Rapeseed meal as a supplementary protein for dairy cows on grass silage-based diet, with the emphasis on the Nordic AAT-PBV feed protein evaluation system

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Academic Dissertation
To be presented with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in the Auditorium B2, Viikki, on November 27, 1992 at 12 noon.

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The effect of rapeseed meal (RSM) supplementation on the performance of dairy cows on direct cut grass silage based diets was studied in five feeding trials. The proportion of RSM varied from 0% to 33% in the concentrate mixture (the grain was an oat-barley mixture of 1:1). In one experiment the treatments were RSM and soybean meal (SBM), while in another experiment forage was either grass silage or barn dried hay cut at the same maturity. In addition, this was compared to the data of other trials in Finland during the last ten years, in which RSM supplementation had been used. Using this data the response in terms of milk yield to RSM supplementation was estimated. The utilization of protein in milk production was estimated by the Nordic AAT-PBV protein evaluation system.

During the experiments (1983-1990) the varieties of turnip rape were changed from high glucosinolate, containing single-zero, to low glucosinolate containing double-zero varieties, while the glucosinolate content was reduced from 40-50 μmoles to 14 μmoles per g of defatted meal. Heat-moisture treatment (™Öpex) further reduced the glucosinolate content by half.

By replacing grain with RSM in the concentrate mixture with *ad libitum* silage feeding, the silage intake increased by 0.43 kg per kg increase in RSM on the basis of dry matter (DM) (non significant). The response in increased milk production was 0.77 kg in milk or 0.70 kg in energy corrected milk (ECM) yield ($P<0.02$), and in protein yield 27 g/d ($P<0.01$) per kg increase in RSM DM. Although the linear effect of the RSM level was significant, the effect on the milk yield was reduced when the level of RSM was over 12-16% of concentrate mixture. The protein content of milk increased by 0.07 g/kg per MJ increase in metabolizable energy intake ($P<0.02$).

Heat-moisture treatment of RSM increased milk production significantly in one experiment (21.9 kg vs. 23.9 kg milk or 23.4 vs. 25.2 kg ECM/d), ($P<0.03$). In two other experiments heat treatment had no noticeable effect on milk yield. In comparing SBM with RSM on the same crude protein basis in the concentrate, no difference in milk yield was observed.

The goitrin content of the milk was reduced when the glucosinolate content RSM, or the level of RSM in the diet, was reduced. With Öpex-treated double zero RSM, the milk contained less than 10 μg/l (sensitivity of analysis 2 μg/l) goitrin.

The utilization of AAT in milk production was also estimated using different constants in the calculations of AAT-PBV values of the feeds. When the proportion of AAT of microbial origin increased, the coefficient of variation of the AAT utilization reduced. This is affected by correcting the microbial-N contamination of the *in sacco* analysis, lowering the estimate for the rumen outflow rate (k-value) from 0.08 to 0.03, and changing the estimate for the efficiency of microbial protein synthesis (MPS). The best model was obtained using the method of VOIGT and PIATKOWSKI (1991) for calculating MPS.

Key words: dairy cows, rapeseed meal, grass silage feeding, protein protection, protein evaluation systems, AAT/PBV

1. Introduction

In Finland milk production accounts for ca. 30% of the gross income from agriculture. The fact that most of the feeds can be produced on the farm, especially for dairy cattle on a grass silage-based diet, works in favour of dairy farming. Hence the proportion of forage in the total feed units averaged 55.4% in 1991 on milk recorded farms, the proportion of silage being 33.1%. Forage was supplemented by grain and protein concentrates, the most important of which were soybean meal and rapeseed meal. In 1991 the combined production of oilseed meals and oil cakes totalled 190 million kilograms, of which 41% was of rapeseed and 59% of soybean. Rapeseed meal is given mainly to cattle, whereas soybean meal is mainly used in pig and poultry feeds, with only 20-25% used in cattle feed. Replacing a part of the soybean meal in cattle feed with rapeseed meal could increase the domestic cultivation of rape, thereby boosting the national self-sufficiency in cattle feeds. Rapeseed cultivation and production of rapeseed meal could increase in the future if rapeseed oil were used as a diesel fuel.

The production of feed protein for ruminants can be increased by using high amounts of nitrogen fertilizer in swards.This was demonstrated in the 1970s by the so-called "Green line" project (Ettala and Lampila 1974, Ettala et al. 1974, Ettala et al. 1978). Grass silage of high crude protein content and high digestibility, supplemented with plain grain concentrate, will satisfy the requirement for digestible crude protein even at high production levels. However, supplementing grain with protein concentrates in a diet of forage with high crude protein content has also increased milk production (Castle and Watson 1976, Gordon and Murray 1979). This indicates that the DCP system has its limitations, and new systems were developed during the 1970s and 1980s. The new feed protein evaluation systems, for instance the Nordic AAT-PBV system (NKJ 1985), have divided the amino acid nitrogen absorbed in the duodenum into that of microbial origin and of feed origin, enabling the protein requirement of the dairy cow to be estimated more exactly than by using the DCP system. In the present study various rapeseed meals were used as supplements to the grass silage based-diet of dairy cows, and their effect on milk production and composition, as well as on the utilization of feed protein, was examined with the AAT-PBV system.

2. Review of literature

2.1 Rapeseed meal

2.1.1. Rapeseed production

2.1.1.1. Origins and cultivation of turnip rape

Among the most commonly cultivated oilseed plants the turnip rape comes third after soybean and linseed (Table 1). Since World War II it has become the most important oilseed plant in the

Table 1. World oilseed production.

	1962-1964	1972-1974	1982-1984	1986/87	1987/88	1988/89	1989/90	1990/91
Soybeans	30.4	53.7	89.9	98.1	103.7	95.4	106.0	104.3
Cottonseed	21.2	24.9	28.8	27.6	31.6	32.6	31.2	33.3
Rapeseed	4.0	7.3	15.3	19.8	23.5	20.4	21.5	24.0
Sunflowerseed	7.2	10.9	16.8	18.8	21.0	20.6	21.7	22.3
Groundnuts	10.7	11.1	13.2	15.0	15.1	16.2	15.6	15.9
World[1)]	79.9	121.3	178.2	194.4	209.5	202.7	211.2	216.1

[1)] Soybeans, cottonseed, rapeseed, sunflowerseed, groundnuts, copra, palmkernels, linseed
Amounts shown are million metric tons; adapted from Toepfer International (1990)

temperate zone. Traditionally rapeseed was cultivated in India, China and Japan. In Europe rapeseed was cultivated since the 14th century, although rape oil has only been used for cooking since the 1940 (SHAHIDI 1990a). Today China, Canada, India, France, Germany, Great Britain and Poland are important producers of rapeseed (Tables 2 and 3). According to the FAO statistics (FAO 1991) there was some rapeseed cultivation in forty countries in 1990.

