Peas as feed for dairy cows

by

David Galméus

Institutionen för husdjurens utfodring och vård
Sveriges lantbruksuniversitet

Department of Animal Nutrition and Management
Swedish University of Agricultural Sciences

Examensarbete 366
30 hp A1E-nivå

Degree project 366
30 credit A1E-level
Uppsala 2012

Peas as feed for dairy cows

by

David Galméus

Handledare/ *Supervisor*: Ingemar Olsson
Examinator/ *Examiner*: Peter Udén

Nyckelord/ *Key words*: Peas, dairy cows

Institutionen för husdjurens utfodring och vård
Sveriges lantbruksuniversitet

Department of Animal Nutrition and Management
Swedish University of Agricultural Sciences

Examensarbete 366
30 hp A1E-nivå
Kurskod EX0136

Degree project 366
30 credit A1E-level
Course code EX0136
Uppsala 2012

1. Abbreviation list *4*

2. Abstract5

3. Introduction *7*

4. Literature review – peas as a feedstuff for ruminants *9*

4.1 Chemical composition and feeding value of peas **9**
4.1.1 Protein content and characteristics 9
4.1.2 Content and nature of starch 12
4.1.3 Content and characteristics of fibre and oligosaccharides 12
4.1.4. Fat content 13
4.1.5 Methods to affect the ruminal starch digestibility and the amino acid supply to the small intestine 14
4.1.5.1 Extrusion 14
4.1.5.2 Steam flaking 15
4.1.5.3 Pressure toasting 16

4.2 Feeding value of peas **16**
4.2.1 Protein value 16
4.2.2 Energy value 17
4.2.3 Milk production and milk composition 17

5. Experiment *18*

5.1 Material and Methods **18**
5.1.1 Animals and experimental design 18
5.1.2 Experimental diets and feed sampling 18
5.1.3 Infusion of markers 21
5.1.4.1 Ruminal measurements 22
5.1.4.2 Digestibility measurements 22
5.1.4.3 Milk recording and sampling 23
5.1.4.4 Sampling of plasma 23
5.1.5 Nylon bag measurements 23
5.1.6 Laboratory analysis 24
5.1.6.1 Determination of DM and sample preparation 24
5.1.6.2 Chemical analyses 24
5.1.6.3 Determination of markers and nucleic acids 25
5.1.6.4 Analyses of milk and plasma 25
5.1.7 Calculations 25
5.1.7.1 Rumen passage rate 25
5.1.7.2 Flow of DM and nutrients 25
5.1.7.3 Rumen undegraded protein and N synthesis efficiency 26
5.1.7.4 Total tract digestibility 26
5.1.7.5 *In situ* ruminal degradability and intestinal digestibility 26
5.1.8 Statistical analysis 26

5.2 Results **27**
5.2.1 Chemical composition and *in situ* digestibility 27
5.2.2 Feed intake 29
5.2.3 Ruminal fermentation 30
5.2.4 Weight of evacuated rumen content, pool size, outflow rates, and flow 32
5.2.5 Composition of rumen microbes 33
5.2.6 Digestibility of DM, OM, fat and carbohydrates 35
5.2.7 Digestibility of N, bacterial N synthesis, and flow of N and AA to the duodenum 37
5.2.8 Milk production and milk composition 39

5.3 Discussion **41**
5.3.1 Chemical composition and *in situ* digestibility 41
5.3.2 Feed intake 42
5.3.3 Ruminal fermentation 42
5.3.4 Influence of treatment on rumen pool size and outflow rates 43

5.3.5 Composition of rumen microbes 43
5.3.6 Digestibility of DM, OM, fat and carbohydrates 44
5.3.7 Digestibility of N, bacterial N synthesis, and flow of N and amino acids to the duodenum 45
5.3.8 Milk production and milk composition 47
6. Conclusions 49
7. Sammanfattning 50
8. References 52

1. Abbreviation list

AA	amino acid
AAN	amino acid Nitrogen
AAT	amino acids absorbed in the small intestine
ADF	acid detergent fibre
ANF	antinutritional factors
ATP	adenosine triphosphate
CP	crude protein
CTP	cytidine 5´-triphosphate
DAPA	2,6-diaminopimelic acid
DM	dry matter
EAA	essential amino acid
ECM	energy corrected milk
EFA	essential fatty acid
EFD	effective fibre degradation
FA	fatty acids
FCM	fat-corrected milk
FFA	free fatty acids
GLM	general liner model
GTP	guanosine 5´-triphosphate
IHA	The Department of Animal and Aquacultural Sciences
LAB	liquid associated bacteria
LDL	lowdensity lipoproteins
NAB	nucleic acid bases
NAN	non-ammonia nitrogen
NDF	neutral detergent fibre
NEAA	non-essential amino acid
NRF	Norwegian Red Breed
OM	organic matter
PBV	ruminal protein balance
PUFA	polyunsaturated fatty acids
RUP	rumen undegraded protein
SAB	solid associated bacteria
SAS	statistical analysis system
SFH	Senter for Husdyrsforsøk
TI	trypsin inhibitors
TIA	trypsin inhibitor activity
UMB	Norwegian University of Life Sciences
UTP	uridine 5´-triphosphate
VFA	volatile fatty acids

Abbreviations for treatment S, PR, R and SPR are given on page 20.

2. Abstract

An approaching overproduction of cereals and a future uncertain supply of non genetic modified vegetable protein feedstuffs have increased the interest in an expansion of the domestic production of protein feedstuffs in Norway. Besides rapeseed (*Brassica campestris*), peas (*Pisum sativum*) may be the most suitable crop for this purpose. Peas are characterized by having a relatively high content of crude protein, ranging between 20-26%, a high content of starch, 42-51%, and a low content of fat. In general, the energy value for peas is higher than for barley but lower than for rapeseed and soybean meal.

Pea protein consists of albumins and globulins to 85-100%, which leads to the fact that a large part of the pea protein is soluble and degradable in the rumen. Starch in peas is on the other hand to a large extent resistant to rumen degradation compared to starch from other starch rich feedstuffs. To decrease the ruminal degradability of dietary protein, and by means of that increase the total flow of amino acids to the small intestine, several processing methods are used. These methods are often based on some kind of heat treatments, which result in so called Malliard reactions. Expanding is one of these heat treatments, which earlier has shown to decrease the ruminal degradation of concentrates consisting of peas. The amino acid profile in peas is characterized by a high content of lysine but a low content of the sulphur containing amino acids cysteine and methionine.

There are no earlier experiences from trials in Norway, where peas are used as a feed for dairy cows. To increase the knowledge of the effect when peas are fed in large amounts to dairy cows, an *in vivo*-trial has been performed within the project "Alternative protein rich concentrate feedstuffs" (Alternative proteinrike kraftfôrråvarer) at the Department of Animal and Aquacultural Sciences at Norwegian Life Science University. The trial was performed with four dairy cows with rumen and intestinal fistulas in a Latin square design with four treatments and four periods. Treatments with extruded and pelleted concentrates were compared, which, with the exception for a base mixture, consisted of
1) 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% of a Ca-bonded fat source (Aco Feed Gigant) (S), 2) 10% full fat rapeseed and 27% peas (PR), 3) 10% full fat rapeseed, 19.9% barley and 6.9% oats (R), and 4) a blend of concentrate mixture S and PR in a ratio

of 50:50 (SPR). The fat content was planned to be equal among all concentrate mixtures and the content of N equal among S, PR and SPR. All experimental concentrates were extruded at 103-107° C, and thereafter pelleted at 70-75° C.

Peas in combination with full fat rapeseed did not affect the dry matter intake of feed. Only a small variation in ruminal pH and fermentation products was detected among concentrate mixtures. The ammonia concentration in milk, which is usually used as an indicator for ruminal protein degradability, was however higher for PR which represented the highest pea content. On the other hand, no higher values of blood urea levels were detected for PR compared to the other treatments. Although the amino acid profile in the dietary protein differed among treatments, there were only a small difference detected of the amino acid profile in the protein in rumen microbes and the total protein fraction flowing to the duodenum. There were only small variations in the digestibility coefficients in the rumen and small intestine and in the flow of single nutrients to the duodenum. However, there was a tendency for increased flow of total N fraction to the duodenum for S than for PR. The exchange of peas on behalf of cereals which was the fact for addition of peas in PR compared to R tended to counteract the depression in ruminal digestibility of dry matter and NDF, which was the fact for R.

The production results were affected by frequent clinical mastitis, and therefore quite insecure. However, on basis of present data, there was a decrease in daily production of ECM and a decrease in milk protein content for R. When cereals were substituted by peas, the daily production of ECM was not increased to the same level as that of the S and SPR. With focus on milk production, the optimal concentrate seemed to be a mixture of soybean meal and cereals or soybean meal and cereals in combination with peas and rapeseed.

3. Introduction

Cereals of Norwegian origin have been a dominating ingredient in feed concentrates within the Norwegian feed industry, supplemented of imported vegetable protein and fat raw materials and some by-products from the fish and food industry. The present legislation for agricultural production in Norway allows production of cereals for use as feedstuffs to a national requirement of traditional livestock (Uhlen *et al.,* 2005).

The production of cereals in Norway, in the period 1999-2010, is calculated to exceed the requirement. There is a risk for this surplus production of cereals to be permanently established, and at time of 2010 to be as large as 68 000 tons of feed cereals per year, corresponding to an area of 18 500 ha. This situation is due to an expected decrease in the concentrate consumption in Norway, a substitution of consumed carbohydrate rich feed raw materials by protein rich feed raw materials, and an increased import of feed raw materials. It is of importance for the Norwegian agriculture industry to reach a balance between production and requirement of domestic feed raw materials in order to avoid expensive market regulations in the plant production. On the other hand, is also of importance to keep the area of plant production at present level (Uhlen *et al.*, 2005).

Besides the increased production of cereals, many of the protein rich feed raw materials used in the Norwegian animal production are now imported. In future, import of protein rich feed raw materials is supposed to be an unsafe source supply to meet the requirement because of the fact that the supply of non genetic modified protein rich feedstuffs is globally decreasing. Furthermore, among protein sources of animal origin, a negative opinion occurs which make these raw materials less attractive as a substitute for the imported feedstuffs (Karlengen *et al*., 2005; Uhlen *et al.,* 2005).

The factors mentioned above, together with an increasing organic animal production, promote the great interest of the domestic Norwegian production of protein rich feed raw materials. Among protein rich feed raw materials that can be produced in Norway, rapeseed (*Brassica campestris*) and peas (*Pisum sativum*) are the most relevant ones (Karlengen *et al.*, 2005). The latter one will be in focus of this thesis. The reason to grow

peas is, besides the climatologic tolerance, that peas contain relatively high levels of protein, ranging between 20-27%, and also high levels of starch, ranging between 42-52%, which makes the energy value favourable for ruminants (Thomke, 1979, Christiansen *et al*., 1985).

The area used for growth of peas covered about 800 ha in 2004, which only represent a very small part of the total Norwegian arable area. Therefore, the growth area for pea production has a great potential to be increased. Theoretically, a possible area for growth of peas is estimated to be about 25 000 ha. However, because of the structure of the Norwegian agriculture, the pea growth would not be that large in reality but, nevertheless, may be considerably increased from the actual level (Uhlen *et al.*, 2005).

In general, peas are known to contain a large proportion of rumen degradable protein while the proportion of the rumen degradable starch is lower than in many cereals. Experimental experiences from peas used as a feedstuff for dairy cows in Norway are lacking. Therefore, at the Department of Animal and Aquacultural Sciences at theNorwegian University of Life Sciences (UMB) trials are now performed with dairy cows in order to get better knowledge about the affection of large proportions of peas within feeding. The project "Alternative protein rich feed raw materials" (Alternative proteinrike kraftfôrråvarer), of which the trial in present thesis is a part of, is a cooperation between the Department of Animal and Aquacultural Sciences (IHA) at UMB, Planteforsk, Graminor AS and Matforsk.

This thesis contains two major parts. The first one is a literature study which aims to give an overview of the characteristics of peas used as a feed raw material for dairy cows what concerns chemical composition, nutrient utilization, how nutrients characteristics affect it, and the feeding value of peas for milk production. The second part deals with an *in vivo* and an *in situ* trial which are intended to evaluate the additional value of peas to full-fat rapeseed and how peas in combination with full-fat rapeseed compared to soybean meal affect feed intake, digestibility and utilization of dry matter (DM) and nutrients, ruminal fermentation and microbial synthesis.

4. Literature review – peas as a feedstuff for ruminants

4.1 Chemical composition and feeding value of peas

Peas (*Pisum sativum*) in general, used as a feedstuff for ruminants, characterizes of relatively high contents of protein, starch and fibre, and low contents of fat and ash (Thomke, 1979). The energy value, chemical composition and digestibility parameters of peas, in relation to barley, full-fat rapeseed and soybean meal are presented in Table 4.1.

Table 4.1 Chemical composition (g kg^{-1}), effective fibre degradation (EFD), effective protein degradation (EPD) and protein digestibility in small intestine (%) of rumen undegraded dietary protein, compared to that in barley, full fat rapeseed, and soybean meal (Spörndly, 1999)

	Peas	Barley	Full fat rapeseed	Soybean meal
Chemical composition				
Crude protein	239	122	210	510
Crude fat	17	28	460	10
Crude fibre	68	60	80	60
NDF	100	246	120	95
Ash	32	27	50	70
Starch	550	556	10	62
Sugar	50	24	10	121
NFE	645	767	200	350
Digestibility coefficients				
EFD	46	53	61	72
EPD	80	78	68	64
Protein digestibility in small intestine	78	69	64	95

4.1.1 Protein content and characteristics

Among sources, the crude protein content in peas range between 20.0-27.5% (Thomke, 1979; Christansen *et al*., 1985; Lallès, 1992; Bastianelli *et al.*, 1995). According to Christiansen and Larsen (1987), in an investigation with chemical analyses of 8 pea cultivars grown in Denmark, the average crude protein content was 26.7%, with a minimum and maximum value of 23.8 and 30.3%, respectively, which tends to be higher than for other sources. As presented in Table 4.1, peas have a total protein content intermediary between that of soybean meal and cereals (Bastianelli *et al*., 1995; Spörndly,

1999). In soybeans the protein content is often twice as high as in peas, with proposed levels of 42% in raw soybeans and 50-55% when defattened (Lallès, 1992). Even rape seed meal contains substantially higher levels of protein than peas, with a proposed value of 41.6% (Spörndly, 1999).

