not only the amount and quality of the protein that are important factors. Since HC is the residue remaining after extracting the oil from the seeds, the extraction procedure will determine the fat content. Although cold-pressing was used on both seed sources, the HC used in the Paper I study had a fat content of 231 g/kg DM while the HC used in the other studies had a fat content of 124-127 g/kg DM. This was probably due to the different press capacities, but factors such as fat and moisture content of the seeds might also have influenced the oil extraction. A high fat content in HC may limit its potential inclusion in the diet, since dietary lipids can disrupt rumen fermentation (Jenkins, 1993).

Since the NDF content of HC was high (284-393 g/kg DM in Paper I-III), the degradability of this fibre fraction becomes important. The content of iNDF determined *in situ* demonstrated that as much as 845 g/kg NDF was indigestible (Paper II). This value was surprisingly high. The iNDF value of rapeseed cake reported by the NorFor Nordic Feed Evaluation System (2010) is 379 g/kg NDF. It is clear that the use of HC as a protein feed for ruminants will be limited by such a high level of iNDF. Since a significant proportion of the feed cannot be digested, the ME content will be low. Furthermore, the content of non-fibre carbohydrates, such as starch, was low in HC. This resulted in a high calculated PBV value (Paper III) due to the low level of fermentable carbohydrates to balance the RDP. It is therefore crucial that the other feed components in the ration contribute fermentable carbohydrates in order to utilise the degradable CP for microbial protein synthesis efficiently.

5.2 Estimates of ruminal protein degradability

Reliable, accurate and practical methods to evaluate protein degradability still need to be found. Although much research has been devoted to this area, no method is without disadvantages. The modification of the original GP technique described by Raab *et al.* (1983) resulted in a new methodology providing estimates of IVDP that could be used for calculations of EPD values for protein feeds (Paper I). The pre-incubation of the rumen fluid included in the new GP technique had two main purposes. First, it should enhance microbial activity and thus avoid an initial fermentation lag time. Second, it should reduce the background ammonia levels in the rumen fluid (and hence, the blanks) and make the method more sensitive to the differences in ammonia-N caused by increased levels of carbohydrates. The improved sampling procedure enabled measurements of ammonia-N from the same flask during the whole incubation time and thereby increased the analytical capacity of the method. However, the method needs to be automated to become appealing for commercial applications of the procedure. It also needs to be further validated using feeds with known *in vivo* protein degradabilities. Estimates of *in vitro* CP degradability could also be correlated to animal responses in production trials in order to evaluate the protein value of feeds.

In the absence of *in vivo* data, it may be of interest to compare prediction results obtained using different techniques. The estimated rumen protein degradation using the new *in vitro* GP technique and the *in situ* technique were compared by Karlsson *et al.* (2010). There were significant linear

relationships between IVDP and *in situ* CP disappearance when the data for the cold-pressed feed cakes were compared separately from the data for the expeller and the meals (Figure 4). The estimated EPD values, using the two techniques, differed for both HC and rapeseed cake, while there were no between-method differences for the other feeds (Karlsson *et al.*, 2010). The estimates of protein degradability provided by the *in situ* technique were considerably higher early in the incubations. This could be explained by loss of undegraded and soluble protein from the bags. All protein disappearing from the bag is assumed to be degraded, even though some particles may pass out from the bags without being degraded, resulting in an overestimation of the degradability (Dewhurst *et al.*, 1995; López, 2005). In addition, studies by Hedqvist & Udén (2006) have shown that soluble proteins vary in degradation rates and cannot, as in the *in situ* technique, be assumed to be completely degraded in the rumen.