The name "rape" in rapeseed was derived from the Latin word for turnip *(rapum)*. Turnip, rutabaga, cabbage, Brussels sprout, mustard and many other common vegetables are closely related to rape. Rape grows in low temperatures and tolerates humidity, thus thriving in such temperature zones where soybean and sunflower do not survive.

DOWNEY and RÖBBELEN (1989) studied the origins of the oilseed plants of the *Brassica* family. Certain species of the *Brassica* family may well be among the earliest known cultivated plant, as some vegetable forms of this family were commonly used as early as in the Neolithic age, and reference was made to oilseed rape and mustard in Indian Sanskrit texts from the 21st to the 16th century B.C. These plants and their medicinal qualities were also mentioned in Greek, Roman and Chinese texts from the 6th to the 3rd century B.C. In Europe rape cultivation did not begin until the early Middle Ages. The commercial cultivation of rape began in the Netherlands as early as in the 16th century. Rape oil was traditionally used as an illuminant (lamp oil) and lubricant for steam engines, gaining a notable market share among food oils in the West only after World War II, thanks to improved varieties and more efficient processing techniques (DOWNEY and RÖBBELEN 1989).

The major varieties of oilseed plants in the *Brassica* family are *Brassica campestris* or turnip rape, and *B. napus* or rape. The former originated in the high plateaus in Turkey, from where it spread two thousand years ago to cover an area from the islands in the western Atlantic Ocean to China and the East coast of Korea, from northern Norway to the Sahara and North India. *Brassica napus* is a cross between *B. campestris* and *B. oleracea*. The latter originates in the Mediterranean region, and it is generally accepted that *B. napus* originated in southern Europe (DOWNEY and RÖBBELEN 1989).

There are spring and winter varieties of both *B. campestris* and *B. napus*. The winter varieties tend to yield more, but their wintering characteristics are poorer than those of winter grain crops.

Table 3. Rapeseed production in Europe.

	1979-1981	1988	1989	1990
France	871	2469	1803	2011
Germany	618	1640	2869	2157
Poland	434	1199	1586	1206
UK	274	1040	976	1231
Denmark	204	504	655	819
Czechoslovakia	165	380	387	380
Sweden	313	305	422	401
Finland	68	121	125	117
Europe, total	3203	8076	8261	8754

Amounts shown are 1000 metric tons; adapted from FAO Yearbook 1990

Table 2. Rapeseed production of some important producers.

	1962-1964	1972-1974	1982-1984	1986/87	1987/88	1988/89	1989/90	1990/91
Canada	0.21	1.22	2.78	3.79	3.85	4.31	3.10	3.26
PR China	1.04	1.20	4.72	5.88	6.61	5.04	5.44	6.55
India	1.19	1.92	2.65	2.61	3.37	4.02	3.80	4.00
Poland	0.28	0.49	0.63	1.30	1.19	1.18	1.58	1.20
EC-12	0.37	0.50	2.91	3.69	5.94	5.18	4.99	5.87

Amounts shown are million metric tons; adapted from Toepfer International (1990)

Breeding of the presently cultivated turnip rape and rape varieties has been pursued especially in Canada. *B. campestris* was imported to Canada in 1936, and a few years later *B. napus* was brought from Argentina. Commercial rapeseed production began in 1942 in Canada, the objective being to produce lubricant for the Allied war machinery. The erucic acid content of rapeseed was high, as it is still today in the Asian countries, where rape oil containing between 22 and 60 per cent of erucic acid is still being produced (HEAR, or high erucic acid rapeseed). The first rapeseed varieties with reduced erucic acid content, yielding rape oil containing less than 5% erucic acid (zero variety, "single low" or "single zero" varieties), were bred in Canada in 1968. These varieties are also known as LEAR (low erucic acid rapeseed). The first varieties low in both erucic acid and glucosinolate were licensed in 1974 (the "double low" or "double zero" varieties), i.a. the "Tower" variety. The low glucosinolate character came from the Polish variety "Bronowski". A double zero yellow seeded variety of turnip rape, "Candle", was developed in 1976. Having a low fibre content it is called a triple low (or triple zero) variety (SHAHIDI 1990b).

The brand name "Canola" was assumed in Canada in 1979 for all double low varieties. Canola was defined as a rapeseed variety yielding oil containing less than 2 per cent erucic acid, from which a defatted rapeseed meal containing less than 30 μmoles/g glucosinolate can be produced. Glucosinolates include, among others, four aliphatic glucosinolates: gluconapin, progoitrin, glucobrassicanapin and napoleiferin (SHAHIDI 1990b).

2.1.1.2. Cultivation of rapeseed in Finland

In practice, the cultivation of oilseed plants began in Finland in 1942 with the sowing of Argentinean linseed flax, although fiber flax and hemp had been traditionally grown (VALLE 1953). To a small extent hemp had been grown for seed and oil production. Linseed flax was being studied from 1924 at the Agricultural Research Center and from 1939 at the Plant Breeding Institute of Hankkija Wholesale Cooperative, resulting in two Finnish linseed flax varieties, "Vaanila" and "Tikkurila" (Maa- ja metsätalousministeriö 1975). From 1947 to 1951 the annual linseed flax cultivation covered an area of 2,000 to 4,000 hectares, the seed harvest averaging only 652 kg per hectare (VALLE 1953).

The cultivation of winter turnip rape began towards the end of the 1940s (Table 4), and by 1959 the area under winter turnip rape cultivation had increased to 18,600 hectares. By 1976 the cultivation of winter varieties had ceased in favour of spring varieties of rape and turnip rape (PAHKALA and SOVERO 1988). Their combined cultivation area was at its largest in 1988, totalling 82,900 hectares (80,300 spring turnip rape and 2,600 spring rape. The respective figures for 1991 were 59,300 and 1,700 hectares (National Board of Agriculture 1991a). The yield of spring turnip rape has averaged 1,500 kg/ha (Table 4), being 1,780 kg/ha in

Table 4. Rapeseed production in Finland.