As for all raw materials, the total crude protein (CP) content in peas is variable (Bastianelli *et al.*, 1995). The influence of environment and cultivation methods on CP content is significant. In fact, the protein level may vary considerably from one sample to another even for the same variety. There are also variations due to cultivar, although they are fewer than those attributable to cultivation methods and environment. However, the variation in content of CP has been reduced among recently registered cultivars. One of the objectives in the selection among plant genetics is to reduce variability of CP content (Christiansen and Larsen, 1987).

Lallès (1992) presents amino acid (AA) profiles in pea, soybean and cow milk protein, which are shown in Table 4.2. Pea protein is richer in lysine than the proteins of soybeans, barley, and rapeseed meal (Lallès, 1992). According to Christiansen and Larsen (1987), the lysine levels in pea protein are high, with a presented mean value of 7.21 g/16 g N (CP = N×6.25). Peas like legumes in general are poor in the two sulphur containing AA cystein and methionine (Thomke, 1979; Lallès, 1992). Hence, the interest to increase the fraction of rumen undegradable protein in peas may vary (Jordbruksverkets informationsenhet, 1999). Christiansen and Larsen (1987) present a methionine level of 0.91 g/16 g N, which is even lower than the presented level in Table 4.2. The tryptophane levels tend to be quite low as well, with the presented value of 0.89 g/16 g N. Both pea and soybean protein contains lower levels of threonine than cow milk, shown in Table 4.2.

Table 4.2 Comparison of the main features of the amino acid (AA) profiles of soybeans, peas and cow milk, expressed in g assayed AA/16 g N (Lallès, 1992)

	Soybean	Peas	Cow milk
Threonine	3.7	3.7	4.6
Proline	5.7	4.1	10.1
Glycine	4.7	4.8	2.0
Alaline	4.8	4.9	3.5
Cystine	1.5	1.5	0.9
Methionine	1.5	1.2	2.6
Isoleucine	5.8	4.8	5.8
Lysine	6.7	7.4	8.5
Arginine	7.8	8.8	3.6
Essential AA	46.5	44.3	47.7
Non-essential AA	62.4	56.3	60.3

The variation in the AA profile is small in pea protein, and no differences are assumed to occur between white and coloured pea cultivars (Christiansen and Larsen, 1987; Thomke, 1979). In peas, protein quality tends to vary with the size of the seeds. Small seeds in general tend to contain a protein of lower quality than seeds of larger sizes (Christiansen and Larsen, 1987).

In ruminants AA absorbed in the small intestine originates from both microbial protein synthesised in the rumen and from dietary AA sources that are not degraded in the rumen (Kung and Rode, 1996; McDonald *et al*., 2002). The degradability of protein in the rumen depends on the relationship between protein fractions with high solubility in water and salt solutions, such as albumins and globulins, and protein fractions with less solubility in water, such as prolamins and gluteins. The pea protein consists to 85-100% of albumins and globulins, which leads to the fact that a large fraction of pea protein is soluble and degradable in the rumen (Bastianelli *et al*., 1995). Goelema *et al*. (1998) showed that the ruminal degradability of N in raw peas was about 75%. When increasing the proportion of raw peas in concentrate blends, containing peas and full fat rapeseed, a greater soluble fraction and a higher degradation rate of N in the rumen have been observed (Chapoutot and Sauvant, 1996).

Pea proteins are predominantly water soluble (85%). This characteristic may not be beneficial for feeding ruminants because of excessive rumen protein degradation of raw peas (Bastianelli *et al*., 1995). According to Ljøkjel *et al.* (2003a), the high proportion of ruminal degradable protein in raw peas results in a low post-ruminally digestibility of rumen undegraded protein (RUP).

4.1.2 Content and nature of starch

Starch is the major storage carbohydrate in peas (Goelema *et al.*, 1998), and provides the most abundant component of peas (Table 4.1), with a variation of 42 to 52% (Bastianelli *et al*., 1995; Spörndly, 1999; Christiansen *et al.*, 1985). The variation of starch may partly be explained by the level of crude protein (Bastianelli *et al*., 1995). The content of amylopectin in peas is similar to that of cereals, with a proportion of about 70% amylopectin (Bastianelli *et al*., 1995). The proportions of amylose and amylopectin, size of starch granules, amylose-lipid complex bounds and protein matrix may have an effect on starch digestibility (Stevnebø *et al*., 2005). Starch from peas is less soluble and degradable in the rumen than from other feedstuffs rich in starch. It is shown that the ruminal degradability of starch is about 60% which indicates that starch from peas is less degradable than starch from barley (Goelema *et al.*, 1998). In ruminal fluid, untreated peas are characterized by a slow degradation rate of starch and a rapid solubility of protein. Hence, after intake, ammonia rises rapidly in the rumen. The lack of a source of easily degradable energy in synergy with the ruminal ammonia level would explain a deficit in microbial protein synthesis when raw peas are fed to ruminants (Chapoutot and Sauvant, 1996).

4.1.3 Content and characteristics of fibre and oligosaccharides

In peas, cellulose and lignin are present in comparatively small amounts. Other fibre components include, principally, pectic substances within the cotelydons and cellulose-hemicellulose complexes within the hull (Bastianelli *et al*., 1995). As presented in Table 4.1, the ruminal fibre digestibility expressed as effective fibre degradation (EFD) tends to be lower for peas than for barley, full-fat rapeseed and soybeans (Spörndly, 1999).

Peas contain small amounts, about 5% of DM, of oligosaccharides and disacharides. Of this part, sucrose represents 30-40%. Oligosaccharide components which are presented in lower amounts are alpha-galactosides, such as raffinose, stachyose and verbascose, which have been proved to cause flatulence in several monogastric animals (Bastianelli *et al*., 1995), and for pre-ruminats such as calves (Lallès, 1992). The variation in presence of different oligosaccharides in peas is low and seems to be, predominantly, of genetic origin (Bastianelli *et al*., 1995).

According to Chapoutot and Sauvant (1996), the NDF fraction in peas is known to be more degradable than that in rapeseed. A higher proportion of peas in relation to full-fat rapeseed in extruded blends increased the digestibility of cell wall components. For example, the *in situ* degradation rate of NDF and acid detergent fibre (ADF) increased, and the digestibility of organic matter (OM) increased non significantly for concentrate blends consisting of rapeseed and peas, when the ration was changed from 40:60 to 20:80. According to Focant *et al*. (1990), no differences were observed among heifers fed 38.5% ground, 39.0% steam-flaked or 39.3% extruded peas of total diet, in ruminal degradation of OM, after correction for bacterial OM synthesised in the rumen. The OM degradability ranged from 62.0 to 62.8% of intake. According to Chapoutot and Sauvant (1996), a higher proportion of peas led to a slightly faster rate of *in situ* total DM degradation and increased the effective degradability of feeds. Extrusion decreased both parameters.

4.1.4. Fat content

The mean value of fat content in feed peas is low and less than 2% (Table 4.1). Peas as a single feed raw material should for that reason not affect the rumen environment negatively. Of the total fat content, 90% occur as triglycerides with a composition similar to those of cereals being polyunsaturated in nature and with a predominance of linoleic acid. There has been some variability observed among cultivars, although a low variability for round cultivars. The risk of oxidation is small since the fat content and the activity of present oxidations enzymes in peas are low (Bastianelli *et al*., 1995).

4.1.5 Methods to affect the ruminal starch digestibility and the amino acid supply to the small intestine

According to Petit *et al*. (1997) and Goelema *et al*. (1998), the degradability of pea protein in the rumen could be a limiting factor for peas to replace all the supplemental protein when the requirement for RUP for high producing dairy cattle is not met. Various methods have been used to increase the supply of protein and AA to the small intestine, including dietary proteins with high values for RUP, and to treat feedstuffs to increase the RUP. It should be mentioned that increasing the amount of RUP does not always increase the total amount of AA reaching the small intestine. The increase in RUP can cause a decrease in microbial protein synthesis, resulting in no net change in the AA flow to the duodenum (Kung and Rode, 1996). Optimal conditions of treatments are generally defined as those which decrease rumen degradability without negatively altering post-ruminal digestion (Goelema *et al*., 1998). For ruminants, different methods to increase the RUP in the diet are common, and most of them are some kind of heat treatments (Kung and Rode, 1996; Goelema *et al*., 1998). Heating causes carbonyl groups of sugars to combine with free amino-groups of protein in the so called Maillard-reaction. The heat treatment increases the flow of AA to the duodenum and also the apparent absorption of AA in the small intestine. Some precautions must be taken during heat treatment of proteins, as excessive heat can extensively damage some essential AA such as lysine, methionine and cysteine (Kung and Rode, 1996).

4.1.5.1 Extrusion

Extrusion of peas, increases the insoluble portion of the protein and the gelatinization of starch, which tends to balance the rate of ammonia production and fermentation of starch. By this method, microbial protein synthesis may therefore be optimized (Focant *et al*., 1990; Petit *et al*., 1997). According to Chapoutot and Sauvant (1996), extrusion reduced the ruminal degradability of N by 20% and the rate of degradation decreased from 15 to 6% per hour. When peas and full-fat rapeseed were extruded in blends, a decrease in ruminal N degradability was detected. This led to an increased flow of dietary AA to the duodenum. But the comparison of crude protein (CP) effective degradation and CP degradation values for different feeds confirmed that a small part of the non-degradable

dietary N after extrusion could remain unavailable in the intestine. According to Focant *et al*. (1990), extrusion of pea caused a significant improvement on the flow of all AA in the duodenum.

According to Chapoutot and Sauvant (1996), extruded feeds containing 60-80% peas present a greater soluble fraction of starch than unprocessed feeds. Extruded mixtures containing peas and full fat rapeseed presented a greater soluble fraction of starch than unprocessed mixtures. When the pea content was increased in extruded blends containing full fat rapeseed, the degradation rate of starch measured *in situ* was increased (Ljøkjel *et al*., 2003b). According to Goelema *et al*. (1998), extrusion of peas in 140° C decreased the rumen degradability of protein from 88% to 66%, while total starch digestibility increased from 87% to 96%.

4.1.5.2 Steam flaking

Although, extrusion has been observed to be an effective method to gelatinize starch of peas, steam flaking under atmospheric pressure, which is an effective heat treatment to gelatinize the starch of cereal seeds, failed to gelatinize starch of peas. This resistance to gelatinization is probably caused by the nature of the starch, with its entrapment in fibrous thick-walled cells which prevents its complete swelling during cooking. Furthermore, the ruminal pH has been observed to be higher with ground and steam-flaked peas than with extruded (Focant *et al*., 1990).

Steam flaking of peas had no effect on AA flow. Total AA flow to the duodenum of heifers fed ground peas was only 78.2% of total AA intake (Focant *et al*., 1990). According to Chapoutot and Sauvant (1996), a higher proportion of pea in the raw blends also led to higher values of CP true digestibility. However, steam flaking of peas only decreased ruminal fluid digestion of N from 69 to 62%, which is considerably lower than for cereals, and it had no effect of total AA flow to the duodenum for heifers. The duodenal flow of bacterial N was observed to increase by 53% with extruded peas than for grounded and steam-flaked peas. This more effective microbial synthesis was assumed to be related to the digestion of carbohydrates (Focant *et al*., 1990).

4.1.5.3 Pressure toasting

Another heat treatment that can be used for legumes is pressure toasting. After this treatment the ruminal degradability of protein decreased of about 29%, although the total tract digestibility of dietary protein was still high and only slightly decreased (Goelema *et al*.,1998).

4.2 Feeding value of peas

4.2.1 Protein value

According to Petit *et al*. (1997), peas appear to be a good feed for lactating cows because of their relatively high protein content. Calculated values for protein quality, AA absorbed in the small intestine (AAT), and ruminal protein balance (PBV), according to the AAT/PBV-system, are presented in Table 4.3. According to Spörndly (1999), the AAT value for peas tends to be similar to that for barley, almost the double as for full-fat rapeseed, but only half compared to soybean meal. On the other hand, the PBV value was considerably higher for full-fat rapeseed and several times higher in soybean meal than in peas.

Table 4.3 Tabulated energy[1] and protein value[2] (g/kg) of peas, compared to that in barley, full fat rapeseed, and soybean meal

	Barley	Full fat rapeseed	Peas	Soybean meal
FEm	1.16	1.94	1.18	1.46
CP	122	210	239	510
AAT	90	56	98	182
PBV	-30	110	80	261

[1] Norwegian feed units of net energy (Ekern *et al*., 1991).
[2] (Spörndly, 1999).

The first limiting AA for the dairy cow differs depending on feed. Methionine is the first limiting AA in diets with legume seeds as a main protein feed, and lysine is the first limiting AA when cereals are used as the main protein feed (Boisen *et al*., 2000). The AA composition in pea protein is of several reasons superior to that in soybeans (Thomke,

1979). The first limiting AA, for young preruminant calves, is the sulphur AA, lysine, threonine and isoleucine. Thus, pea protein should be adequate, after methionine addition, for covering calf requirement of AA, although the utilization has to be considered (Lallès, 1992).

4.2.2 Energy value

Peas are an energy rich feed component for ruminants as shown from digestibility of organic matter, which is identical to that of soybean meal (Bastianelli *et al*., 1995). According to Norwegian feed tabular values (table 4.3), peas have a slightly higher energy content than barley, but considerably lower energy content than in soybean meal and much lower than in full-fat rapeseed. The major storage component in peas consists of starch. Starch is therefore the main energy source in peas which is further fermented to VFA by the rumen micro organisms (Bastianelli *et al.,* 1995).

4.2.3 Milk production and milk composition

There are limited numbers of reports presented concerning the influence of peas on milk yield and milk composition. According to Öster and Thomke (1978), there were no differences observed for milk yield with a lactation level averaging 19.0 kg d^{-1} when soybean and rapeseed meal were substituted by 30 % peas on concentrate basis. However, the energy intake, in relation to the milk yield in fat-corrected milk (FCM), was higher for peas than for soybean and rapeseed meal for cows in the second or latter lactations.