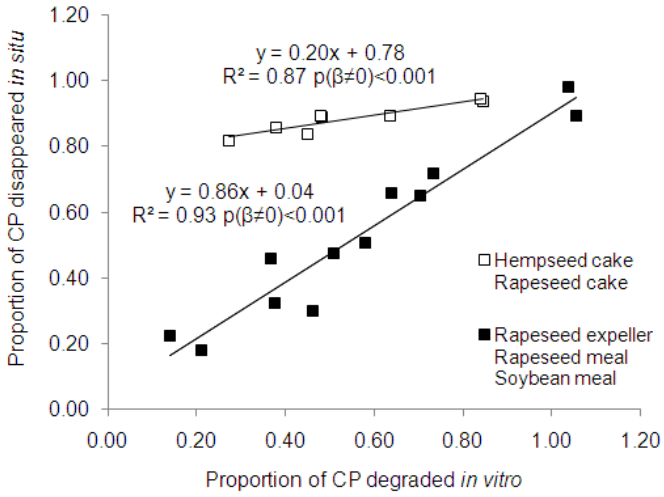

Figure 4. Relationships between *in vitro* degradable crude protein (CP) and *in situ* CP disappearance estimated after 4, 8, 12 and 24 h of incubation, from Karlsson *et al.* (2010).

The differences in estimated protein degradability using the new GP technique and the *in situ* technique were feed-dependent. The two cold-pressed feed cakes had a considerably higher fat content than the other feeds and the lipids might have adversely affected the protein degradation in the closed *in vitro* system compared to when the feed was incubated in the rumen. In a review, Doreau and Ferlay (1995) concluded that there was no experimental evidence demonstrating the effects of lipids on N degradation

in the rumen. However, lipid supplementation has been shown to decrease the degradability of N compounds estimated in batch culture *in vitro*, probably due to a decrease in bacterial growth or to specific inhibitions of proteolytic bacteria strains, while *in situ* estimations have shown no variation or an increase in N degradation (Doreau & Ferlay, 1995). The *in vitro* gas technique has also been used to assess effects of anti-nutritional factors in feeds (Makkar *et al.*, 1995), indicating that the system is susceptible to detect inhibition of rumen microbial activity.

The HC was the feed with the largest between-method differences, with an EPD value of 0.33 when estimated using the new GP technique and 0.84 when estimated using the *in situ* technique (Paper I). The EPD values of the HC used in the studies for Papers III and IV estimated using the *in situ* technique were 0.71 and 0.74, respectively. Since *in vivo* data are lacking, it is difficult to say whether the results from one method are closer to the actual values than those from the other. The methods represent two different ways of measuring protein degradation: by CP disappearance or by ammonia release. Based on the discussion above, the EPD values obtained using the *in situ* method are probably overestimates, while the EPD obtained using the new GP method might be an underestimate of the CP degradation of HC.

5.3 Amino acids and their digestibility

The most abundant AA found in HC (glutamic acid, arginine and aspartic acid) were the same as those reported in earlier studies (Callaway, 2004; Wang *et al.*, 2008). Of the total AA in HC, approximately 48% were EAA. The EAA profile (excluding tryptophan) for milk, rumen bacteria and protein feeds derived from NRC (2001) are compared to the EAA profile of HC in Figure 5. The biggest difference can be seen for arginine, where the HC contains a much higher proportion relative to rapeseed and soybean meal, which in turn contain a higher proportion than rumen bacteria and milk. Since methionine and lysine are often considered to be the most limiting AA with respect to milk production (Kung & Rode, 1996; NRC, 2001), and histidine has been shown to be the most limiting AA for dairy cows fed grass silage-based diets (Vanhatalo et al., 1999), the content of these AA in a protein feed may be of special interest. In addition, for growing cattle, methionine has been shown to be the most limiting AA when most of the absorbed AA comes from microbial protein (Schwab *et al.*, 2005). Generally, the AA requirements of sheep for growth and milk production do not differ greatly from those of beef and dairy cattle. One