	1948-1955	1956-1960	1961-1965	1966-1970	1971-1975	1976-1980	1981-1985	1986-1990
Area, 1000 ha	10.8	9.9	6.7	5.1	10.1	31.7	61.1	76.6
Yield, kg/ha	1210	1120	1140	1440	1430	1530	1470	1530
Production, 1000 t/year	12.5	11.3	7.5	7.6	14.6	47.8	88.3	115.4

Adapted from National Bord of Agriculture, Helsinki, Finland.

1990, when the corresponding figure for spring rape was 2,090 kg/ha (National Board of Agriculture 1991a).

The first single zero varieties were brought to Finland in 1976, followed later by double zero varieties. In 1982 fifteen per cent of the spring turnip rape cultivated was of double zero varieties, whereas the corresponding share of spring rape was as high as nearly 70 per cent. At that time turnip rape covered 89.5% of the total rapeseed cultivation area. By 1988 the double zero varieties covered some 70 per cent of all spring turnip rape (varieties "Kova" and "Valtti"), and almost all spring rape ("Topas") (National Board of Agriculture 1991b).

2.1.2. Composition of rapeseed

2.1.2.1. Whole seed

The properties of the whole seed of the turnip rape have been reviewed (e.g. RÖBBELEN and THIES 1980, BELL 1984, BERTRAM et al. 1986, HENKEL and MOSENTHIN 1989). The seeds of rape and turnip rape are normally black, reddish brown, or occasionally yellow in colour, and round, with a diameter ranging from 1.5 to 3.2 mm. Winter rape has the largest seeds. The ripe rapeseed comprises an embryo (84 to 86 per cent of the dry weight) and a hull with a single cell layer of adhering aleuron as the only remains of the endosperm. The embryo consists mainly of two large cotyledons with an oil content of about 50 per cent. The cotyledons contain protein granules similar to those in the aleuron layer (BENGTSSON et al. 1972).

The proportion of the hull is between 13.0% and 18.7% of the dry weight of the rapeseed (APPELQVIST 1972, BERTRAM et al. 1986), or 27% to 30% of rapeseed meal after the extraction of oil. The black or brown seeds have thicker hulls than the yellow seeds. The hull still contains 9 to 13 per cent hexane-soluble fat and 15 to 18 per cent protein in addition to fibre, its main ingredient. The dark colour of the hull is mainly due to condensed polyphenols. Of the undesirable constituents of the seed, glucosinolate and sinapine are mainly found in the core, whereas tannins are in the hull (BERTRAM et al. 1986, HENKEL and MOSENTHIN 1989).

The oil content of the rapeseed varies from one species or variety to another. In winter rape it typically ranges from 42 to 50 per cent and in spring rape from 37 to 47 per cent of the dry matter. The respective figures for turnip rape are 40 to 48 per cent and 36 to 46 per cent (APPELQVIST 1972). After the extraction of fat the rapeseed meal contains roughly 40 per cent crude protein, depending on the species and variety. For instance, in 1969 in Canada the protein content of the defatted meal (DM) varied from 33.0 to 47.9 per cent (APPELQVIST 1972).

2.1.2.2. Rapeseed meal

Industrially the oil can be removed from the rapeseed either by pressing or by pressing and extracting with hexane. Before pressing, the crushed rapeseed is, when necessary, first dried, then purified, flaked and cooked in a stacked cooker in a temperature between 75° and 85°C, usually for 20 to 40 minutes (UNGER 1990). Continuous action expellers or screw presses are normally used for pressing most of the oil from the seed. The fat content of the remaining rapeseed cake varies between 15 and 18 per cent. The rapeseed cake is then subjected to an extraction process using hexane in a temperature of about 55°C. After extraction the rapeseed meal (RSM), which contains from 1.5% to 5% fat, is toasted at 100°C, dried and allowed to cool before storage (CARR 1989).

Rapeseed meal differs from rapeseed in that most of the oil has been removed. The removal of oil radically alters the composition of rapeseed, as the remaining fractional ingredients are then concentrated. In terms of a normal proximate analysis of feeds, roughly 12% of rapeseed meal consists of crude fibre, mainly in the form of the hulls (BELL and JEFFERS 1976).

The crude fat content of rapeseed meal depends on the quantity of oil left in the meal. The crude fat content, on average 4.1% of DM, includes the

remaining oil, the impurities removed from the oil during purifying, and other compounds, the largest group of which being phosphatides (gums) (BELL and JEFFERS 1976).

The crude protein content of rapeseed meal (N x 6.25) is roughly 40% of the defatted meal. The crude protein content of the hull ranges from 12% to 16% depending on the proportion of remaining embryonic matter, whereas that of the embryonic matter is about 52% (BELL 1984). The average composition of Finnish rapeseed meal and cake is shown in Table 5.

2.1.2.3. Rapeseed protein

The main proteins in the seed are water-soluble albumin and salt-soluble globulin. Albumins form a major part of the metabolically active, vitally important protein in the cell. Albumins can also function as storage proteins. Regarding their amino acid composition, albumins are better dietary than storage proteins, especially as far as dietary sulphur-containing amino acids are concerned (NORTON 1989). The storage protein is located in the so-called protein bodies or aleuron grains (APPELQVIST 1972).

The majority of rapeseed proteins are storage proteins without enzymatic activity. Between 18% and 28% of the crude protein in the seed consists of sedimentation coefficient 12 S, a globulin also known as crucipherin (BHATTY et al. 1968, SCHWENKE et al. 1973b). It is a neutral protein, soluble in sodium chloride, with a molecular weight of 300,000 to 360,000 (SCHWENKE 1990). The 2 S proteins are water soluble, alkaline albumins known as napins. Their molecular weight ranges from 12,000 to 14,000. They account for about 40% of the protein content of the seed (SCHWENKE et al. 1973b). Albumins are relatively rich in sulphurous amino acids (cystine 6.9%) and lysine (9.0%) (SCHWENKE et al. 1973a). A newly identified group of proteins, oleosines, make up ca. 20% of the protein content of the rapeseed. They are believed to be efficient emulsifying agents in the dry seeds (MURPHY et al. 1991).