According to Syrjälä-Qvist *et al.* (1981), no effects on milk production, milk composition or milk nutrients were observed when 35% peas on concentrate basis were substituting soybean meal to lactating dairy cows. Furthermore, there were no differences in energy and protein utilization depending on peas in this trial.

According to Thomke (1984), available production results for peas show that peas in combination with forage of normal quality can be used to substitute soybean or rapeseed meal and cereal grains. Furthermore, no effects on pregnancy have been observed that could be related to peas.

5. Experiment

5.1 Material and Methods

The experiment was performed at the Department of Animal and Aquacultural Sciences (IHA) at UMB from February 16, to June 6, 2004.

5.1.1 Animals and experimental design

The experimental design was a Latin square with four periods and four treatments (diets). The animals were four dairy cows of Norwegian Red Breed (NRF), ranging from 45 to 95 days postpartum at the start of the first experimental period, in 3rd to 5th lactation, weighing from 532 to 670 kg. The animals were fitted with rumen flexible cannula (Bar Diamond Inc., Parma, ID, US; 100 mm i.d.) and closed T-type polyethylene cannula (ANKOM Inc., Fairport, NY, US; 25 mm i.d.) in the proximal duodenum 50-60 cm distal to pylorus (distal to the bile duct entrance). Two of the animals were also equipped with a T-type polyethylene cannula (25 mm i.d.) in the terminal ileum 40-50 cm proximal to the ileoceacal junction. The cows were housed in tie-stalls in a research barn. The experiment was approved by the Norwegian Animal Research Authority, and animal care was conducted according to laws and regulations controlling experiments with live animals in Norway.

Each period lasted for 21 days. Within period, the first 14 days were used as an adjustment period, whereas sampling took place from day 15 to 21. However, for one animal the treatment, which was planned for period one, was moved to an extra period after the main trial because of illness.

5.1.2 Experimental diets and feed sampling

The forage consisted of grass silage, which was ensiled in a tower silo and produced and distributed by Senter for Husdyrsforsøk (SFH) at UMB. The required amount of silage was taken out of the tower silo each day until day 12 of the first period, and then all the

silage used in the rest of the trial was taken out and frozen. At the time of silage freezing, representative random samples were taken out. These random samples were blended and new samples of this material were taken. The silage was continuously taken out and thawed between three to five days before feeding. Within all treatments, silage was fed *ad lib*, which implied that a minimum level of 10% forage residues was permitted on a daily basis. This was measured by weighing the feed residues before feeding each morning at 06.00. If necessary the amount of forage was corrected for the coming feeding times at 14.00 and 22.00 the same day, and the next day at 06.00.

Ingredient composition of the experimental concentrate mixtures is presented in Table 5.1. The concentrates were manufactured by Felleskøpet Øst-Vest in Norway. Treatments with extruded and pelleted concentrates were compared, which, except for a base mixture, consisted of 1) 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% of a Ca-bonded fat source (Aco Feed Gigant) (S), 2) 10% full fat rapeseed and 27% peas (*Pisum sativum*) (PR), 3) 10% full fat rapeseed (*Brassica campestris*) and 19.9% barley and 6.9% oats (R), 4) a blend of concentrate mixture S and PR in a ratio of 50:50 (SPR), corresponding to 13.5% peas, 5% full fat rapeseed, 6% soybean meal, 7.85% barley, 2.7% oats and 1.9% Aco Feed Gigant. The fat content was planned to be equal among all concentrate mixtures and the content of N equal among S, PR and SPR. All experimental concentrates were extruded at 103-107° C, and thereafter pelleted at 70-75° C.

The animals were fed a fixed amount of concentrate, individually adjusted to maintenance and milk production at the start of the first experimental period. The concentrate ration varied between 11.0 and 13.0 kg per day depending on animal. These amounts were kept during the whole trial.

Table 5.1 Ingredient composition, calculated chemical composition and feeding value from chemical analysis of ingredients of experimental concentrate mixtures

	Treatments			
	S	PR	R	SPR[1]
Feed raw materials in experimental mixtures (%)				
Soybean meal (extracted)	12.0	-	-	6.0
Rape seed (full-fat)	-	10.0	10.0	5.0
Peas	-	27.0	-	13.5
Barley	15.6	-	19.9	7.8
Oats	5.4	-	6.9	2.7
”Ako Feed Gigant”	3.8	-	-	1.9
Feed raw materials in base mixtures (%)				
Barley	40.2	40.2	40.2	40.2
Oats	14.1	14.1	14.1	14.1
Molasses	5.0	5.0	5.0	5.0
Urea - ”Rumisan”	0.5	0.5	0.5	0.5
Limestone meal – ”Visnes”	1.5	1.4	1.5	1.5
Mono ammonium phosphate	0.2	0.1	0.17	0.2
Magnesium phosphate	0.8	0.8	0.8	0.8
Sodium chloride	0.7	0.7	0.7	0.7
Sodium sulphate	0.1	0.1	0.1	0.1
”Mikro Storfe”	0.1	0.1	0.1	0.1
”Vitamine-5”	0.1	0.1	0.1	0.1
Chemical composition and feeding value (g kg^{-1})				
Crude protein	147.0	148.0	119.0	147.5
Fat	56.0	56.0	60.0	56.0
Starch	383.0	425.0	413.0	404.0
FEm (pr 100 kg)[2]	100.6	102.6	100.2	101.6
AAT[3]	93.6	79.6	82.6	86.6
PBV[4]	1.8	15.6	-1.6	8.7

[1] Calculated mean values from S and PR.
[2] Norwegian feed units of net energy (Ekern *et al.*, 1991).
[3] Amino acids absorbed in the small intestine, according to the AAT/PBV-system (Spörndly, 1999).
[4] Protein balance in the rumen, according to the AAT/PBV-system (Spörndly, 1999).

5.1.3 Infusion of markers

Distribution of marker infusion and sampling are presented in Table 5.2. Markers were infused through the rumen fistula from day 1 to 21 for each period (Table 5.2). The marker solution consisted of the liquor marker, Co-EDTA, consisting of 13.9% Co, and the particle marker, Yb-Acetate, consisting of 41.0% of Yb, dissolved in distilled water to a concentration of 9.0 g Co-EDTA and 3.0 g Yb-acetate per litre. To the solution, 10 ml of acetate buffer was added. The infusion started with a dose equivalent to the amount of one day's infusion to get a quick stable concentration in the rumen. The concentration of markers was maintained by continuous infusion through the rumen fistula by PVC tubes coupled to a peristaltic pump. Once a day the cans with marker solution were weighed at 08.00, in order to measure the amount of infused solution. The infusion was calculated to give a corresponding amount of Co-EDTA and Yb-Acetate of 24.75 respectively 8.25 g per day to each animal.

Table 5.2 Management and distribution of sample collection for each period

Day	Infusion of markers	Collection and sampling of faeces and urine	Sampling of rumen liquor	Sampling of duodenal and ileal digesta	Rumen emptying	Blood sampling	Milk registration	Milk sampling
1	x (start)						x	
(1-15)	x						x	
15	x	x					x	x
16	x	x	x	x			x	x
17	x	x	x	x			x	x
18	x	x			x	x	x	x
19	x				x		x	x
20	x						x	x
21	x						x	x

5.1.4 Sampling

5.1.4.1 Ruminal measurements

Rumen liquor (Table 5.2) was collected day 16 at 06.00, 10.00, 17.00 and 21.00, and day 17 at 08.00, 12.00, 15.00 and 19.00. Directly after collection, pH was measured and 10 ml of rumen liquor were put in a centrifuge tube with 0.5 ml formic acid. The samples were stored at 4° C until analysis.

In connection to complete rumen emptying, samples of rumen contents were taken day 18 at 12.00 and day 19 at 16.00. The material was separated in liquid and particle phases by using a sieve with 6.0 mm openings. Each phase was weighed, and samples with a representative part of liquor and particle phase were taken out and recombined for direct DM analysis in 103° C in 24 hours. After sampling the material was brought back to the rumen. After the rumen emptying at day 19, the microbial mass of liquid associated bacteria (LAB), solid associated bacteria (SAB) and protozoa were determined. The procedure to isolate protozoa was based on the method described by Martin *et al.* (1994), using 50 min. flocculation time. LAB and SAB were isolated as described by Volden and Harstad (1998), without addition of formaldehyde. The microbial fractions were transferred to plastic beakers and immediately frozen.

5.1.4.2 Digestibility measurements

Approximately 500 ml of duodenal digesta and 250 ml of ileal digesta were collected day 16 at 06.00, 10.00, 17.00 and 21.00, and day 17 at 08.00, 12.00, 15.00 and 19.00 (Table 5.2). If the flow of digesta was low, the collection was stopped after one hour and a smaller amount was accepted. Immediately after collection, pH was measured in each sample, and 500 g of duodenal digesta and 250 ml of ileal digesta were frozen. One can was used for each cow and period and frozen between each collection. After the experiment, the collected material was slowly thawed, blended, and new samples were taken out.

Faeces and urine (Table 5.2) were quantitatively collected in one mixed fraction from day 15 to day 18 at 08.00, 14.00 and 22.00. The material was mixed and weighed for each day, and representative samples of about 10% of the total mixed material were taken out and frozen. After the trial, the frozen samples were thawed, blended, and new samples were taken out for analysis.

5.1.4.3 Milk recording and sampling

The cows were milked twice a day, at 06.00 and 15.30. The milk yield was measured for each milking. Milk samples were collected each day from day 15 to day 21 for each cow and period and 2-Bromo-2nitropane-1,3 diol (D&F Control Systems Inc. USA) was added to the milk samples. After the sampling period, the material was warmed to 39º C, blended, and representative samples of 40 ml were taken for chemical analysis and 10 ml in centrifuge tubes for determination of urea concentration.

5.1.4.4 Sampling of plasma

Blood samples were taken from the jugular vein (10 ml in heparinized tubes) day 18 at 08.00, 10.00 and 12.00. The samples were immediately centrifuged at 500×*g* and the plasma was kept at -20° C until analysis.

5.1.5 Nylon bag measurements

Nylon bag measurement of ruminal degradation characteristics of dietary N, starch and NDF as well as intestinal digestion of dietary N and starch in experimental concentrates were performed as described by Madsen *et al*. (1995) and Prestløkken and Harstad (2001). Three dry NRF cows fitted with rumen flexible cannula (Bar Diamond Inc., Parma, ID, US; 100 mm i.d.) of which two were fitted with closed T-type polyethylene cannula (ANKOM Inc., Fairport, NY, US; 25 mm i.d.) in the proximal duodenum 50-60 cm distal to pylorus (distal to the bile duct entrance) were used. The animals were fed 2.0 kg grass hay and 0.8 kg concentrate (Favør 20, by Felleskøpet Øst-Vest) twice a day, at 06.00 and 14.00. The experimental concentrates were incubated in 0, 2, 4, 8, 16, 24, 48 and 96 hours

in the rumen. Residues from 16 hours ruminal incubation were dried and applied in the duodenal cannula and collected in the faeces after a maximum time of 24 hours after application.

5.1.6 Laboratory analysis

5.1.6.1 Determination of DM and sample preparation

The DM content of silage, rumen recombined material, and blended urine and faeces was determined by oven drying at 103° C per 24 hours. Before analysis of chemical composition the sample materials, except for milk and plasma, were freeze dried and milled to pass a 1.0 mm screen.

5.1.6.2 Chemical analyses

Samples of experimental mixtures, rumen recombined material, rumen bacteria and protozoa, duodenal and ileal digesta, and blended faeces and urine, were determined analysed for ash, Kjeldahl-N (rumen bacteria and protozoa were analysed for N), crude fat, NDF, and starch. Feeds, rumen bacteria and protozoa, and duodenal digesta were also analysed for the content of AA, including 2,6-diaminopimelic acid (DAPA). The content of ash and Kjeldahl-N was determined in the feed, rumen recombined material, duodenal and ileal digest, and urine and faeces, according to AOAC (1990). In rumen bacteria and protozoa, N was determined by the Dumas method (AOAC, 1990) on a Leco Nitrogen Analyser (Leco Corporation, St. Joseph, MI, USA). Nitrogen in residues after ruminal and intestinal incubation was determined according to Dumas method (AOAC, 1990), using the Fison EA 1108 Elementar Analyser. Crude fat was determined by extraction with petroleum ether after HCl-hydrolysis (AOAC, 1990). NDF was determined as described by Goering and Van Soest (1970), following a sequential analysis of hemicellulose, cellulose and lignin (with sodium sulphite, without amylase). The method of McCleary *et al.* (1994) was used for determination of starch without correction for sugar.

5.1.6.3 Determination of markers and nucleic acids

Analyses of Co and Yb, in duodenal and ileal digesta and in blended faeces and urine, were carried out as described by Siddons *et al*. (1985). The concentrations were determined by atomic absorption analysis (GBC 906 Atomic Absorption Spectrophotometer, GBC Ltd., Melbourne, Australia). The content of nucleic acids, in the duodenal and ileal digesta and rumen bacteria and protozoa, was determined according to Makkar and Becker (1999).

5.1.6.4 Analyses of milk and plasma

Milk samples were analysed for fat, protein and lactose by infrared analysis (MilkoScan 255 A/B; Foss Electric Inc., Hillerød, Denmark). Milk and blood urea was determined with the Cobas MIRA S auto-analyser (Roche AG, Basel, Switzerland).

5.1.7 Calculations

5.1.7.1 Rumen passage rate

Rumen passage rate (k_p) of DM and NDF was estimated from rumen evacuation and duodenal flow data according to the equation (Stensig *et al*., 1998):

K_p, h^{-1} = [(flow to duodenum, kg d^{-1}) / (rumen pool size, kg)] / 24

5.1.7.2 Flow of DM and nutrients

Duodenal and ileal flow were calculated from the infused amount of Co and Yb and their concentrations in pooled samples, assuming a steady state dilution of the rumen marker pools into the intestines. Daily flow was calculated using the average flow estimates for the two markers. Faecal and urinal recovery of markers was calculated, as the amount of markers excreted in faeces and urine in percent of the infused amount of markers. The

flow of bacterial N to the duodenum was estimated using nucleic acid bases (NAB) and DAPA as markers, and a mixture of LAB and SAB in duodenal digesta was calculated according to Volden (1999) assuming that LAB and SAB constitute 40 and 60% of the rumen bacterial biomass (Legay-Carmier and Bouchard, 1989; McAllister *et al.*, 1994). Total duodenal flow of bacteria N (g d^{-1}) was calculated by dividing the corrected duodenal flow of DAPA-N (g d^{-1}) with the DAPA-N:N ratio of the bacteria. Duodenal flow of bacteria N was also calculated based on content of NAB according to Makkar and Becker (1999).