exception is the greater requirement for sulphur-containing AA to support growth of wool, which contains high levels of cystine (NRC, 2007). The diagram (Figure 5) indicates that HC is a good source of methionine and histidine but a poor source of lysine, in comparison to both rapeseed and soybean meal. The lysine:methionine ratio in HC (1.4:1) was considerably lower than the optimal level for metabolisable protein (2.8-3.0:1) to achieve maximum milk protein yield (Schwab, 2010). The high ruminal degradability of AA in combination with the low intestinal digestibility of undegraded AA indicated that the contribution of intestinally available dietary AA from unheated HC was low (Paper IV). The proportion of AA in the RUP in concentrates (0.85) and the intestinal digestibility of the undegraded AA (0.82) suggested in the PBV/AAT system (Madsen *et al.*, 1995) would clearly overestimate the AAT value of HC. When calculating the AAT value of HC for Paper III, the intestinal CP digestibility obtained with the three-step procedure was used, resulting in a low AAT value of 32 g/kg DM. Using the digestibility of CP or total AA from Paper IV would result in an even lower AAT value. However, one can speculate that an overestimate of ruminal AA degradation using the *in situ* method would result not only in an underestimate of the amount of undegraded AA, but also of their digestibility.

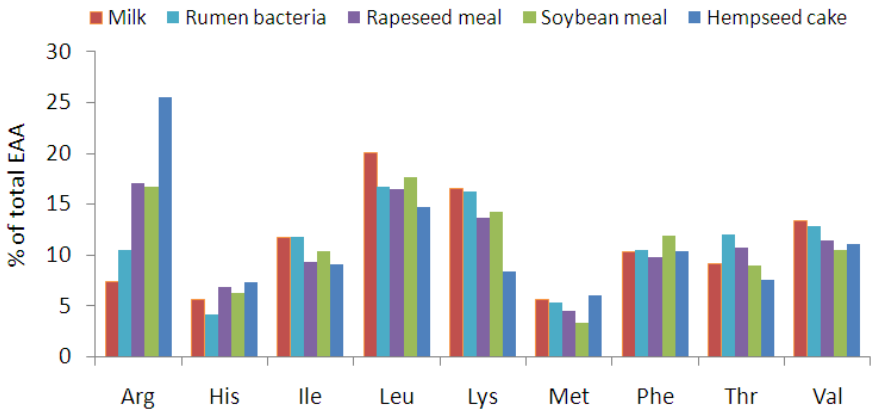

Figure 5. Comparison of the essential amino acid (EAA) profiles of milk, rumen bacteria and protein feeds. Values other than hempseed cake are derived from a comparison made by NRC (2001).

Nevertheless, moist heat treatment of HC could effectively be used for shifting the site of CP and AA digestion from the rumen to the small intestine (Paper IV). The highest temperature resulted in the largest amount

of RUP and undegraded total AA with the highest intestinal digestibility. Hence, moist heat treatment for 30 min at a temperature of 130°C did not overprotect the protein. If the amount of indigestible CP is not increased, a larger amount of RUP will logically result in higher intestinal digestibility. Several other studies have shown that it is possible to shift the protein digestion of oilseed feedstuffs from the rumen to the small intestine by heat treatment without decreasing the post-ruminal availability (McKinnon *et al.*, 1995; Dakowski *et al.*, 1996; Mustafa *et al.*, 1999a). However, heat treatment of rapeseed meal at temperatures of 140°C (Dakowski *et al.*, 1996) and 145°C (McKinnon *et al.*, 1995) resulted in heat damaged protein and, hence, a decreased intestinal CP digestibility.

5.4 Effects of hempseed cake on animal performance

5.4.1 Dry matter intake

The intake potential of a feed is an important factor. Despite high levels of fibre and fat in HC, inclusion of 318 g/kg DM did not have any detrimental effects on DM intake of dairy cows (Paper II). Similarly, the lambs fed HC, peas, rapeseed cake or the control diet exhibited no differences in DM intake (Paper III). This is in agreement with other studies that found no negative effects on DM intake of feeding hempseed meal (Mustafa *et al.*, 1999b), full-fat hempseed (Gibb *et al.*, 2005) or HC (Hessle *et al.*, 2008). Furthermore, a higher NDF intake and a lower starch intake by steers fed HC compared to those fed a mix of soybean meal and barley resulted in an improved rumen function measured as a lower number of long particles in the faeces and higher faecal DM and visual consistency score (Hessle *et al.*, 2008).