Rapeseed meal includes more amino acids (AA) containing sulphur and slightly less lysine than soy-

Table 5. Composition of the Finnish rapeseed meal and cakes 1989[1)].

		Rapeseed meal Crude fat < 5 %	Crude fat 5-7 %	Crude fat >7 %	Rapeseed cake
No of samples		13	23	9	31
In dry matter (%)					
Ash	x̄	8.3	8.1	7.8	7.1
	CV	5.0	23	2.3	5.2
Crude protein	x̄	37.9	37.9	36.5	33.8
	CV	5.2	4.4	2.9	5.8
Crude fat	x̄	4.1	5.3	7.7	17.2
	CV	20.8	17.4	9.6	14.4
Crude fibre	x̄	13.8	13.3	13.3	11.1
	CV	6.2	7.9	7.4	8.0

[1)] Mean and coefficient of variation (%)
Adapted from Valtion maatalouskemian laitos (1989)

Table 6. Essential amino acid composition of rapeseed meal and rapeseed hulls compared with soybean meal (BELL 1984).

	In dry matter (%)			In crude protein (N x 6.25)		
	Rapeseed hulls[a)]	meal[b)]	Soybean meal	Rapeseed hulls	meal	Soybean meal
Arginine	0.28	2.50	3.26	2.49	6.11	6.44
Histidine	0.11	1.15	1.21	0.98	2.81	2.40
Isoleucine	0.45	1.63	2.37	4.00	3.98	4.69
Leucine	0.62	2.85	3.79	5.51	6.97	7.49
Lysine	0.66	2.45	3.14	5.87	5.98	6.22
Methionine	trace	0.73	0.71	trace	1.78	1.40
Cystine	0.24	0.50	0.33	2.13	1.23	0.65
Tryptophfan	0.05	0.48	0.61	0.40	1.16	1.20
Phenylalanine	0.43	1.64	2.43	3.82	4.01	4.80
Valine	0.67	2.09	2.53	5.96	5.11	5.00
Threonine	0.69	1.84	1.92	6.13	4.50	3.80
Protein (Nx6.25, %)	11.25	40.95	50.57	100	100	100

a) FINLAYSON 1974, b) CLANDININ et al.(1981), SARWAR et al. 1981

bean meal (Table 6). The amino acids of Finnish rapeseed meal have been found to contain 5.4% to 5.9% lysine, 4.3% to 4.4% threonine, 2.3% to 2.5% methionine and 2.3% to 2.5% cystine (percentage of crude protein) (NÄSI 1991, NÄSI and SILJANDER-RASI 1991).

2.1.2.4. Rapeseed fat

The rapeseed fats are predominantly, more than 90%, triglycerides. In addition, there are less than 4% phospholipids, the most important one being lecithin (Table 7).

Table 7. Fat composition of low erucic rapeseed (ZADERNOWSKI & SOSULSKI 1978).

Triglycerides	92.1 %
Diglycerides	1.2
Free fatty acids	0.5
Sterol esters	1.1
Sterols	0.6
Phospholipids	3.6
Glycolipids	0.9

The fatty acid composition of rapeseed fats depends primarily on the variety (Table 8). Formerly varieties high on erucic acid, with erucic acid contents ranging from 45% to 50% of all fatty acids, were cultivated. In the 1960s rapeseed oil caused an unusually high incidence of cardiac defects in laboratory animals, presumably due to erucic acid. As this phenomenon was not observed in humans, the nutritional risk may have been exaggerated (ACKMAN 1990). The EEC Commission set the upper limit for erucic acid content at 15% in 1976, the present limit of 5% having been introduced in 1979. Low erucic acid rapeseed, i.e. single zero varieties, have an erucic acid content of less than 2% of all fatty acids (WARD et al. 1985). In Finland erucic acid content has been below 1% for several years.

Rapeseed oil contains less linoleic acid, but more α-linolenic acid than soybean oil. Linoleic acid, also known as Vitamin F, tends to reduce the blood cholesterol level. The proportion of linoleic acid is higher in low erucic acid rapeseed varieties. A high linolenic acid content is an undesirable trait, as this

Table 8. Comparison of major fatty acids in some edible vegetable oils of commerce (w/w% fatty acids).

Fatty acids	HEAR[a]	LEAR[b]	Candlec	Tower[d]	Soybean	Corn	Safflower	Sunflower	Peanut	Olive	Linseed
14:0	-	0.04	0.05	0.05	0.1	-	0.1	-	0.1	-	-
16:0	4	3.48	3.55	3.88	10.8	11.4	6.5	6.2	10.0	11.0	5.5
18:0	1	1.50	1.38	1.56	4.0	1.9	2.3	4.7	2.3	2.2	4.3
20:0	1	0.42	0.43	0.50	-	-	-	-	-	-	-
22:0	<1	0.27	0.20	0.28	-	-	-	-	-	-	-
Total saturated	6	5.71	5.61	6.46	15.1	13.3	10.4	10.8	17.8	13.5	9.8
16:1	-	0.22	0.28	0.29	0.2	-	0.4	-	0.1	0.8	-
18:1	15	61.65	55.58	64.02	23.8	25.3	12.2	20.4	47.1	75.8	21.1
20:1	10	1.38	1.78	1.24	0.2	-	-	-	1.4	0.3	-
22:1	45	0.44	1.63	0.08	-	-	-	-	-	-	-
Total monounsaturated	70	63.69	59.27	65.80	24.3	25.3	12.6	20.4	48.6	77.1	21.1
18:2n-6	14	19.69	21.87	18.79	53.3	60.7	77.4	68.8	33.6	8.3	13.3
18:3n-3	9	10.65	12.99	8.59	7.1	0.7	0.4	-	-	0.6	55.7
Total polyunsaturated	13	30.34	34.86	27.61	60.6	61.4	77.8	68.8	33.6	8.8	69.0

Adapted from ACKMAN (1990)

[a] HEAR =high erucic acid rapeseed; [b] LEAR = low erucic acid rapeseed, *B. campestris var.* Torch

[c] B. *campestris var.* Candle; [d] B. *napus var.* Tower

acid oxidates easily, giving the oil an unpleasant odour. The oleic acid content of rapeseed oil is notably higher than that of soybean, the former resembling olive oil in this respect. Oleic acid has assumed increasing significance in recent studies on nutrition (ACKMAN 1990).