5.1.7.3 Rumen undegraded protein and N synthesis efficiency

Rumen undegraded protein (RUP) including endogenous protein was calculated by subtracting bacterial N from non-ammonia N (NAN).

5.1.7.4 Total tract digestibility

Total tract digestibility was determined from total collections of blended urine and faeces.

5.1.7.5 *In situ* ruminal degradability and intestinal digestibility

In situ ruminal degradation of N was calculated according to Ørskov and McDonald (1979), assuming a fractional passage rate of 8% h^{-1} and by using the determined passage rate of DM. *In situ* intestinal digestibility of rumen escape N was calculated according to Hvelplund *et al*. (1992).

5.1.8 Statistical analysis

Data was analysed as a Latin square with four periods and four treatments using the general liner model (GLM) procedure of the statistical analysis system SAS (2001). The model contained effects for period, animal and treatment. Effect of treatment (S, PR, R and SPR) was tested on chemical composition and AA-profile of concentrate mixtures, feed intake, ruminal fermentation products and pH, rumen parameters, chemical

composition and AA-profile within rumen bacteria and protozoa, intake and ruminal digestibility of DM, OM, fat, starch, NDF, and N, flow of AA to the duodenum, milk production and composition, and urea concentration in milk and blood. Means were separated by least-squares and the *P*-difference procedure, using the model:

$$Y_{ijk} = \mu + A_i + P_j + C_k + e_{ijk}$$

where μ, A, P, C, and , e_{ijk} are means, the effect of animal ($i = 1 – 4$), period ($j = 1 – 4$), treatment ($k = 1 – 4$), and errors, respectively. Statistical differences were considered to exist at $P<0.05$, and a tendency was considered to exist at $0.05 \leq P<0.10$.

5.2 Results

5.2.1 Chemical composition and *in situ* digestibility

Chemical composition and *in situ* digestibility of experimental feedstuffs and grass silage are presented in Table 5.3. Only small variations were observed for the content of DM, OM and starch among the concentrate mixtures. In contrast, the content of fat was observed to be significantly higher in S, than in the other concentrate mixtures. The fat content ranged from 65.0 to 78.0 g kg^{-1}. The content of N and NDF was observed to be significantly lower respectively higher in R than in the other concentrate mixtures. Moreover, the chemical composition on grass silage agreed with Scandinavian feed tabular values.

Table 5.3 Content of dry matter (g kg^{-1}), chemical composition (g kg^{-1} DM), ruminal degradability and intestinal digestibility of N (%) measured *in situ* of the experimental concentrate mixtures where treatment S = 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% Aco Feed Gigant, PR = 10% full fat rapeseed and 27% peas, R = 10% full fat rapeseed, 19.9% barley and 6.9% oats, SPR = a blend of concentrate mixture S and PR in a ratio of 50:50, and silage

	Treatment					
	S	PR	R	SPR[1]	SEM	Silage
Dry matter	910.3	915.0	917.8	912.6	9.7	258.0
Organic matter	940.2	938.9	939.3	939.5	0.9	949.1
Fat	78.0[a]	65.0[c]	66.5[bc]	71.5[b]	2.5	41.5
NDF	155.7[b]	146.5[b]	174.6[a]	151.1[b]	5.2	538.0
Starch	413.1	430.3	437.1	421.7	12.7	0.0
Nitrogen	27.9[a]	27.3[b]	23.7[c]	27.6[ab]	0.2	21.7
In situ ruminal N degradability	54.7	61.7	58.1	58.2	-	-
In situ intestinal N digestibility	95.9	95.7	95.0	95.8	-	-
In situ ruminal starch degradability	87.6	78.5	85.2	83.1	-	-
In situ intestinal starch digestibility	93.9	98.6	97.9	96.2	-	-
In situ ruminal NDF degradability	68.6	63.9	56.4	66.2	-	-

[a, b, c] Different letters among treatments indicates statistical differences ($P<0.05$).
[1] Calculated from S and PR in a ratio of 50:50.

The content of AAN and the AA profile (% of total AAN) in concentrates and silage are presented in Table 5.4. The content of total AAN was lower for R than for the others. The proportion of essential amino acid (EAA) significantly differed among all concentrates. PR showed highest proportion of EAA followed by SPR, S and R, and the opposite range for non-essential amino acid (NEAA). Except for threonine and tyrosine, there was a large variation of single AA among concentrates. The proportion of lysine was highest in PR, while the proportion of methionine and cystein was significantly higher in R than in the other concentrates. The highest proportions of single AA were observed for arginine and glutamic acid in all concentrates, ranging from 14.7 to 17.1% and 13.9 to 16.3%, respectively. The lowest proportions were observed for tyrosine and the sulphur containing methionine and cysteine, ranging from 1.9 to 2.0%, 1.0 to 1.2% and 1.9 to 2.3%, respectively.

Table 5.4 Content of amino acid N (AAN) (g kg^{-1} DM) and amino acid profile (LS means % AAN of total AAN) of experimental concentrate mixtures, where treatment S = 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% Aco Feed Gigant, PR = 10% full fat rapeseed and 27% peas, R = 10% full fat rapeseed, 19.9% barley and 6.9% oats, SPR = a blend of concentrate mixture S and PR in a ratio of 50:50, and silage

	Treatment					
	S	PR	R	SPR	SEM	Silage
AAN	18.1[a]	18.2[a]	14.0[b]	18.1[a]	0.1	15.4
EAA						
EAA sum	50.6[c]	52.1[a]	48.7[d]	51.3[b]	0.1	50.4
Arginine	15.9[c]	17.1[a]	14.7[d]	16.5[b]	0.1	8.2
Histidine	5.3[ab]	5.3[a]	5.2[b]	5.3[5.3ab]	0.1	5.3
Isoleucine	3.7[a]	3.5[c]	3.4[c]	3.6[b]	0.0	3.0
Leucine	6.2[a]	5.9[c]	6.2[a]	6.1[b]	0.0	6.2
Lysine	7.0[c]	8.1[a]	6.2[d]	7.5[b]	0.0	10.3
Methionine	1.1[b]	1.0[b]	1.2[a]	1.0[b]	0.0	1.6
Phenylalanine	3.4[a]	3.2[c]	3.4[a]	3.3[b]	0.0	3.7
Threonine	3.3	3.3	3.3	3.3	0.0	4.8
Valine	5.0[b]	4.8[b]	5.2[a]	4.9[b]	0.1	7.1
NEAA						
NEAA sum	49.4[b]	47.9[d]	51.3[a]	48.7[d]	0.1	49.6
Alanine	5.2[c]	5.3[b]	5.4[a]	5.3[b]	0.0	11.0
Aspartic acid	7.1[b]	7.2[a]	6.0[c]	7.2[ab]	0.0	8.8
Cysteine	2.0[b]	1.9[b]	2.3[a]	2.0[b]	0.0	0.6
Glutamic acid	15.2[b]	13.9[d]	16.3[a]	14.6[c]	0.1	8.4
Glycine	6.3[c]	6.5[ab]	6.6[a]	6.4[b]	0.1	9.1
Proline	6.7[b]	6.3[b]	6.5[b]	8.0[a]	0.2	5.1
Serine	5.0[a]	4.8[c]	4.9[bc]	4.9[ab]	0.1	5.2
Tyrosine	2.0	2.0	1.9	2.0	0.0	1.5

[a, b, c, d] Different letters among treatments indicates statistical differences ($P<0.05$).

5.2.2 Feed intake

Total DM intake and the intake of silage and concentrate are presented in Table 5.5. There were only small variations among the experimental treatments. The intake averaged 21.3 kg d^{-1} for total DM, and 10.2 kg d^{-1} for silage, which is analogous to a mean forage concentrate ratio of 47.6:52.4%.

Table 5.5 LS means of feed intake (kg DM d^{-1}), where treatment S = 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% Aco Feed Gigant, PR = 10% full fat rapeseed and 27% peas, R = 10% full fat rapeseed, 19.9% barley and 6.9% oats, SPR = a blend of concentrate mixture S and PR in a ratio of 50:50

	Treatment				
	S	PR	R	SPR	SEM
Total intake of feed	21.4	21.0	21.6	21.3	0.9
Intake of concentrate	11.2	11.0	11.4	11.2	0.3
Intake of silage	10.2	10.1	10.2	10.1	0.8

5.2.3 Ruminal fermentation

Ruminal pH and concentrations of fermentation products are presented in Table 5.6. There was a variation of NH_3 concentrations among diets, a tendency for higher concentration for PR than R ($P<0.06$). The maximum concentration levels of total VFA, (not shown in Table 5.6) were detected 2 hours after feeding for PR and SPR, and 3 hours after feeding for S and R. Furthermore, there were just a few significant differences depending on treatment. As shown in Table 5.6, of a single VFA, butyrate showed significantly higher proportion for R compared to S of 12.6 and 10.8 mol%, respectively. Of single observations, observed 1 hour after feeding (not presented in Table 5.6) the concentration of propionate significantly increased when R was substituted by SPR. Furthermore, two hours after feeding, the concentration of ammonia and isovalerate significantly increased when R was substituted by PR and valerate when R was substituted by SPR.

Table 5.6 LS means of rumen concentration of ammonia (NH_3) and total volatile fatty acids (VFA) (mmol $l^{-1)}$, VFA pattern (molar%), and pH, where treatment S = 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% Aco Feed Gigant, PR = 10% full fat rapeseed and 27% peas, R = 10% full fat rapeseed, 19.9% barley and 6.9% oats, SPR = a blend of concentrate mixture S and PR in a ratio of 50:50

	Treatment				
	S	PR	R	SPR	SEM
VFA and NH3 concentration					
Total VFA	110.4	110.2	103.0	110.2	6.0
NH_3	94.0	114.8	87.8	100.6	16.4
VFA pattern					
Acetate	64.6	63.6	64.0	65.8	2.1
Propionate	21.1	21.0	21.2	19.6	2.5
I-buturate	0.8	0.8	0.8	0.7	0.1
Buturate	10.8^{b}	13.2^{ab}	12.6^{a}	11.6^{ab}	0.7
I-valerate	1.3	1.1	1.0	1.0	0.3
Valerate	1.4	1.4	1.3	1.3	0.1
pH					
pH min^{1}	6.0	5.9	5.8	5.7	-
pH max^{1}	6.5	6.5	6.5	6.4	-
pH mean	6.2	6.2	6.1	5.9	0.2

[a, b] Different letters among treatments indicates statistical differences ($P<0.05$).[1] Data consists of mean values.

Ruminal pH-values are presented in Table 5.6 and in Figure 5.1. The highest levels were detected immediately before feeding for all treatments and the lowest values 3 hours after feeding for S and PR, and 4 hours after feeding for R and SPR.

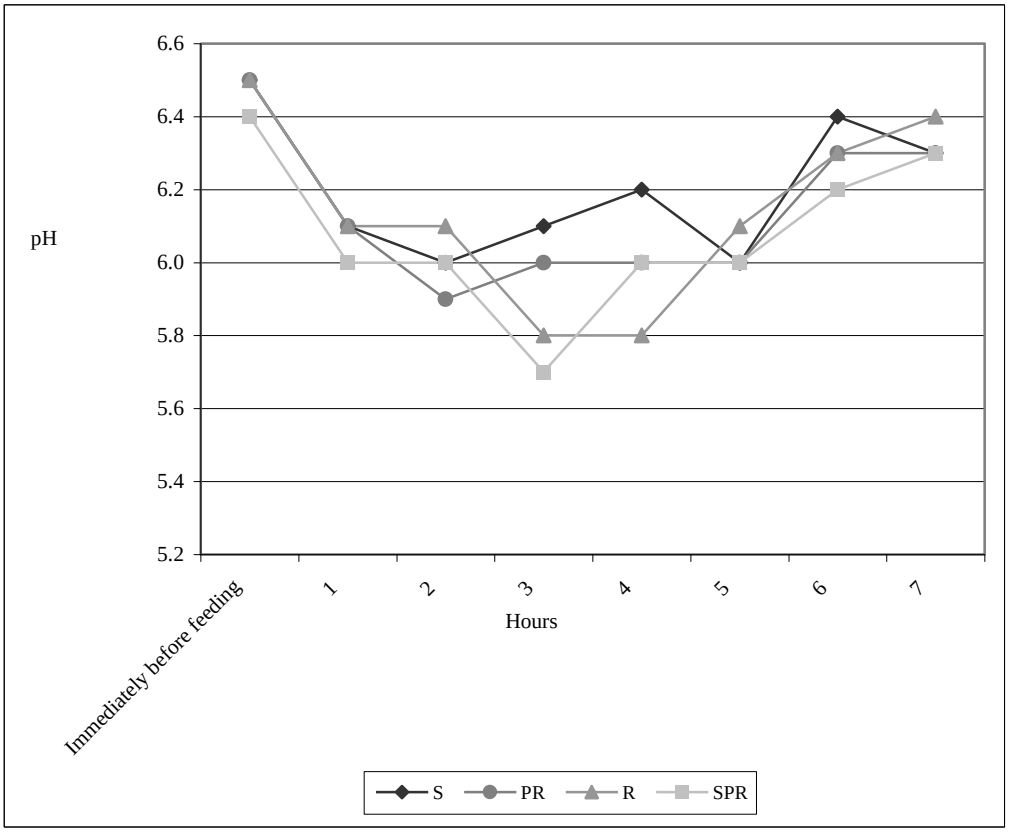

Figure 5.1 Rumen pH variations measured before feeding and 1-7h after feeding, where treatment S = 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% Aco Feed Gigant, PR = 10% full fat rapeseed and 27% peas, R = 10% full fat rapeseed, 19.9% barley and 6.9% oats, SPR = a blend of concentrate mixture S and PR in a ratio of 50:50.