5.4.2 Milk yield and composition

Inclusion of HC in dairy cow diets was expected to result in an increased milk yield. We believed that the increase in yield would diminish with an increasing proportion of HC, but the clear negative effects on production reported in Paper II were unexpected. When formulating the diets we did not know about the high amounts of iNDF, which resulted in the low calculated ME content. Furthermore, the increased proportion of HC in the diet resulted not only in higher CP concentrations, but also in a higher NDF and fat concentration and a lower starch concentration. The inclusion of larger proportions of HC probably did not produce the expected increase in AAT supply to the animals. First, the lower intake of fermentable carbohydrates with increasing HC proportion may have limited microbial

protein synthesis. Second, the intestinal digestibility of RUP and undegraded AA was found to be low (Papers III and IV).

The AA profile of HC discussed above appears to be adequate except with respect to lysine, which may be limiting for milk protein synthesis. However, a good AA profile is of little benefit if the amount of intestinally available dietary CP is as low as 90 or 47 g/kg CP (Papers III and IV). The possibility of using heat treatment to protect the CP from degradation and increase the amount of intestinally available dietary CP and AA is interesting. *In vivo* measurements are required to provide data on the effects of the heat treatment of HC on animal performance.

In trials, the response of dairy cows to RUP supplements has been highly variable. In a meta-analysis conducted by Ipharraguerre and Clark (2005a), the milk production responses to RUP supplements relative to soybean meal ranged from -2.5% to +2.75%. Santos *et al.* (1998) concluded, from a literature review covering 12 years of publications, that increased RUP often resulted in decreased microbial protein synthesis and that there was no consistent improvement in lactation performance. In a meta-analysis conducted by Huhtanen & Hristov (2009), ruminal CP degradability did not appear to be a significant factor in predicting milk protein yield. The best prediction models were based on total digestible nutrients and CP intakes.

To be able to evaluate the effects of CP in isolation (Paper II), the diets would have needed to be reformulated to compensate for any additional differences in nutrient composition. This was not done and, instead, the results represent the production responses that would occur on a commercial farm when simply replacing a certain amount of compound feed in the diet with HC. It can be concluded that the inclusion of 143 g HC/kg DM resulted in the highest production results. As the experiment was conducted in the form of a dose-response trial with HC as the only protein feed, the production response relative to other protein feeds could not be assessed. This was considered, instead, in the study described in Paper III, in which three protein feeds were evaluated.

The inclusion of HC in ruminant diets may be limited by its fat content, which is high in PUFA (Callaway, 2004). Supplements containing high levels of PUFA can have a negative impact on microbial fibre digestion, resulting in a reduced acetate:propionate ratio and reduced synthesis of milk fat (Fredeen, 1996). Due to human health concerns, however, there is an interest in increasing the concentration of PUFA in dairy products, and this may be achieved by feeding supplements rich in PUFA. Even though this research area was not included in the work reported herein, a pilot study

evaluating the effects of HC on the fatty acid concentration in the milk was integrated into the feeding trial with dairy cows (Paper II). The results (unpublished data) showed that increased proportions of HC in the diet increased the concentrations of unsaturated fatty acids in the milk, while the concentrations of saturated fatty acids decreased.