2.1.2.5. Carbohydrates of rapeseed meal

RSM comprises about 50% crude fibre and fractions of nitrogen-free extract. Hulled RSM contains roughly one third carbohydrate, one half protein (% N x 6.25), and various other minor constituents. There are relatively small quantities of monosaccharides and disaccharides, starch being almost non-existent. The composition of carbohydrates in hulled rapeseed meal is 14.5% pectin, 7% cellulose residues, 4.5% amyloid, 2% arabinan, and 1% arabinogalactan (BELL 1984). Hulled rapeseed meal also contains protein (52%), lignin (2.6%), sinapine (2.4%), ash (3.7%), phytates (2%) and glucosinolates (1%). Dry RSM matter contains about 10% soluble sugars. THEANDER and ÅMAN (1976) reported 6.8% to 7.5% sucrose, 2.4% stachyose, 0.3% raffinose and 0.2 - 0.5% fructose in a moisture-free defatted turnip rape meal. α-galactocides of sucrose, containing raffinose and stachyose, cannot be absorbed by the human digestive system, as it lacks the enzyme α-galactosidase required for their hydrolysis. This has been observed to cause flatulence in humans and animals (NACZK and SHAHIDI 1990).

Hull mass amounts to about 16% of the weight of the full seed, and 30% of the oil-free RSM. The hulls contain 34% N-free extract, 44% crude fibre, 14.5% pentosans, 32% cellulose, 3.8% sugars, 12% to 24% lignin, 6% to 12% polyphenols and 1.5% tannins (BELL 1984). Lignin content is lower in triple zero rapeseed varieties. THEANDER et al. (1977) reported 7.9% lignin in the hulls of yellow-hulled turnip rapeseed. The digestibility of hull energy has been found to be very low in pigs, ranging from 0% (brown hulls) and 30% (yellow hulls) (BELL and SHIRES 1982).

2.1.2.6. Minerals

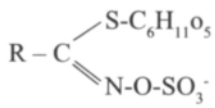
The mineral content of RSM was reviewed by BELL (1984). RSM dry matter contained 7.4% to 7.6% ash, 0.68% calcium and 1.2% to 2% phosphorus, of which nearly two thirds was phytin-bound phosphorus. In addition, the RSM dry matter contained 1.3% potassium, 0.64% magnesium, 0.8% to 1.7% sulphur and only 0.026% sodium (SUMMERS et al. 1983). The phytin content of the rapeseed calls for careful attention when formulating a diet, as is the case with cereal grain and oilseed meal in general. Availability of RSM phosphorus to young chickens does not exceed 30% (SUMMERS et al. 1983).

2.1.2.7. Glucosinolates

2.1.2.7.1. Structure and classification of rapeseed glucosinolates

The presence of glucosinolates and phenolic choline esters in rapeseed limits its use as a feed for many animal species. Glucosinolates are an integral group of sulphur-containing anions present in nature, the structure of which was studied by ETTLINGER and LUNDEN (1954). More than 100 different glucosinolates are known today (SØRENSEN 1990).

The basic structure of glucosinolates is as follows:



Glucosinolates can be classified as alkenylglucosinolates and indolylglucosinolates on the basis of their side chain. In the former the R is straight-chained, in the latter it is heterocyclic. Glucosinolates are hydrophilic and strongly acidic compounds, isolated and handled as salts. The glucosinolates of the species *Brassica* are derived biosynthetically from three amino acids: methionine, phenylalanine and tryptophan. About 30 different glucosinolates have been found in the seed of *Bras-*

sica napus (SØRENSEN 1990). Glucosinolates numbered 1 to 15 in Table 9 can be derived from methionine, 14 to 22 from phenylalanine, and 23 to 27 from tryptophan (SØRENSEN 1990).

The following glucosinolates present in *B. napus* are quantitatively the most prominent (SANG and SALISBURY 1988):

I Gluconapin
II Progoitrin
III Glucobrassicanapin
IV Napoleiferin
V Glucobrassicin
VI 4-hydroxyglucobrassicin
VII Sinigrin
VIII Gluconasturtiin

In addition to the four alkenylglucosinolates (gluconapin, progoitrin, glucobrassicanapin and napoleiferin) defined in the Canola standard, the quantities of the indolylglucosinolates glucobrassicin and 4-hydroxyglucobrassicin are included in the total glucosinolate content as defined in the EEC standard (EEC 210/55, ref. SCHNUG and HANEKLAUS 1988). After 1991 the total glucosinolate content of the rapeseed produced in the EEC countries may not exceed 20 μmoles/g in air-dry seed. In the double zero varieties the quantity of alkenylglucosinolate is reduced, resulting in a relative increase in indolylglucosinolate content (mainly 4-hydroxyglucobrassicin) (Table 10).

Glucosinolates are not toxic as such, but become so during enzymatic degradation. The release of myrosinase enzymes (thioglucoside glucohydrolase, EC 3.2.1), present in plant cells and synthe-

Table 9. Selected glucosinolates important for the quality of rapeseed and other cruciferous corps (SØRENSEN 1990).