The numbers of recorded pH values below 6.0 were 0, 1, 2 and 1 for S, PR, R and SPR, respectively, which implies that pH did not sink to the same extent for S compared to the other treatments. Only one significant difference depending on treatment was detected among pH from immediately before feeding to 7 hours after feeding. Two hours after feeding, PR showed lower pH-value than R, of 5.9 and 6.1, respectively. However, the mean value of pH over time did not differ although R showed a nominally lower pH-value than the other treatments.

5.2.4 Weight of evacuated rumen content, pool size, outflow rates, and flow

Weight of evacuated rument content, pool size and outflow rates of dry matter, NDF and starch, and flow of duodenal and ileal digesta are summarized in Table 5.7. There were no effects of treatments on these parameters. Weight of evacuated rumen content averaged 80.9 kg, and the pool size of OM, NDF and starch 8.8, 5.8 and 0.4 kg respectively. The mean flow of duodenal and ileal digesta was 393.0 and 100.1 l d^{-1}, respectively.

Table 5.7 LS means of evacuated rumen content (kg), duodenal and ileal flow of digesta (l d^{-1}), rumen pool size (kg), and rumen outflow rate (% h^{-1}) where treatment S = 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% Aco Feed Gigant, PR = 10% full fat rapeseed and 27% peas, R = 10% full fat rapeseed, 19.9% barley and 6.9% oats, SPR = a blend of concentrate mixture S and PR in a ratio of 50:50

	Treatment				
	S	PR	R	SPR	SEM
Evacuated rumen content	73.3	83.4	82.1	84.9	17.6
Duodenal flow	404.6	379.9	403.3	384.1	21.7
Ileal flow	97.1	115.8	97.6	90.0	13.4
Rumen pool size					
Dry matter	9.0	8.8	8.6	9.0	1.2
NDF	6.0	5.7	5.7	5.8	0.7
Starch	0.3	0.4	0.3	0.4	0.2
Rumen outflow rate					
Dry matter	7.2	7.0	7.6	7.0	0.8
NDF	2.0	2.1	2.6	2.2	0.4
Starch	8.0	9.7	10.6	8.3	2.5

5.2.5 Composition of rumen microbes

Chemical composition and AA profile (% individual AAN of total AAN) of the rumen bacteria and protozoa is presented in Table 5.8. No significant differences were detected among treatments for the content of AAN, neither for protozoa nor bacteria. The proportion of total EAA and NEAA in protozoa did not vary among treatments. For both bacteria and protozoa, there were only significant variations found for two AA, both essential, among treatments. For SPR, there was a significantly higher proportion of isoleucine in rumen bacteria and protozoa found than for PR and R. In protozoa the proportion of lysine was significantly higher for R than for the other treatments. The highest and lowest proportions of single AA tended to alter almost equally among S, PR and R. In general the proportion of isoleucine and lysine was higher in protozoa than in bacteria, while opposite range was observed for arginine and histidine. Of the sulphur containing AA, the proportion of methionine was equal, and of cysteine slightly lower in protozoa than in bacteria. For both bacteria and protozoa, the highest presence of single AA was observed for arginine and glutamic acid, and the lowest presence was observed for tyrosine and the sulphur containing cysteine and methionine.

Table 5.8 LS means of chemical composition (g kg^{-1} DM) and amino acid profile (LS means of AAN % of total AAN) of rumen bacteria and protozoa (VFP) where treatment S = 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% Aco Feed Gigant, PR = 10% full fat rapeseed and 27% peas, R = 10% full fat rapeseed, 19.9% barley and 6.9% oats, SPR = a blend of concentrate mixture S and PR in a ratio of 50:50

	Bacteria					VFP				
	Treatment					Treatment				
	S	PR	R	SPR	SEM	S	PR	R	SPR	SEM
Chemical composition										
Nitrogen	68.4	70.0	66.8	69.1	2.4	59.4	63.8	62.3	62.8	2.9
Fat	139.0	121.1	132.5	130.3	7.3	61.5	66.0	55.8	55.3	7.9
Starch	64.3	57.8	62.3	76.3	14.6	368.4	319.0	342.3	332.0	27.0
DAPA-N	0.24	0.24	0.24	0.23	0.03	0.08	0.08	0.07	0.08	0.01
AAN (g 100 g N^{-1})	46.8	48.0	46.6	46.2	1.8	45.4	46.2	49.5	48.0	2.6
EAA										
EAA sum	52.7	53.2	52.6	51.0	2.0	56.4	56.2	56.6	56.2	0.2
Arginine	11.9	12.0	12.0	10.6	1.4	11.7	11.5	11.5	11.5	0.2
Histidine	3.6	3.8	3.6	3.3	0.3	3.7	3.8	3.9	3.7	0.1
Isoleucine	4.7^{b}	4.8^{ab}	4.8^{b}	5.1^{a}	0.2	5.5^{ab}	5.5^{bc}	5.5^{c}	5.6^{a}	0.0
Leucine	6.2	6.2	6.2	6.4	0.3	6.1	6.1	6.1	6.1	0.1
Lysine	10.9	10.9	10.8	9.7	1.5	15.3^{b}	15.4^{b}	15.7^{a}	15.4^{b}	0.1
Methionine	1.8	1.8	1.8	1.9	0.1	1.7	1.7	1.7	1.7	0.1
Phenylalanine	3.1	3.4	3.1	3.2	0.4	3.3	3.3	3.3	3.3	0.1
Threonine	4.8	4.7	4.8	4.9	0.2	4.3^{a}	4.3^{a}	4.1^{b}	4.3^{a}	0.1
Valine	5.8	5.8	5.7	6.0	0.2	4.7	4.8	4.8	4.7	0.1
NEAA										
NEAA sum	47.3	46.9	47.4	49.1	2.0	43.6	43.9	43.5	43.8	0.2
Alanine	8.7	8.7	8.7	8.8	0.5	5.5	5.5	5.3	5.5	0.1
Aspartic acid	9.3	9.3	9.2	9.6	0.3	9.9	9.9	9.9	9.9	0.1
Cysteine	1.3^{b}	1.3^{b}	1.3^{b}	1.4^{a}	0.1	1.6	1.6	1.6	1.6	0.01
DAPA	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Glutamic acid	9.5	9.3	9.4	9.9	0.4	10.0	10.0	10.1	10.1	0.1
Glycine	8.0	8.1	8.1	8.3	0.4	6.4	6.4	6.3	6.4	0.1
Proline	3.3	3.0	3.3	3.5	0.4	3.0	3.3	3.2	3.2	0.3
Serine	4.6	4.5	4.5	4.6	0.2	4.2	4.2	4.2	4.2	0.2
Tyrosine	2.8	2.9	2.9	3.1	0.2	2.9	3.0	3.0	2.9	0.1

[a, b, c] Different letters among treatments indicates statistical differences ($P<0.05$).

5.2.6 Digestibility of DM, OM, fat and carbohydrates

Ruminal and intestinal digestibility of DM, OM and fat are summarized in Table 5.9. Intake of DM and OM did not differ among treatments. The fat intake was, however, observed to be significantly higher for S than for PR and R. The ruminal digestibility of DM, OM, and fat did not differ among treatments. The negative ruminal digestibility of fat indicated a microbial net synthesis and endogenous addition of fat, which was nominally highest for R. Treatments did neither cause any significant differences on the digestion of DM and OM in rumen, nor in total gastrointestinal tract. There was, however, a tendency of higher ruminal DM digestibility for PR and SPR than for S and R. The small intestine digestibility was not analysed statistically, but nominally PR showed a lower digestibility of DM and OM than the other treatments. The small intestine digestibility of duodenal flow of DM ranged from 47.1 to 59.1%, and total tract digestibility of intake of DM ranged from 65.7 to 67.9%. The small intestine digestibility of fat averaged 71.9% of duodenal flow, and for total gastrointestinal tract digestibility 63.7% of intake.

Table 5.9 LS means of intake (kg d^{-1}) and digestion of dry matter, organic matter and fat in the rumen (% of intake)[1], small intestine (% of duodenal flow)[2], and total gastrointestinal tract (% of intake) S = 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% Aco Feed Gigant, PR = 10% full fat rapeseed and 27% peas, R = 10% full fat rapeseed, 19.9% barley and 6.9% oats, SPR = a blend of concentrate mixture S and PR in a ratio of 50:50

	Treatment				
	S	PR	R	SPR	SEM
Intake					
Dry matter	21.4	21.0	21.6	21.3	0.9
Organic matter	20.0	19.8	20.3	20.0	0.9
Fat	1.3[a]	1.2[b]	1.2[b]	1.3[ab]	0.04
Ruminal digestion[1]					
Dry matter	19.4 (20.1)	23.8 (23,4)	17.9 (9.4)	24.0 (23.1)	6.3
Organic matter	31.1 (31.7)	34.6 (35.0)	28.2 (20.2)	34.4 (34.7)	6.5
Fat	-25.7 (-19.6)	-24.3 (-21.4)	-34.2 (-45.5)	-20.9 (-21.4)	9.5
Small intestine[2] (ileal) digestion					
Dry matter	53.2	47.1	59.1	52.3	-
Organic matter	50.7	37.8	58.2	49.9	-
Fat	72.9	72.8	85.3	66.1	-
Total tract[3] digestion					
Dry matter	68.7	68.8	68.6	67.6	3.7
Organic matter	70.2	68.5	70.5	69.1	3.6
Fat	65.7	68.8	60.3	59.9	4.8

[a, b] Different letters among treatments indicates statistical differences ($P<0.05$).
[1] Data within parenthesis consists of mean values from the two animals with ileal fistulas.
[2] Data consists of mean values from the two animals with ileal fistulas.
[3] Data consists of values from analysis of feaces and urine mixed in one fraction.

Intake, ruminal digestibility, digestibility in small intestine and total gastrointestinal tract of the carbohydrate fractions NDF and starch are presented in Table 5.10. Among treatments, neither for intake nor for digestion parameters, there were no significant differences. The intake of NDF and starch averaged 7.8 and 4.8 kg d^{-1}, respectively. Even when there were no significant differences of ruminal digestibility of NDF there was a trend of higher digestibility for S and PR than for R ($P<0.10$ and $P<0.07$, respectively). The small variations observed for total digestibility of NDF implies a compensatory fermentation of fibre in the large intestine. Although, the ruminal starch digestibility showed small variations among treatments, the intestinal digestibility in R was nominally higher than in PR. Because of the modest amount of data at hand, the small intestine digestibility was not analysed statistically but showed a tendency of higher, respectively

lower, digestibility of starch for R and SPR than the other treatments. The total gastrointestinal tract digestibility of starch was nearly 100% for all treatments with a very small variation.

Table 5.10 LS means of intake (kg d^{-1}) and digestion of carbohydrates in the rumen (% of intake)[1], small intestine (% of duodenal flow)[2], and total gastrointestinal tract (% of intake) where S = 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% Aco Feed Gigant, PR = 10% full fat rapeseed and 27% peas, R = 10% full fat rapeseed, 19.9% barley and 6.9% oats, SPR = a blend of concentrate mixture S and PR in a ratio of 50:50

	Treatment				
	S	PR	R	SPR	SEM
Intake					
NDF	7.8	7.5	8.0	7.7	0.5
Starch	4.6	4.7	5.0	4.7	0.2
Ruminal digestion[1]					
NDF	60.0 (60.7)	61.0 (63.4)	51.0 (43.8)	61.4 (62.1)	6.5
Starch	84.8 (86.4)	84.0 (84.6)	82.0 (77.4)	83.3 (84.3)	3.4
Small intestine[2] (ileal) digestion					
NDF	-30.2	-77.5	-14.8	-38.0	-
Starch	72.9	72.8	85.3	66.0	-
Total tract[3] digestion					
NDF	59.7	57.3	57.0	56.0	5.7
Starch	98.5	98.4	98.7	98.5	0.2

[1] Data within parenthesis consists of mean values from the two animals with ileal fistulas.
[2] Data consists of mean values from the two animals with ileal fistulas.
[3] Data consists of values from analysis of feaces and urine mixed in one fraction.

5.2.7 Digestibility of N, bacterial N synthesis, and flow of N and AA to the duodenum

Intake, duodenal flow, and small intestinal digestibility of N are presented in Table 5.11. Among treatments, there were no significant differences of N intake. The nominally highest level of N intake was, however, recorded for S and the lowest for R, of 554 and 512, respectively. In general, S showed the highest and R the lowest duodenal flow for all N fractions, with the exception of the flow of AAN, where PR and SPR showed a significantly higher flow than R. Duodenal flow of bacterial N estimated from NAB was in general substantially lower than estimated from DAPA. On the other hand, the amount of RUP was higher estimated from NAB compared to DAPA.

Table 5.11 LS means of nitrogen intake (g d^{-1}), flow to the small intestine (g d^{-1}), and small intestine digestibility of non ammonia N (NAN), bacterial N, N, rumen undegraded protein N (RUP N) (g d^{-1}), rumen escape of N measured *in situ* (%) for concentrates, and bacterial N synthesis where S = 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% Aco Feed Gigant, PR = 10% full fat rapeseed and 27% peas, R = 10% full fat rapeseed, 19.9% barley and 6.9% oats, SPR = a blend of concentrate mixture S and PR in a ratio of 50:50

	Treatment				
	S	PR	R	SPR	SEM
N intake	554	538	512	548	23
Flow to the duodenum					
Total, N	733	706	683	708	42
NAN	678	647	620	649	34
Bacterial N (NAB)	305	291	290	284	35
Bacterial N (DAPA)	482	457	414	512	61
Amino acid N	459[ab]	465[a]	446[b]	465[a]	6
RUP N (NAB)	373	356	330	366	25
RUP N (DAPA)	197	189	206	142	59
In situ rumen escape N	45	38	42	42	-
Small intestine (ileal) digestion					
NAN[1]	97	96	97	96	-
Amino acid N[1]	96	109	89	102	-

[a, b] Different letters among treatments indicates statistical differences ($P<0.05$).
[1] Data consists of mean values from the two animals with ileal fistulas.