5.4.3 Growth performance

The requirements of growing animals for metabolisable protein relative to that of ME are highest at birth and will then decrease with age and body weight (Schwab *et al.*, 2005). In the feeding trial with growing lambs (Paper III), the HC was evaluated as a protein feed for young animals with high protein requirements, where the protein supplement could comprise a large proportion of the diet. Considering that the ADG of the lambs fed HC was similar to the lambs fed the control diet, HC cannot be recommended as an alternative protein feed for growing lambs. The reason for the low growth response when fed on HC may be related to its low ME content and the low digestibility of RUP, as discussed earlier. However, Hessle *et al.* (2008) concluded that HC is a viable alternative as a protein feed for intensively fed growing cattle. They did not find any differences in growth performance of calves and steers supplemented with either HC or a mix of soybean meal and barley. However, the calves fed HC had a higher intake of DM, ME and CP. For the steers there were no differences in DM or ME intake but the CP intake was higher for those on the HC diet. Furthermore, the proportion of protein supplement was considerably smaller in the steers' diets (1.4 kg HC out of a total DM intake of 11.1 kg) compared to the lambs' diets (Paper III). Levels of full-fat hempseed up to 14% of dietary DM have been shown to have no detrimental effects on growth performance or feed efficiency of cattle, compared to those fed a standard barley-based diet (Gibb *et al.*, 2005).

5.5 Efficiency of protein utilisation by ruminants

The efficiency of CP utilisation by ruminants is generally low but a rather large variation indicates that improvements are possible. The linear decrease in CP efficiency (milk protein/CP intake) with increasing HC proportion in the diet (Paper II) is in agreement with the findings of other feeding trials (Broderick, 2003; Olmos Colmenero & Broderick, 2006) and the meta-analysis conducted by Huhtanen & Hristov (2009), who found that efficiency decreased with increasing dietary CP concentration. It seems that maximising CP efficiency occurs at the expense of production performance.

An increasing milk yield could increase CP efficiency provided that dietary CP concentration is not increased, but the effect seems to be considerably smaller than the effect of reducing CP intake (Huhtanen & Hristov, 2009). Ipharraguerre & Clark (2005b) concluded that it is possible to reduce the CP intake of high productivity dairy cows without compromising the supply of metabolisable protein if the sources of RUP and RDP are properly matched with available carbohydrates in the diet. This would increase the productivity and improve the conversion of feed CP into milk protein.

Feeding CP in excess will result in both higher feed costs and a negative environmental impact in terms of higher N emission (Huhtanen & Hristov, 2009). The N emission associated with milk production can be estimated from MUN, and dietary CP content has been shown to be the most important nutritional factor influencing MUN (Nousiainen *et al.*, 2004). Increasing the proportions of HC in the diet of dairy cows resulted in an expected increase in milk urea concentrations (Paper II). However, the urea level of 5.1 mM for the cows fed the highest HC proportion was lower than expected. Their diet contained 195 g CP/kg DM, a large proportion of HC with a high PBV value (Paper III) and possibly also limited amounts of fermentable carbohydrates. Relative to the relationship between MUN and CP (Nousiainen *et al.*, 2004), there seemed to have been less excess N from the HC diets. These results support the findings of the *in vitro* study (Paper I), indicating low rumen CP degradability for HC, and also data from an earlier *in situ* study by Mustafa *et al.* (1999b).

Estimated ADG/CP intake (g/g, data not shown) of the lambs (Paper III) was lower for animals fed the B-HC diet, with a value of 0.86 compared to 1.19, 1.36 and 1.56 for those fed the B-0, B-P and B-RC diets, respectively. It seems likely that ADG in the study was more related to energy intake, since the linear relationship between ADG and ME intake (R^2=0.76 for treatment means) was stronger than between ADG and the intake of either CP or AAT (R^2=0.36 and 0.39 respectively, for treatment means). It was only the lambs fed B-P that fulfilled the ME requirements according to NRC (2007) for an ADG of 250 g/day, and the lambs fed B-P and B-HC that reached the recommended CP intake.