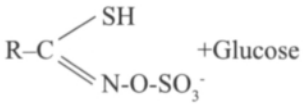
No.	Trivial names	Semisystematic names
Glucosinolates derived from methionine:		
1	Sinigrin	Allylglucosinolate
2 CE	Gluconapin	But-3-enylglucosinolate
3 CE	Glucobrassicanapin	Pent-4-enylglucosinolate
4 CE	Progoitrin	(2R)-2-Hydroxybut-3-enylglucosinolate
5	Epiprogoitrin	(2S)-2-Hydroxybut-3-enylglucosinolate
6 CE	Napoleiferin	(2R)-2-Hydroxypent-4-enylglucosinolate
7	Glucoibervirin	3-Methylthiopropylglucosinolate
8	Glucoerucin	4-Methylthiobutylglucosinolate
9	Glucoberteroin	5-Methylthiopentylglucosinolate
10	Glucoiberin	3-Methylsulphinylpropylglucosinolate
11	Glucoraphanin	4-Methylsulphinylbutylglucosinolate
12	Glucoalyssin	5-Methylsulphinylpentylglucosinolate
13	Glucoraphenin	4-Methylsulphinylbut-3-enylglucosinolate
14	Glucocheirolin	3-Methylsulphonylpropylglucosinolate
15	Glucoerysolin	4-Methylsulphonylbutylglucosinolate
Glucosinolates derived from phenylalanine:		
16	Glucotropaeolin	Benzylglucosinolate
17	Gluconasturtiin	Phenethylglucosinolate
18	Glucobarbarin	2-Hydroxy-2-phenylethylglucosinolate
19	Glucolepigramin	m-Hydroxybenzylglucosinolate
20	Sinalbin	p-Hydroxybenzylglucosinolate
21	Glucolimnanthin	m-Methoxybenzylglucosinolate
22	Glucoaubrietin	p-Methoxybenzylglucosinolate
Indol-3-ylmethylglucosinolates biosyntetically derived from typtophan:		
23 E	Glucobrassicin	Indol-3-ylmethylglucosinolate
24	Neoglucobrassicin	N-Methoxyindol-3-ylmethylglucosinolate
25	Sulphoglucobrassicin	N-Sulphoindol-3-ylmethylglucosinolate
26 E	4-Hydroxyglucobrassicin	4-Hydroxyindol-3-ylmethylglucosinolate
27	4-Methoxyglucobrassicin	4-Methoxyindol-3-ylmethylglucosinolate

C: Glucosinolates standard by Canola Council
E: Glucosinolates standard by EEC

Table 10. Content of glucosinolates in some rapeseed cultivars (SANG & SALISBURY 1988).

Glucosinolate	Brassica campestris		Brassica napus	
	Candle (Canada)	Torpe (Sweden)	Oro (Canada)	Regent (Canada)
	Glucosinolates (percentage of total amount)			
Gluconapin	26	31	12	18
Progoitrin	34	25	68	54
Glucobrassicanapin	21	26	6	3
Napoleiferin	7	6	5	2
Glucobrassicin	tr	tr	tr	4
4 - hydroxyglucobrassicin	7	7	5	17
Sinigrin	0	0	2	2
Gluconasturtiin	4	6	2	tr
Total glucosinolates (μmoles/g defatted meal)	52	42	111	41

sized by microbes in the digestive tract, e.g. as rapeseed tissue degrades, causes disintegration of glucosinolates. The products of this disintegration are glucose and aglucon (RÖBBELEN and THIES 1980):



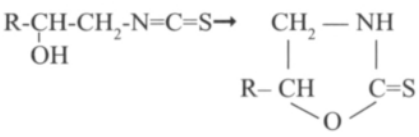
In a neutral environment aglucon releases sulphates, and isothiocyanate (trivial name mustard oil) is generated:

$$R - N = C = S^{-} + HSO_4$$

In a mildly acidic environment, or when coming into contact with ferrous ion, aglucons generate nitriles and elemental sulphur:

$$R - C \equiv N + S$$

Isothiocyanates carrying a hydroxyl group in the β-position form spontaneously cyclic compounds, oxazolidine-2-thiones.



In a neutral or mildly alkaline environment isothiocyanates of indolylglucosinolate origin form thiocyanate ions (Table 11).

Table 11. Principal degradation products of the rapeseed glucosinolates (HENKEL & MOSENTHIN 1989).

Glucosinolate	Primary degradation products	Secundary degradation products
Progoitrin	Goitrin	pH-7: nitriles (traces) pH<7: nitriles and isothiocyanates
Gluconapin	Isothiocyanate	
Glucobrassicanapin	Isothiocyanate	
Napoleiferin	5-allyl-2-thiooxazolidon	
Glucobrassicin	ph>7: thiocyanate or 3-hydroxymethylindole	pH<7: nitriles

2.1.2.7.2. Analysis of glucosinolates in RSM

Enzymatic degradation of glucosinolates by myrosinases produces substances, the identification and measuring of which can be utilized in assessing glucosinolate content. These substances include isothiocyanates, goitrin (5-vinyl-oxazolidine-2-thione), nitriles and thiocyanate. The total glucosinolate content can be determined from the released glucose (MCGREGOR et al. 1983).

Far more sophisticated methods for analysing glucosinolates have been developed during the last two decades, enabling their classification according to the following criteria.

Single glucosinolates can be identified by gas chromatography using tri-methylsilylised desulphoglucosinolates (TMS derivative) (UNDERHILL and KIRKLAND 1971, THIES 1976). This is also the reference method of the EEC. Reversed-phase liquid high performance chromatography has made it possible to identify all known glucosinolates either as non-degraded glucosinolates (HELBOE et al. 1980) or as desulphoglucosinolates (SANG and TRUSCOTT 1984).

The coloured complex formed by a glucosinolate and palladium ion can be measured by means of spectrophotometry - a fast and sensitive method for determining total glucosinolate content (THIES 1982, MØLLER et al. 1985). The X-ray fluorescence method was developed recently for determining total glucosinolate content by means of measuring the sulphur content of an organic specimen. The method is based on the close relationship of the glucosinolate and sulphur contents of the rapeseed (SCHNUG 1987). Near infrared reflectance analysis is not precise enough, not even for screening purposes (MCGREGOR 1990).

2.1.2.7.3. Physiological effects of glucosinolates

The degradation products of glucosinolates have various physiological effects, mainly affecting the thyroid. Degradation of glucosinolates is due to the release of myrosinase from degrading rapeseed tissue. The enzyme is also synthesized by intestinal

bacteria in the gastrointestinal tract. Hence, inactivating the enzyme, e.g. by means of heating rapeseed products, will not eliminate the harmful effect of glucosinolates (MARANGOS and HILL 1974).

Degrading glucosinolates produce isothiocyanate, which decreases thyroxine synthesis by means of reducing the amount of iodine used in the synthesis. This deficiency can be eliminated by adding iodine to the feed (ANKE 1980). Goitrin causes a more significant functional disorder by preventing the oxidation of iodide into chemically active iodine and its binding with aromatic tyrosine (GMELIN 1969, BERGNER and SCHMIDT 1972, FENWICK and HEANEY 1983). The increased supply of iodine cannot eliminate the deficiency. Instead, the thyroid gland begins to grow, thereby increasing the production and availability of tyrosine. The goitrogenic effect can be counteracted by adding trace elements to the diet (MENZEL 1983).