AA profile (% individual AAN of total AAN) of the AAN flow to the duodenum is summarized in Table 5.12. Among treatments, there were no significant differences of the proportion of EAA and NEAA of the AAN reaching the duodenum. However, there was a nominally higher proportion of EAA for S than for R, of 49.4 and 47.9, respectively. Only a few significant differences on proportion AAN from single AA were observed. The proportion of histidine significantly decreased when SPR was substituted by R. For threonine and alanine, S showed a higher proportion than PR and for alanine there was a higher proportion observed for S than for PR and R. The highest proportion of AAN was represented of glycine and arginine, and the lowest proportion of methionine and cysteine for all treatments, averaging 14.7, 10.8, 1.2 and 1.5% of total AAN, respectively.

Table 5.12 Amino acid profile of individual amino acids N (LS means AAN% of AAN) in digesta to the duodenum where S = 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% Aco Feed Gigant, PR = 10% full fat rapeseed and 27% peas, R = 10% full fat rapeseed, 19.9% barley and 6.9% oats, SPR = a blend of concentrate mixture S and PR in a ratio of 50:50

	Treatment				
	S	PR	R	SPR	SEM
EAA					
Sum EAA	49.4	48.2	47.9	48.4	1.0
Arginine	11.1	10.5	10.8	10.8	0.4
Histidine	4.2[ab]	4.2[ab]	4.1[b]	4.3[a]	0.06
Isoleucine	4.2	4.3	4.2	4.1	0.2
Leucine	6.1	6.0	5.9	5.9	0.3
Lysine	9.6	9.4	9.3	9.5	0.1
Methionine	1.1	1.2	1.2	1.2	0.04
Phenylalanine	3.0	2.9	2.9	2.9	0.1
Threonine	4.4[a]	4.2[b]	4.3[ab]	4.4[ab]	0.1
Valine	5.8	5.7	5.3	5.6	0.4
NEAA					
Sum NEAA	50.6	51.8	52.1	51.6	1.0
Alanine	7.3[a]	7.1[b]	7.0[b]	7.1[ab]	0.06
Aspartic acid	8.3	7.9	8.0	8.1	0.2
Cysteine	1.5	1.4	1.4	1.5	0.1
Glutamic acid	9.5	9.1	9.4	9.3	0.2
Glycine	12.8	15.6	15.5	14.7	0.2
Proline	3.9	3.8	3.7	3.8	0.1
Serine	4.9	4.6	4.7	4.8	0.2
Tyrosine	2.4	2.4	2.5	2.5	0.2

[a, b] Different letters among treatments indicates statistical differences ($P<0.05$).

5.2.8 Milk production and milk composition

Milk production, milk composition and concentration of milk and blood urea are presented in Table 5.13. It should be mentioned that occurrences of clinical mastitis were observed several times during the trial. There was a significantly higher production of energy corrected milk (ECM) for S and SPR than for PR and R. There was also a non-significant tendency of increased milk production when SPR was substituted by S ($P<0.07$). The production of milk protein and lactose was affected by treatment. Furthermore, there was a large variation within production levels as well as concentrations

of milk protein and lactose. Both protein production and milk protein concentration were significantly higher for S and SPR than for PR and R. Lactose production and lactose concentration were highest for SPR and lowest for R. Of the lactose content there was, except for the significant differences, a tendency of lower lactose concentration for PR than S (*P<0.06*).The milk urea concentration significantly increased when SPR was substituted by PR, and there was also a tendency of increased concentration for PR compared to R (*P<0.06*). On the other hand, the urea concentration in blood showed significantly higher concentrations for SPR than for R, and a tendency of higher concentration for R than for S and PR.

Table 5.13 LS means of milk production (g d^{-1}), milk composition (%), and concentration of urea in milk and blood (mmol l^{-1}) where S = 12% soybean meal, 15.6% barley, 5.4% oats and 3.8% Aco Feed Gigant, PR = 10% full fat rapeseed and 27% peas, R = 10% full fat rapeseed, 19.9% barley and 6.9% oats, SPR = a blend of concentrate mixture S and PR in a ratio of 50:50

	Treatment				
	S	PR	R	SPR	SEM
Production					
Milk, (kg d^{-1})	32.9	32.4	31.7	34.8	2.3
ECM[1], (kg d^{-1})	33.7[a]	29.7[b]	29.9[b]	33.0[a]	1.4
Fat	1311.2	1154.9	1208.0	1287.5	79.0
Protein	1118.4[a]	969.7[b]	941.7[b]	1075.8[a]	38.0
Lactose	1682.0[a]	1505.0[b]	1454.9[b]	1675.5[a]	68.4
Composition					
Fat	3.8	3.6	3.8	3.7	0.2
Protein	3.2[a]	3.0[b]	3.0[b]	3.1[a]	0.1
Lactose	4.8[ab]	4.6[bc]	4.6[c]	4.8[a]	0.1
Concentration					
Milk urea	4.3[ab]	5.0[a]	4.2[ab]	4.0[b]	0.4
Blood urea	4.2[ab]	4.1[ab]	3.4[b]	4.4[a]	0.4

[a, b, c] Different letters among treatments indicates statistical differences (*P<0.05*).
[1] According to Spörndly (1999).

5.3 Discussion

5.3.1 Chemical composition and *in situ* digestibility

The experimental concentrates were composed such to achieve equivalent content of fat and, except for R, even equivalent content of N. Thus, no differences in chemical composition of the concentrate mixtures were expected (Table 5.1). There are no known explanations for the higher fat and N content in SPR than in the other concentrate mixtures (Table 5.3).

All concentrate mixtures in the trial were extruded at 103-107º C. According to Ljøkjel *et al.* (2003b), starch in peas may be gelatinized to a less extent when heated under the same moisture condition as cereals. Heat treatment may also increase the solubility of fibre (Vranjes and Wenk, 1995; Shinnick *et al*., 1998), causing NDF to decrease. On the other hand, excessive heat treatment may cause lignin to decrease the solubility of fibre (Van Soest & Mason, 1991), causing NDF to increase. Thus, the effect of the expander treatment on the fibre and starch content may be variable within analysis.

The observed lower content of AAN in R was due to the planned lower content of N. The variation within AA-profile among experimental concentrates may be explained by the proportions of AA within the included feed raw materials. Lysin constituted a larger proportion of AAN when peas were included, which agree with Bastianelli *et al.* (1995) and Lallès (1992). Even the lower proportions of sulphur containing AA, when peas were included in the concentrate mixtures, are in agreement with other sources. The highest proportions of sulphur containing AA were presented when only rapeseed was included. This could be explained by the higher content of cereals for this concentrate mixture in comparison when peas were included. The proportions of sulphur containing AA are higher in cereals than in peas (Boisen *et* al., 2000). The increased proportion of total EAA in the concentrate mixtures, when increasing the content of peas, agrees with the statement by Lallès (1992), that pea protein has a higher biological value than protein in soybeans.

The increased *in situ* ruminal degradability of N for pea concentrates was also observed by Ljøkjel *et al*. (2003a). *In situ* ruminal degradability of NDF was observed to increase when peas were added to rapeseed which is in agreement to data presented by Ljøkjel *et al.* (2003b). When same amount of rapeseed was included in both PR and R, the decreased degradability of NDF for R must be explained by the fact that the NDF fraction in peas is more degradable than NDF in cereal grains.

5.3.2 Feed intake

The concentrates were all well consumed and the small variation of feed DM intake among diets in present trial agrees with Chen *et al.* (2003) when heifers were fed raw peas, and with Reed *et al,* (2004a) when rolled peas were fed to growing steers. According to Syrjälä-Qvist *et al*. (1981), the DM intake was slightly higher for dairy cows fed raw peas than soybean meal. Data from present trial indicates a similar ruminal disappearance in DM among treatments, which corresponds to a similar feed intake.

5.3.3 Ruminal fermentation

The ability to resist microbial degradation differs among raw materials (Herrera-Saldana *et al.,* 1990; Nocek and Tamminga, 1991). In general starch in peas is more resistant than starch in cereals (Ljøkjel *et al.,* 2003b). Normally, heat treatment results in gelatinizing of starch, rendering starch more accessible to microbial breakdown (Van Soest, 1994). According to Ljøkjel *et al*. (2003b), heat treatment, except for high temperatures, caused a less gelatinizing for starch in peas than for starch in cereals.

VFA are a major end product of ruminal fermentation (Reed *et al*., 2004b). The small variation in ruminal starch degradability among treatments could explain similar concentrations of total VFA. The numerical low concentration of total VFA for R could be explained by the low ruminal degradable protein for this treatment rather than the amount of degradability of fermentable carbohydrates. The similarity in proportions of single VFA, except for butyrate, could be explained by the similarity in chemical composition and ruminal degradability after extrusion among treatments. The small variation of pH is a consequence due to the similarity of VFA concentrations among treatments. The lack of

variation in pH among treatments, furthermore, corresponds to the small variation within the ruminal digestibility of starch.

Ruminal NH_3 concentration was nominally highest when peas were included in the diet (Table 5.6). The ruminal NH_3 concentration has a high correlation to the ruminal degradability of N, which is further described by Chapoutot and Sauvant (1996). The increased NH_3 concentration for pea diets could be explained by the high correlation between the protein solubility and degradability which are higher for peas than for soybeans (Bastianelli *et al*., 1995) and for rapeseed (Ljökjel *et al.,* 2003). In present trial, even after extrusion, there was an increased *in situ* ruminal degradability of N for pea containing diets. Similar data is reported by Reed *et al*. (2004a) who observed a cubically increased NH_3 concentration when rolled corn was substituted by rolled peas for steers.

5.3.4 Influence of treatment on rumen pool size and outflow rates

Feeding peas did not change the weight of evacuated rumen content and DM pool size (Table 5.7). These similarities are probably resulting from the fact that treatments with soybeans and peas, combined with full-fat rapeseed, did not alter ruminal pH, and the rumen cellolytic activity was equal. According to de Visser *et al.* (1998), the animal can probably handle a decreased disappearance of NDF without reducing feed intake as long as maximum rumen pool size of NDF is not reached. Furthermore, Mertens (1994) proposes a limitation of rumen pool size of NDF of about 11 to 13 g NDF kg^{-1} body weight. In the present trial, the rumen pool size of NDF corresponded to 13 g kg^{-1} body weight, and thus the feed intake may be limited by the ruminal pool size of NDF.

5.3.5 Composition of rumen microbes

Composition of the microbial biomass in the duodenal digesta is determined by the respective proportions of LAB, SAB, and protozoa which leave the rumen (Ushida *et al*., 1991; Lallès *et al.,* 1992). Concentrations of N and AAN differ between microbial groups. This implies that changes in microbial populations could alter the relation between N and AAN leaving the rumen. Except for S, ruminal pH sometimes was reduced below 6.0 (Table 5.6, Figure 5.1). At rumen pH below 6.0, cellulolytic bacteria (in which SAB

probably is a main part) and the protozoa usually decrease in number (Hoover, 1986). In contrast, the amolytic bacteria probably increase in number. However, when peas did not alter rumen pH and VFA-pattern to a larger extent, there were probably no large changes within microbial population among treatments.

In present trial, treatments did neither seem to affect the microbial flora in the way to change chemical composition nor AA-profile in the rumen bacteria and protozoa. The similarity of starch degradability among diets could be a regulating factor since the N degradability rate in the pea diet was higher than for the other treatments, indicated by the increased NH_3 in rumen liquor and blood.

5.3.6 Digestibility of DM, OM, fat and carbohydrates

Low ruminal pH reduces the ability for cellulolytic bacteria to degrade fibre (Hoover, 1986). Several authors report that increased amounts of rapidly degraded carbohydrates have reduced ruminal degradability of fibre (Ørskov, 1986). A nominal reduction in NDF degradability was observed for R. When the content of starch was similar (Table 5.3) as well as the ruminal starch digestibility (Table 5.10) and the pH (Table 5.6), low pH from a fast fermentation rate of starch may not be the explanation for the decreased NDF degradability. Instead the content of an unsaturated fat source from the full-fat rapeseed might be the explanation, which is further described by Jenkins (1993). The addition of peas to full-fat rapeseed tended to increase the ruminal digestibility of NDF. The explanation for this must be the positive effect on microbial growth and fermentation from the increased support of AA and peptide N sources (Dewhurst *et al.*, 2000). The similarities in total tract NDF digestion indicate a compensatory fermentation of NDF in the large intestine. Therefore, the decreased ruminal fermentation in the rumen for the full-fat diets is probably not affected by a changed potential ruminal degradable NDF fraction. This is also an evidence that the content of unsaturated fat in the rumen might have affected the digestibility of NDF. The total tract digestion of starch was high and nearly 100% for all diets. This may indicate that extrusion of peas at 103-107º C may have decreased the variations within total tract digestibility of starch between peas and cereals.

5.3.7 Digestibility of N, bacterial N synthesis, and flow of N and amino acids to the duodenum

Microbial N, dietary N escaping ruminal degradation and endogenous N constitute the AA entering the small intestine. According to Stokes *et al.* (1991), the amount of endogenous N is correlated to DM intake or flow of OM to the duodenum. No differences in DM intake or flow of OM to the duodenum were observed among treatments. The flow of total N was however higher than the intake of dietary N for all treatments (Table 5.7). This should be caused by an endogenous support of dietary N to the rumen microbes. The flow of all measured N fractions was lower for R than for the other treatments, although only significant for flow of AAN. This indicates that the endogenous addition of N did not fully compensate the nominally lower intake of dietary N which was the fact for this treatment. Among the other treatments, there were no differences neither of intake of dietary N nor of OM flow. Rumen available energy and N are the nutritional factors that most often limit microbial growth (Hoover and Stokes, 1991). However, several other factors are important and within the rumen complex interactions exist, and the efficiency of microbial growth in the rumen may vary greatly (Titgemeyer, 1997). The efficiency may also vary to a large extent depending on marker used for estimation. Protozoa does not contain DAPA, except for engulfed bacteria (Rahnema and Theurer, 1986). Thus, DAPA could be used as a marker for rumen microbes (Robinson *et al*., 1996).