6 Conclusions

The studies that this thesis is based upon demonstrate that it is possible to use cold-pressed HC as a protein feed for ruminants, but that its feed value is limited. A moderate level of HC in the diet of dairy cows gave the highest production results, while higher levels resulted in reduced yields of milk and milk components. Including HC in lambs' diets did not improve their growth performance compared to lambs fed a control diet with no protein supplement. The HC had an unusually high iNDF content, contributing to a low estimated ME content, and its inclusion caused a concomitant decrease in ME for the total ration. The estimated ruminal CP degradablilty of HC differed depending on which method of analysis that was used. When estimated using the new *in vitro* GP technique the EPD value of HC was low, but estimated with the *in situ* technique, EPD was considerably higher. The intestinal CP digestibility of unheated HC determined using the three-step *in vitro* procedure was low. The HC seemed to contain adequate proportions of the EAA methionine and histidine but lysine levels were limited. The AA had a high *in situ* degradability and a low *in vitro* digestibility and, hence, HC only contributed small amounts of intestinally available dietary AA. However, moist heat treatment could effectively be used to shift the site of CP and AA digestion from the rumen to the small intestine. Heat treatment may be a way to improve the protein value of HC, but, even so, the high proportion of iNDF limits its nutrient value.

7 Future perspectives

The environmental impact of animal production systems will continue to be an important issue. In order to produce as much feed as possible on the farm and to be less dependent on imported feeds, further research on protein crops suited to production in Northern Europe is needed. The potential for alternative crops, such as hemp, is dependent on both their agronomic performance and their nutritional value. The future of hemp cultivation for seed production at high latitudes is uncertain due to the limited number of varieties that produce reasonable seed yields. Continuous breeding for improved varieties or the development of hemp as a multifunctional crop could increase the economic profit. Hempseed oil is interesting from a human health perspective and has potential for increased sales. Today, HC is an expensive alternative protein source for ruminants. The cost needs to be comparable to other protein feeds before it becomes a viable alternative on commercial farms.

From a nutritional point of view, it is of interest to reduce the amount of iNDF in HC. The amount and degradability of NDF in different hempseed varieties should be determined to examine whether these results are consistent, or if there are varieties that contain less NDF or NDF that is more digestible. It may also be possible to breed for these traits. Since the seed shell probably contains the majority of the fibre, it would be interesting to investigate the possibility of shelling the seeds. Heat treatment of hemp is an option for increasing the RUP and the intestinally available CP of the feed. Techniques to heat treat oilseed feeds are already well established and should be possible to apply to HC on a large scale. Such additional treatments will increase the cost of the feed and the achievable benefits in terms of animal performance should be evaluated. The use of hempseed as a fat supplement in ruminant feeding could also be further investigated. Due to increasing concern for human health, it is desirable to increase the PUFA

and reduce the saturated fat in animal products and by feeding hempseed or HC such effects on fatty acid concentrations in milk and meat could be achieved.

To increase the utilisation of CP by ruminants and minimise the N emission from these production systems, biological efficiency rather than maximum production needs to be considered. Feeding management will continue to play an important role: feed rations must meet, but not exceed, protein requirements of the animals. To accomplish this, accurate evaluation systems that predict the protein value of feeds are needed. The new *in vitro* GP technique has potential to become a useful method for evaluating CP degradation in the rumen and will be further developed in the near future. Focus will be on standardisation of the rumen fluid to reduce the background ammonia levels as well as automation of the sampling procedure. This will make the technique more accurate and practical and increase the possibilities for it being used more extensively.

8 Populärvetenskaplig sammanfattning

Det finns ett stort intresse att öka användningen av lokalproducerat proteinfoder inom nordeuropeiskt lantbruk. Detta skulle minska beroendet av importerat proteinfoder som t.ex. sojabönor, men kräver grödor med högt proteininnehåll av god kvalitet som är anpassade till ett nordligt växtklimat. Hampa (*Cannabis sativa* L.) har varit en viktig kulturväxt i många århundraden och kan odlas för fiber- eller fröproduktion även i norra Europa. Vid kallpressning av hampfrö, för att utvinna hampolja, fås en proteinrik pressrest (hampfrökaka). Målet med denna avhandling var att utvärdera möjligheterna att använda hampfrökaka som proteinfoder till idisslare. Detta undersöktes genom laboratoriestudier och utfodringsförsök.