Glucosinolates are very prone to affect the palatability of feeds in monogastrics. HENKEL and KALLWEIT (1989, ref. HENKEL and MOSENTHIN 1989), for example, gave chicks a feed mixture containing 20% single zero rapeseed meal (*B. campestris*). After a few days the birds refused to feed on it. The glucosinolate content of new double zero varieties is so low that such rapeseed meal may be given as the sole protein supplement feed (20% to 25% of the diet) to chicks and pigs (CLASSEN et al. 1991, CAMPBELL and SLOMINSKI 1991). In dairy cows glucosinolate-content affects the palatability of rapeseed meal less, and the meal of double zero varieties does not affect feed intake, not even in relatively high concentrations (EMANUELSON 1989).

In monogastrics glucosinolates induce enlargement or cirrhosis of the liver, retarded fertility, and thyroidal enlargement, as well as frailty of legs in chicks (HILL 1979). These symptoms have been significantly reduced by using double zero rapeseed meal (CAMPBELL and SLOMINSKI 1991), but its detrimental effect on the fertility of sows remains to some extent, depending on glucosinolate content (ETIENNE et al. 1991). The double zero rapeseed products have also had a negative effect on bovine fertility (EMANUELSON 1989).

2.1.2.7.4. Glucosinolates in milk

When feeds containing glucosinolates are given to cows, hydrolysis products are also secreted in the milk. VIRTANEN et al. (1959) and VIRTANEN (1963) reported that 0.05% of the goitrin (5-vinyl-oxazolidone-2-thione) present in the feed was secreted into the milk. Consequently BACHMANN et al. (1985) found that 0.1% of the goitrin in *B. napus* rapeseed meal was secreted into the milk.

In Finland, ARSTILA et al. (1969) reported goitrin contents ranging from 35 to 100 µg per litre of milk. They assumed that the goitrin was derived from weeds of the *Cruciferae* family. The following year milk samples from the same area were found to be free of goitrin, presumably due to chemical weed killers used on the farms. In a Canadian study of six western and three eastern dairies the goitrin content of milk did not exceed 2 µg per litre (BENNS et al. 1979). In Switzerland, BACHMANN et al. (1985) measured goitrin contents ranging from 37 to 707 µg per litre, when 0.1 to 1.0 kg as *B. campestris* rapeseed meal was given daily. The extracted rapeseed meal contained 46 µM goitrin per gram, with a total glucosinolate-content of 77 µg per gram. In Finland the goitrin content of dairy milk was measured in 1983 from 224 samples from various regions in the country. The goitrin content exceeded the minimum discernible level of 2 µg per litre in only 19 samples, averaging 6.4 µg goitrin per litre in these samples (range 2 to 31 µg/l) (RAURAMAA 1983).

The role of milk in endemic goitre has been the subject of much discussion. PELTOLA (1960) gave rats milk collected "from a moderately severe goitre endemic area" (Orimattila, Finland) and milk from "a non-goitrous district" (Porvoo, Finland). The milk from the "goitrous area" induced enlargement of the thyroid gland, which could not be prevented by supplemental iodine. This result could not, however, be obtained by VIRTANEN (1963).

KRUSIUS and PELTOLA (1966) found that 0.1 µg/d goitrin induced nearly significant, and 0.5 µg/d significant, thyroid growth in rats. That means a ratio of 0.5 to 2.3 µg/d/kg metabolic live weight and 12

to 59 µg/d per 75 kg. IVARSSON and NILSSON (1973) gave rats milk from cows that had been feeding on concentrates containing none, 4.2% or 8.1% RSM (69 µM isothiocyanate and 81 µM goitrin per g of DM). In rats thyromegaly could be prevented by increasing the intake of iodine, but the combined effect of goitrin and other goitrogenic substances could not be excluded. There is relatively little information about the quantity at which goitrin becomes harmful to man. ARSTILA et al. (1969) stated that 200 µg/d will suffice to inhibit the normal accumulation of radioiodine in the thyroid gland, and this dose may also induce enlargement of the thyroid gland.

2.1.2.8. Sinapine

Sinapine is a choline ester of sinapine acid (3,5-dimethoxy-4-hydroxycinnamic acid). Sinapine is a cation, and in the rapeseed it is combined with the glucosinolate sinalbin (p-hydroxybenzylglucosinolate), thus substituting potassium. The sinapine content of rapeseed varies between 0.78 and 1.33 per cent (APPELQVIST 1972), being lower in *B. campestris* than in *B. napus* (MUELLER et al. 1978). In an alkaline environment sinapine is hydrolysed to sinapic acid and choline. Contrary to the free choline present in feeds, or choline chloride or hydrolyzed sinapine, which are absorbed in the small intestine, bound choline is not absorbed in that manner (GOH et al. 1979). In the large intestine the intestinal bacteria converted sinapine to trimethylamine (HOBSON-FROHOCK et al. 1973, MARCH and MACMILLAN 1978), which, when absorbed, is converted to trimethyloxide by the liver and kidneys, and secreted. Certain species of poultry (those laying brown eggs) have a genetic defect, resulting in an insufficient level of the TMA-oxydase activity for all the trimethylamine to be degraded, the remaining trimethylamine being deposited in the egg, affecting its flavour (PEARSON et al. 1979). TMA-oxydase synthesis may also be adversely affected by free goitrin and tannin (FENWICK et al. 1981, GOH et al. 1985). Similarly, a fishy smell has been observed in milk (ANDERSEN 1985).

2.1.2.9. Phytic acid

THOMPSON (1990) has produced a comprehensive review of phytic acid. It is a *myo*-inositol (1,2,3,4,5,6-hexakis-dihydrogen phosphate) by structure. Phytic acid acts as the primary reservoir of phosphorus, binding between 60 and 90 per cent of the phosphorus present in the rapeseed. Phytic acid also binds cations, and protects the plant from oxidative defects and molding during storage by binding zinc. Phytic acid is normally present in rapeseed as salts of Ca, Mg and K. The phytic acid content of extracted *B. campestris* or *B. napus* rapeseed meal varies between 3.7% and 4.8%, averaging 4.4% of the defatted meal (ANJOU et al. 1977).