In present trial DAPA, as a marker, was compared to NAB. There was a large difference in the proportion of Bacterial N and RUP depending on marker. In average for all treatments, the proportion of bacterial N of NAN was about 45% and 72% when estimated from NAB and DAPA, respectively. These results differ from data found by Lund (1997), who did not find any differences in N synthesis using DAPA or RNA. According to Volden (1999), when feeding diets consisting of barley and oats together with grass silage, the proportion of bacterial N of total NAN flowing to the duodenum was 62% and 75% estimated from DAPA and purines, respectively. Thus, the variation between DAPA and NAB seems to be larger than for DAPA in comparison with purines. According to Dewhurst *et al.* (2000) there can be a change in the proportion in the nucleic acids N ratio in microbes after isolation. This fact can be an explanation for the difference between the use of NAB and DAPA for estimation of bacterial N and RUP N (Table 5.11). Compared with data of N supply, presented by Reed *et al*. (2004b), from steers fed raw peas and *in*

situ ruminal degradability of extruded pea and full-fat rapeseed mixtures by Ljøkjel *et al*. (2003a), DAPA should in this case be the more adequate marker for estimation of the bacterial N:RUP N ratio in the duodenal flow.

It has been shown that depending on form of dietary N, large differences in microbial growth rate and, consequently, cell yield can occur. The ruminant animal is unique in its ability to survive on a diet consisting entirely of non-protein N. However, the efficiency of microbial growth is enhanced by both the addition of AA and peptides to their growth medium (Dewhurst *et al*., 2000). This fact may be the explanation for the increased flow of AAN to the duodenum when peas replaced grain in the full-fat rapeseed treatment. In this case, the fat source in the pea rapeseed treatment did not affect the flow of AAN to the duodenum negatively compared to the treatment with soybean meal, cereals and a saturated fat mix. As discussed, the higher concentrations of NH_3 indicate an increased degradability rate of N in the rumen, for PR compared to R, which may imply an increased supply of N substrate for microbial protein synthesis. Furthermore, microbial N greatly affects AA profile of duodenal digesta (Hvelplund and Madsen, 1989). Nevertheless, since the AA profile of dietary N and microbial N differed (Table 5.4 and 4.6), treatments were expected to change the AA profile of duodenal digesta. This was however not the case. PR seem to be similar in comparison with S in the support of essential AA.

At some limit, a low rumen pH reduces cellulolytic activity in the rumen (Russel and Wilson, 1996). Low rumen pH itself negatively affects the efficiency of synthesis (Hoover and Stokes, 1991). When both pH and bacterial N synthesis were similar among treatment, pH did not seem to affect rumen microbial synthesis when feeding extruded peas and rapeseed in comparison to soybean meal, grain and a saturated fat mix (Table 5.6 and Figure 5.1). Synchronized rumen release of energy and N is considered to increase efficiency in microbial synthesis (Herrera-Saldana *et al*., 1990). Furthermore, the lack of variations of bacterial N synthesis indicates that rumen release of energy and N is comparable for extruded peas and rapeseed and soybean meal and grain. According to Clark *et al*. (1992), the main contributor to NAN flow to the duodenum originates from bacterial N. Furthermore, in a summary from 152 dietary treatments, microbes supplied an average of 59% of NAN. In present study, NAN contributed of about 72% (estimated from DAPA N) of bacterial N which tends to be a high value. The ratio of bacterial N

showed small variations among treatments but was nominally highest for PR. And thus, proportionally, ruminal synthesis of bacterial N was not largely affected by peas in relation to soybean meal. It should, however, be mentioned that the treatment with soybean meal represented the largest flow of total N to the duodenum. This fact, together with the small variations in digestibility of NAN as well as the AA-profile in the AAN reaching the duodenum among treatments, indicates that the standard treatment with soybean meal represented the greatest contribution of AA to the animal. However, the data concerning small intestine digestibility varied largely for AAN and was for treatment RP and SPR more than 100 % which is hardly believable. The data concerning small intestine digestibility should therefore be considered as an indication of a general high digestibility rather than definite facts. There are no known explanations for the variations in the small intestine digestibility values among treatments. Dewhurst *et al.* (2000) describes some problems to achieve representative samples from intestinal t-cannulas. However, if this had been the case, it should also have appeared even for the other nutrients.

5.3.8 Milk production and milk composition

Although the major objective of the trial was not to study milk production, it is interesting to observe that milk yield counted as ECM was decreased for both PR and R. Since the yield seemed to be at a maximum for S and SPR, the optimal diet may consist of soybean meal and cereals or soybean meal and cereals in combination with peas and full-fat rapeseed.

It has been shown that increased amount of readily fermented carbohydrates in the diet reduces milk fat concentration (Sutton, 1998). In present trial, the lack of variation of milk fat concentration corresponds to the small variation of starch intake and ruminal degradability among treatments. The statement that increased production of butyrate may increase the milk fat concentration (Thomas and Martin, 1988) was not observed in present trial. The increased butyrate production for full-fat rapeseed neither resulted in increased milk fat concentration nor total milk fat production. Furthermore, this increase of butyrate was rather proportional than quantitative. The milk production and milk protein content were not increased when peas replaced grain in the rapeseed diet. The flow

of AAN to the duodenum could not explain the differences in milk protein concentration, when the flow was similar among S, PR and SPR but the milk protein concentration in similarity to R also was lower for PR. On the other hand, the lower milk protein concentration might indicate a decreased digestibility of AAN and uptake of AA in the small intestine for PR. A higher absorption of AA in the intestine may therefore be an explanation of the positive response in milk protein content for S and SPR.

There are several factors that could have influenced the milk production and milk composition. The number of animals in present trial was only four and, therefore, health disturbances could have a relatively large effect on production level and milk composition. There were in present trial several observations made on clinical mastitis as well as variations within numbers of cells (not presented). However, the recordings of these parameters were too insufficient to be included in the study. Another parameter that could affect the production level was the fact that for one animal the treatment R was moved from period one to an extra period after the main trial. This could have influenced the milk yield negatively for this treatment, when the milk recording for this treatment was done at a later average lactation stage than for the other treatments.

6. Conclusions

Peas contain relatively high levels of protein and energy which make them useful within feeding of dairy cows. The large fraction of rumen degradable protein and the more limited fraction of rumen degradable starch may be a limiting factor for high lactating animals because of an insufficiently synchronized release of feed nutrients for microbial synthesis. Peas contain different types and amounts of ANF, but these do not tend to be a limiting factor within feeding for grown cattle such as dairy cows.

In the *in vivo* trial, treatments with extruded concentrates with the exception of cereals, containing large proportions of peas in combination with full-fat rapeseed, changed neither feed intake, digestibility parameters, nor flow of nutrients to the small intestine compared to soybean meal in combination with grain and a saturated fat mix or full-fat rapeseed in combination with grain. Peas did not affect the ruminal fermentation pattern or the rumen pH to a large extent. For the treatments which included rapeseed, the flow of total N was not significantly affected when cereals were substituted by peas. On the other hand, there was a significantly higher flow of AAN when peas were included. Furthermore, peas in combination with full-fat rapeseed neither changed the AA-profile in rumen microbes nor in duodenal AAN flow compared to full-fat rapeseed in combination with cereals or soybean meal in combination with cereals and a saturated fat mix.

The decreased milk production and milk protein content for the treatment with full-fat rapeseed in combination with cereals was increased by the substitution of grain for peas. This effect could have been due to an increased flow of AAN to the duodenum, because of the increased supply of dietary AA and peptide-N by the addition of peas. With a focus on ECM production, the optimal concentrate seems to be a mixture of soybean meal and cereals or a mixture of peas and full-fat rapeseed in combination with soybean meal and cereals.

7. Sammanfattning

En stundande överproduktion av spannmål och en framtida osäker tillgång av icke genmodifierade vegetabiliska proteinfodermedel har medfört ett intresse för en utökning av den inhemska produktionen av proteinfodermedel i Norge. Förutom rybs (*Brassica campestris*) anses ärter *(Pisum sativum)* vara den mest aktuella grödan för detta ändamål. Ärter karaktäriseras av ett relativt högt innehåll av råprotein, varierande mellan 20-26%, ett högt innehåll av stärkelse, 42-51%, och ett lågt innehåll av fett. Generellt är energivärdet i ärter högre än i korn, men lägre än i rybsfrö och i sojamjöl.

Protein i ärter utgörs till 85-100% av albuminer och globuliner vilket medför att en stor del av proteinet är lösligt och nedbrytbart i våmmen. Stärkelsen i ärter är däremot i större utsträckning beständig mot våmnedbrytning än stärkelse i andra stärkelserika fodermedel. Aminosyrasaprofilen i ärter karaktäriseras av en hög andel lysin medan andelen av de svavelhatiga aminosyrorna cystin och metionin är låg. För att minska våmnedbrytningen av foderprotein hos idisslare, och på så sätt öka det totala flödet av aminosyror till tunntarmen, används ett flertal olika processmetoder. Dessa innefattar ofta någon form av värmebehandling vilken åstadkommer så kallade Malliard-reaktioner. Expandering är ett exempel på en värmebehandling vilken tidigare har visats sänka våmsmältbarheten hos protein i kraftfoderblandningar innehållande ärter.

I Norge finns inga tidigare försök genomförda med ärter som fodermedel till mjölkkor. För att öka kunskaperna om påverkan av större mängder ärter inom utfodring till mjölkkor har ett *in vivo*-försök genomförts som en del av projektet "Alternative proteinrike kraftfôrråvare" vid Institutt før Husdyr og Akvakulturvitenskap vid Universitetet før Miljø och Biovitenskap i samarbete med Planteforsk, Graminor AS och Matforsk. I försöket, vilket var upplagt som en romersk kvadrat, användes 4 våm- och tarmfistulerade mjölkkor av rasen Norsk Rødt Fe (NRF). Extruderade och pelleterade kraftfoder, vilka förutom en basblandning, innehöll: 1) 12% sojamjöl, 15,6% havre, 5,4% korn och 3,8% Ca-bunden fettmix (Aco Feed Gigant) (S), 2) 10% rybs (helfrö) och 27% ärter (PR), 3) 10% rybs, 19,9% havre och 6,9% korn (R) samt 4) en blandning av S och PR i förhållandet 50:50 (SPR), jämfördes.

Tillsats av ärter på bekostnad av spannmål hade inga effekter på foderintag. Likaså påvisades i försöket endast små variationer i våm-pH och fermentationsprodukter i våmvätska mellan behandlingarna. Ammoniakkoncentrationen i mjölk, som vanligtvis används som en indikator för våmnedbrytbarheten av foderprotein, var däremot högre för PR. Emellertid kunde inga skillnader gällande ureakoncentration i blodet påvisas mellan PR och de andra behandlingarna. Trots att aminosyraprofilen varierade mellan proteinet i de olika kraftfoderblandningarna, påverkade detta inte nämnvärt aminosyraprofilen hos varken proteinet i våmmikrober eller hos det protein som passerade till tunntarmen. Passagen av enskilda näringsämnen till tunntarm samt nedbrytningskoefficienter i våm och tunntarm var likartade för PR och S. Emellertid ökade passagen av total N-fraktion till duodenum nominellt för S. Tillsats av ärter på bekostnad av spannmål, som i fallet med PR jämfört med R, motverkade den nominellt negativa effekt som R hade på nedbrytning av torrsubstans och NDF i våmmen. Produktionsresultaten får anses vara relativt osäkra på grund av frekventa observationer av klinisk mastit under försöket. Med befintliga data visades i alla fall en sänkning av dygnsproduktionen av ECM för R, innehållande rybsfrö och spannmål. Tillsättning av ärter på bekostnad av spannmål, som i fallet med PR jämfört med R, medförde ingen höjning av ECM eller av mjölkproteinhalten till den nivå som uppnåddes för S. Optimalt ur avkastningssynpunkt verkar vara att även inkludera en viss mängd sojamjöl vid användning av ärter i kombination med rybsfrö. Emellertid måste konstateras att soja i kombination med spannmål, utan inblandning av ärter och rybs, medförde bäst produktionsresultat i försöket.

8. References

AOAC, 1990. *Official Methods of Analysis (15th edition).* Association of Official Analytical Chemists, Washington, D.C., USA

Bastianelli, D., Carrouée, B., Grosjean, F., Peyronnet, C., Revol, N., Weiss, P., 1995. *Peas Utilisation in Animal Feeding.* In: Wiseman, J. (editor), Faculty of Agricultural and Food Sciences, Nottingham, UK. ISBN 2.9508706.1.9.

Boisen, S., Hvelplund, T., Weisbjerg, M.R., 2000. *Ideal amino acid profiles as a basis for feed protein evaluation.* Livestock Production Science 64:239-251.

Chapoutot, P., Sauvant, D., 1996. *Nutritive value of raw and extruded pea-rapeseed blends for ruminants.* Anim. Feed Sci. Techn. 65:59-77.

Chen, J.-Q., Okine, E.K., Price, M.A., Khorasani, G.R., 2003. *Feeding value of peas for backgrounding beef heifers.* Can. J. Anim. Sci. 83:779-786.

Christiansen, K.E., Knudsen, B., Jacobsen, I., Eggum, B.O., 1985. *Næringsværdien i forskellige ærtesorte.* Statens Husdyrbrugsforsøg Meddelelse 587, Copenhagen, Denmark.

Christiansen, M.P., Larsen, T. 1987. *Nutritive Value of Different Pea Varietes with Emphasis on Protein Quality*. Wissenschaftliche Zeitschrift der Wilhelm-Pieck-Universität, Rostock, DDR. ISSN 0863-1204.

Clark, J.H., Lusmeyer, T.H., Cameron, M.R., 1992. *Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows*. J. Dairy Sci. 75:2304-2323.

De Visser, H., Klop, A., van der Koelen, C.J., van Vuuren, A.M., 1998. *Starch supplementation of grass harvested at two stages of maturity prior to ensiling: intake, digestion and degradability in dairy cows.* J. Dairy Sci. 81:2221-2227.

Dewhurst, R.J., Davies, D.R., Merry, R.J., 2000. *Microbial protein supply from the rumen.* Anim. Feed Sci. Techn. 85:1-21.

Ekern, A. et al., 1991. *A new system of energy evaluation for ruminants. Norsk landbruksforskning* 5:273-277. ISSN 0801-5333.

Focant, M., van Hoecke, A., Vanbelle, M., 1990. *The effect of two heat treatments (steam flaking and extrusion) on the digestion of Pisum sativum in the stomachs of heifers.* Anim. Feed Sci. Technol. 28:303-313.