En analysmetod, baserad på mätningar av gasproduktion, vidareutvecklades för att bestämma proteinnedbrytningen i våmmen hos olika proteinfoder. Hampfrökaka samt ytterligare fyra proteinfoder inkuberades i våmvätska och mätningar av gasproduktion och ammoniakkväve användes för att beräkna nedbrytningen av protein. Hampfrökaka hade den lägsta andelen nedbrutet protein på 0.33, jämfört med 0.36, 0.46, 0.59 och 0.67 för rapsmjöl (värmebehandlat), rapsexpeller, rapskaka och sojamjöl. Resultaten tyder på att en stor del av hampprotein passerar våmmen utan att brytas ner. Detta är ofta önskvärt för proteinfoder till högproducerande idisslare för att öka mängden protein som tas upp i tunntarmen. Utvärdering med *in situ*-metoden, där hampfrökakan vägdes in i små påsar och inkuberades i våmmen på kor, visade däremot att proteinnedbrytningen i våmmen var hög. Laboratoriestudier av de icke nedbrutna foderresterna visade att proteinets smältbarhet i tunntarmen var låg. Genom att värmebehandla hampfrökakan i 30 minuter i en autoklav kunde proteinnedbrytningen i våmmen minskas. En större mängd foderprotein passerade därmed våmmen utan att brytas ned, samtidigt som dess smältbarhet i tunntarmen ökade. Den högsta temperaturen (130°C) som

användes i studien gav störst effekt. Studier av fibernedbrytningen med *in situ*-metoden visade att hampfrökakan innehöll en stor mängd icke nedbrytbar fiber som inte kunde utnyttjas av djuren. Detta bidrog till att det beräknade energivärdet för hampfrökan var lågt.

Ett utfodringsförsök med 40 mjölkkor pågick under fem veckor för att studera hampfrökakans effekt på mjölkproduktion och mjölksammansättning. Fyra olika foderstater bestående av gräsensilage och kraftfoder utvärderades. Kornbaserat färdigfoder ersattes med 0, 143, 233 eller 318 g kallpressad hampfrökaka/kg torrsubstans (ts). Koncentrationen av råprotein i foderstaterna varierade från 126 till 195 g/kg ts. Inblandning av hampfrökaka i foderstaten ledde, förutom till en högre andel råprotein, till en högre andel fett och fibrer, en lägre andel stärkelse samt en sänkning av foderstatens beräknade energiinnehåll. Ökad inblandning av hampfrökaka resulterade i en kurvlinjär (först stigande sedan avtagande) respons på avkastningen av mjölk, mjölkprotein, mjölkfett och laktos där de högsta produktionsresultaten observerades för kor som fick en inblandning på 143 g/kg ts. Koncentrationerna av mjölkprotein och mjölkfett samt effektiviteten att omvandla konsumerat foderprotein till mjölkprotein minskade, medan mängden urea i mjölken ökade, med en ökad inblandning av hampfrökaka.

I ett annat utfodringsförsök studerades tillväxten på lamm som under 8 veckor utfodrades med olika proteinfoder i kornbaserade foderstater. I försöket ingick 48 lamm, indelade i 16 grupper som tilldelades en av fyra olika foderstater. Kontrollfoderstaten innehöll inget proteinfoder medan de övriga foderstaterna innehöll antingen ärtor, rapskaka eller hampfrökaka och hade en råproteinkoncentration på 160-162 g/kg ts. Lammen som utfodrades ärtor hade den högsta tillväxten, följt av de som utfodrades rapskaka. Däremot kunde ingen skillnad i tillväxt ses mellan de lamm som utfodrats hampfrökaka och de som fick kontrollfoderstaten.

Resultaten från denna avhandling visar att hampfrökakans fodervärde är begränsande för dess användning som proteinfoder till idisslare. Framför allt behövs lösningar för problemet med den stora andelen icke nedbrytbar fiber.

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