Goelema, J.O., Spreeuwenberg, M.A.M., Hof, G., van der Poel, A.F.B., Tamminga, S., 1998. *Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs.* Anim. Feed Sci. Technol. 76:35-50.

Goering, H.K., Van Soest, P.J., 1970. *Forage fiber analysis (apparatus, reagents, procedures, and some applications).* Agric. Handbook No. 379 ARS-USDA, Washington, DC.

Herrera-Saldana, R.E., Gomez-Alarcon, R., Torabi, M., Huber, J.T., 1990. *Influence of synchronising protein and starch degradation in the rumen on nutrient utilisation and microbial protein synthesis.* J. Dairy Sci. 73:142-148.

Hoover, W.H., 1986. *Chemical factors involved in ruminal fiber digestion.* J. Dairy Sci. 69:2755-2766.

Hoover, W.H., Stokes, S.R., 1991. *Balancing carbohydrates and proteins for optimum rumen microbial yield.* J. Dairy Sci. 74:3630-3644.

Hvelplund, T., Madsen, J., 1989. *Prediction of individual amino acid passage to the small intestine of dairy cows from characteristics of the feed.* Acta Agric. Scand. 39:65-78.

Hvelplund, T., Weisbjerg, M.R., Andersen, L.S., 1992. *Estimation of the true digestibility of rumen undegraded dietary protein in the small intestine of ruminants by the mobile bag technique.* Acta Agric. Scand. Section A - Anim. Sci. 42:34-39.

Jenkins, T.C., 1993. *Symposium: Advances in ruminant lipid metabolism.* J. Dairy Sci. 76:3851-3863.

Jordbruksverkets informationsenhet, 1999. *Ärter och annan trindsäd,* Jordbruksinformation 7. Jordbruksverket, Informationsenheten, Jönköping, Sweden.

Karlengen, I.J., Galméus, D., Taugbøl, O., Harstad, O.M., 2005. *Erter til mjølkeku.* Institutt for husdyr- og akvakulturvitenskap, UMB. Husdyrforsøksmøtet 2005, Sarpsborg. ISBN: 82-7479-018-9, 375-378.

Kung, L. Jr., Rode, L.M., 1996. *Amino acid metabolism in ruminants.* Animal Feed Science Technology 59:167-172.

Lallès, J.P., 1992. *Nutritional and antinutritional aspects of soyabean and field peaproteins used in veal calf production: a review.* Livestock Production Science, 34 (1993) 181-202, INRA, Rennes, France.

Lallès, J.P., Poncet, C., Toullec, R., 1992. *Composition en acides amines des bactéries libres et des bactéries fixées aux particules alimentaires du reticulo-rumen du veau sevré et du monton recevant différentes rations.* Ann. Zootech. 41:75-76.

Legay-Carmier, F., Bouchart, D., 1989. *Distribution of bacteria in the rumen contents of dairy cows given a diet supplemented with soya-bean oil.* Br. J. Nutr. 61:725-740.

Ljøkjel, K., Harstad, O.M., Prestløkken, E., Skrede, A., 2003a. *In situ digestibility of protein in barley grain (Hordeum vulgare) and peas (Pisum sativum L.) in dairy cows: influence of heat treatment and glucose addition.* Anim. Feed Sci. Technol. 107:87-104.

Ljøkjel, K., Harstad, O.M., Prestløkken, E., Skrede, A., 2003b. *In situ digestibility of starch in barley grain (Hordeum vulgare) and peas (Pisum sativum L.) in dairy cows: influence of heat treatment and glucose addition.* Anim. Feed Sci. Technol. 107:105-116.

Lund, P., 1997. *Varmebehandling av kraftfoder til kvæg - Effekt på omsætning af protein, individuelle aminosyrer og stivelse [Heat treatment of concentrated feedstuffs for cattle - Effects on utilisation of protein, individual amino acids and starch].* M. Sc. Thesis, The Royal Veterinary and Agricultural University of Denmark, pp. 146.

Madsen, J., Hvelplund, T., Weisbjerg, M.R., Bertilsson, J., Olsson, I., Spörndly, R., Harstad, O.M., Volden, H., Tuori, M., Varvikko, T., Huhtanen, P., Olafsson, B.L., 1995. *The AAT/PBV protein evaluation system for ruminants.* A revision. Norw. J. Agric. Sci, Suppl. No. 19:33.

Makkar, H.P.S., Becker, K., 1999. *Purine quantification in digesta from ruminants by spectrophotometric and HPLC methods.* Br. J. Nutr. 81:107-112.

Martin, C., Williams, A.G., Michalet-Doreau, B., 1994. *Isolation and characteristics of protozoal and bacterial fractions from bovine ruminal contents.* J. Anim. Sci. 72:2962-2968.

McAllister, T.A., Bae, H.D., Jones, G.A., Cheng, K.-J., 1994. *Micorbial attachment and feed digestion in the rumen.* J. Anim. Sci. 72:3004-3018.

McCleary, B.V., Solah, V., Gibson, T.S., 1994. *Measurement of total starch in cereal flours and products.* J. Cereal Sci. 20:51-58.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., 2002. *Animal nutritio (6th edition).* Pearson Education Limited, U.K.

Mertens, D.R., 1994. *Regulation of forage intake.* In: Fahey, G.C. Jr. (editor), Forage Quality, Evaluation, and Utilization. American Society of Agronomy Inc., Madison, WI, pp. 450-493.

Nocek, J.E., Tamminga, S., 1991. *Site of digestion of starch in the gastrointestinal tract of dairy cows and its effects on milk yield and composition.* J. Dairy Sci. 74:3598-3629.

Petit, H.V., Rioux, R., Quellet, D.R., 1997. *Milk Production and Intake of Lactating Cows Fed Raw or Extruded Peas.* J. Dairy Sci. 80:3377-3385.

Prestløkken, E., Harstad, O.M., 2001. *Effects of expander-treating a barley-based concentrate on ruminal fermentation, bacterial N synthesis, escape of dietary N, and performance of dairy cows.* Animal Feed Science and Technology 90:227-246.

Rahnema, S.H., Theurer, B., 1986. *Comparison of various amino acids for estimation of microbial nitrogen in digesta.* J. Amin. Sci. 63:603-612.

Reed, J.J., Lardy, G.P., Bauer, M.L., Gilbery, T.C., Caton, J.S., 2004a. *Effect of field pea level on intake, digestion, microbial efficiency, ruminal fermentation, and in situ disappearance in beef steers fed growing diets.* J. Anim. Sci. 82:2123-2130.

Reed, J.J., Lardy, G.P., Bauer, M.L., Gilbery, T.C., Caton, J.S., 2004b. *Effect of field pea level on intake, digestion, microbial efficiency, ruminal fermentation, and in situ disappearance in beef steers fed forage-based diets.* J. Anim. Sci. 82:2185-2192.

Russel, J.B., Wilson, D.B., 1996. *Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH?* J. Dairy Sci. 79:1503-1509.

SAS User's Guide: *Statistics, Version 8 Edition*, 2001. SAS Inst. Inc., Cary, NC.

Shinnick, F.L., Longacre, M.J., Ink, S.L., Marlett, J.A., 1988. *Oat fiber: composition versus physiological function in rats*. J. Nutr. 118:144-151.

Siddons, R., Paradine, C.J., Beever, D.E., Cornell, P.R., 1985. *Ytterbium acetate as a particulate-phase digesta flow marker.* Br. J. Nutr. 54:509-519.

Spörndly, R., 1999. *Fodertabeller för idisslar Rapport 247.* Institutionen för husdjurens utfodring och vård, SLU, Uppsala, Sweden.

Stensig, T., Weisbjerg, M.R., Hvelplund, T., 1998. *Evalutation of different methods for the determination of digestion and passage rates of fibre in the rumen of dairy cows.* Acta Agric. Scand., Sect. A, Anim. Sci., 48:141-154.

Stevnebø, A., Sahlström, S., Anker-Nilssen, K., Svihus, B., 2005. *Egenskaper ved stivelsen i bygg som påvirker tilgjengeligheten.* Institutt for husdyr- og akvakulturvitenskap, NLH. Husdyrforsøksmøtet 2005, Sarpsborg.
ISBN: 82-7479-018-9, 379-382.

Stokes, S.R., Hoover, W.H., Miller, T.K., Blauweikel, T., 1991. R*uminal digestion and microbial utilisation of diets varying in type of carbohydrate and protein.* J. Dairy Sci. 74:871-881.

Sutton, J.D., 1988. *Concentrate feeding and milk composition.* In: Haresign, W., Cole, D.J.A. (editors), Recent Developments in Ruminant Nutrition 2. Butterworths, London, UK, pp. 97-110.

Syrjälä-Qvist, L., Setälä, J., Tuori, M., 1981. *Field peas as a protein source for high-production dairy cows on grass silage and hay based feeding.* Journal of the Scientific Agricultural Society of Finland 53:307-313.

Thomas, P.C., Martin, P.A., 1988. *The influence of nutrient balance on milk yield and composition.* In: Garnsworthy, P.C. (editor), Nutrition and Lactation in the Dairy Cow. Butterworths, London, UK, pp. 97-118.

Thomke, S., 1984. *Ärter som fodermedel – näringsvärde, begränsningsfaktorer och användbarhet.* Institutionen för husdjurens utfodring och vård, SLU, Uppsala, Sweden.

Thomke, S., 1979. *Ärter och åkerböna som foder.* Aktuellt från lantbruksuniversitetet 271, Husdjur, SLU, Uppsala, Sweden.

Titgemeyer, E.C., 1997. *Design and interpretation of nutrient digestion studies.* J. Anim. Sci. 75:2235-2247.

Uhlen, A.K., Abrahamsen, U., Gullord, M., 2005. *Potensiale og framtidsmuligheter for norsk fôrkornproduksjon.* Institutt for plante- og mijløvitenskap, NLH. Husdyrforsøksmøtet 2005, Sarpsborg. ISBN: 82-7479-018-9, 383-386.

Ushida, K., Jouany, J.P., Demeyer, D.I., 1991. *Effects of presence or absence of rumen protozoa on efficiency of utilisation of concentrate and fibrous feeds: physiological aspects of digestion and metabolism in ruminants.* In: Tsuda, T., Sasaki, Y., Kawashima, R. (Eds.), Proceedings of VIIth International Symposium on Ruminant Physiology. Academic Press, London, UK, 625-654.

Van Soest, P.J., 1994. *Nutitional Ecology of the Ruminant (2nd edition).* Comstock, Cornell University Press, Ithaca, NY, USA, 476.

Van Soest, P.J., Mason, V.C., 1991. *The influence of the Maillard reaction upon the nutritive value of fibrous feeds.* Anim. Feed Sci. Technol. 32:45-53.

Volden, H., 1999. *Effects of level of feeding and ruminally undegraded protein on ruminal bacterial protein synthesis, escape of dietary protein, intestinal amino acid profile, and performance of dairy cows.* J. Anim. Sci. 77:1905-1918.

Volden, H., Harstad. O.M., 1998. *Chemical composition of bacteria harvested from the rumen of dairy cows fed three diets differing in protein content and rumen degradability at two levels of intake.* Acta Agric. Scand., Sect. A, Anim. Sci. 48:202-209.

Vranjes, M.V., Wenk, C., 1995. *The influence of extruded vs. untreated barley in the feed, with and without dietary enzyme supplement on broiler performance.* Anim. Feed Sci. Technol. 54:21-32.

Ørskov, E.R., 1986. *Starch digestion and utilization in ruminants.* J. Anim. Sci. 63:1624-1633.

Ørskov, E.R., McDonald, I., 1979. *The estimation of preotein degradability in the rumen from incubation measurements weighted according to rate of passage.* J. Agric. Sci. (Cambridge), 92:499-503.

Öster, A., Thomke, S., 1978. *Försök med ärter till mjölkkor.* Research Information Centre, SLU, Uppsala, Sweden. The Research Conference 1978. ISSN 0347-9684.

Nr	Titel och författare	År
358	Metoder för reduktion av halten lättlösliga kolhydrater i vallfoder och jämförelse av analysmetoder Methods for the reduction of soluble carbohydrate levels in conserved roughages and the comparison of analytical methods 30 hp A2E-nivå Emma Pettersson	2011
359	Vilopuls hos 2-åriga varmblodiga travhästar i träning Resting heart rate in 2-year old Standardbreds in training 30 hp A2E-nivå Johanna Berg Johansson	2011
360	The effect of silage quality on gross energy losses 30 hp A2E-nivå Irfan Sakhawat	2011
361	Äggkvalitet kopplat till produktion, ekonomi och djurvälfärd hos svenska värphöns Egg quality and its connection to production, economy and animal welfare amongst Swedish layers 30 hp A2E-nivå Sofia Hollstedt	2011
362	Ättider i olika system att tillföra hästar grovfoder 30 ph A2E-nivå Michaela Lindbäck	2012
363	Deltidsbete i stall med automatisk mjölkning – rastbete jämfört med produktionsbete Part-time grazing in automatic milking systems - exercise pasture compared to production pasture 30 hp A2E-nivå Sara Andersson	2012
364	Nursing technique and growth environment of Rabbit fish (Siganus guttatus) in the area of Tam Giang lagoon, Thua Thien Hue 30 hp A2E-nivå Cecilia Stattin	2012
365	Vallfoder till slaktgrisar – effekter på tillväxt och social beteende vid utfodring Forage in slaughter pig production With focus on growth and social behaviour by feeding 30 hp A2E-nivå Anna Skogar	2012

I denna serie publiceras examensarbeten (motsvarande 15, 30, 45 eller 60 högskolepoäng) vid Institutionen för husdjurens utfodring och vård, Sveriges lantbruksuniversitet. En förteckning över senast utgivna arbeten i denna serie återfinns sist i häftet. Dessa, samt tidigare arbeten, kan i mån av tillgång erhållas från institutionen.

In this series Degree projects (corresponding 15, 30, 45 or 60 credits) at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, are published. Earlier numbers are listed at the end of this report and may be obtained from the department as long as supplies last.

DISTRIBUTION:
Sveriges Lantbruksuniversitet
Institutionen för husdjurens utfodring och vård
Box 7024
750 07 UPPSALA
Tel. 018-67 28 17