Phytic acid has a detrimental effect on the utilization of zinc, although the high fibre content is also inversely related to the utilization of minerals (NWOKOLO and BRAGG 1977). On the other hand, SUMMERS and LEESON (1985) found no difference between soybean or rapeseed meal in the utilization of minerals by chickens on normal diets. Phytic acids also bind proteins, but no effect on *in vivo* digestibility has been demonstrated. As a nutrient phytic acid is believed to reduce the incidence of cancer of the colon in man.

2.1.2.10. Tannins

KOZLOWSKA et al. (1990) studied the tannins present in rapeseed. Tannins are complex phenolic compounds with a molecular weight ranging from 500 to 3000. The tannin content of extracted rapeseed meal is 2 to 3 per cent (FENWICK and HOGGAN 1976). Containing, for instance, tannic acid, tannins are bitter in taste and therefore obviously have, together with glucosinolates, a detrimental effect on palatability. The high tannin content of certain varieties of sorghum reduces the utilization of protein in poultry, but a similar combined effect of tannin and protein has not been observed in the rapeseed (KOZLOWSKA et al. 1990).

2.2. Rapeseed meal as a protein supplement for dairy cows

The use of rapeseed meal in dairy cattle was reviewed e.g. by THOMKE (1981) and HILL (1991). In the past the use of rapeseed meal or cakes as a protein supplement was limited due to the high glucosinolate content of single zero varieties of rapeseed. The addition of between 0.5 and 1.5 kg of RSM or cakes to the diet of dairy cows had no noticeable effect on milk production, compared to other protein supplements (BÜNGER et al. 1940, POIJÄRVI 1944, JARL 1951, NORDFELDT 1958, ASPLUND and MCELROY 1961, LINDELL and KNUTSSON 1976, LINDELL 1976, LAARVELD and CHRISTENSEN 1976, SYRJÄLÄ-QVIST et al. 1982). Reduced palatability (NORDFELDT 1958) or milk yield was observed in some experiments, especially at high RSM levels (20 - 30% in the concentrate) (ASPLUND and MCELROY 1961, INGALLS et al. 1968, WALDERN 1973).

Low glucosinolate double zero rapeseed cultivars have made RSM equal to other vegetable supplements in the diet of dairy cows (INGALLS and SHARMA 1975, SHARMA et al. 1977, PAPAS et al. 1978, SANCHEZ and CLAYPOOL 1983, MURPHY et al. 1985, VINCENT and HILL 1988, VINCENT et al. 1990).

Long term studies have produced some evidence of lowered fertility due to 0-RSM (LINDELL 1976, LINDELL and KNUTSSON 1976), and with 00-RSM on primiparous cows (AHLIN et al. 1985, EMANUELSON 1989, EMANUELSON et al. 1991). This negative effect on primiparous cows was confounded with the type of rapeseed, as the glucosinolate content was highest in the beginning of the experiment, being reduced to one half in the course of the three-year experiment (EMANUELSON 1989).

2.3. New systems for evaluating feed protein in dairy cattle

The protein evaluation of ruminant feeds has traditionally been based on digestible crude protein. During the last ten years many new systems have been reported, all taking into account the protein metabolism of both the rumen and the animal. ALDERMAN (1987) has listed six new systems for evaluating feed protein:

i. The British RDP/UDP system (rumen degraded and rumen undegraded protein) of ROY et al. (1977), subsequently published (1980) and revised by ARC (1984), which describes feeds in terms of truly absorbed amino nitrogen (TAAN). It is to be converted to metabolizable protein for practical purposes (MP=6.25 TAAN) (WEBSTER 1987, 1992).

ii. The French PDI system (protein digested in the intestine) (INRA 1978, VÉRITÉ et al. 1979, VÉRITÉ and PEYRAUD 1989).

iii. The Swiss API (absorbable protein in the intestine), developed from the PDI system (BICKEL and LANDIS 1987).

iv. The German system of measuring the duodenal flow of crude protein (Ausschuss für Bedarfsnormen 1986, ref. ALDERMANN 1987, ROHR 1987). PIATKOWSKI et al. (1990) and VOIGT and PIATKOWSKI (1991) have corrected the efficiency of microbial protein synthesis by taking into account the negative effect of the proportion of UDP in the feed protein.

v. The Nordic[1] AAT-PBV system (absorbable amino acids in the duodenum; protein balance in the rumen) (MADSEN 1985).

vi. The American AP system (absorbed protein) (NRC 1986).

2.3.1. AAT-PBV feed protein evaluation system

The AAT measures intraduodenally true absorbable amino acids, which originate in the microbial protein synthesized in the rumen and the undegraded feed protein (UDP) in the rumen. PBV is the balance between rumen degraded protein (RDP) and the protein requirement of microbes in the rumen (MADSEN 1985, HVELPLUND and MADSEN 1990). The system for calculating the AAT-PBV values for feeds is presented in Appendix 1.

[1] The Nordic countries include Scandinavia and Finland

2.3.1.1. Effective degradation of feed protein

Degradation of feed protein is measured using the *in sacco* method (MEHREZ and ØRSKOV 1978), bearing in mind the recommendations of the CEC-EAAP workshop (OLDHAM 1987) and the Nordic Working Group (MADSEN 1985, LINDBERG 1985, HVELPLUND 1990). The effective protein degradation (EPD) is calculated according to a system devised either by ØRSKOV and MCDONALD (1979), MCDONALD (1981) or KRISTENSEN et al. (1982). The method reported by ØRSKOV and MCDONALD (1979) is presented in Appendix 1.

The correct EPD value, determined with the *in sacco* method, is always only an estimate, as it is affected by many factors in the feed and the rumen, e.g., microbial nitrogen contamination of feed residues in incubated samples, loss of particles from the bag, lower microbial density in the bag than outside it in the rumen, and the value used to calculate the rate of outflow from the rumen. The *in sacco* method is not suitable for feeds containing highly soluble protein, if all soluble protein is not degraded in the rumen. The *in vitro* method, whereby inhibitors are used to stop the fermentation, may be useful, but it requires automated laboratory equipment (BRODERICK 1987, BRODERICK and CLAYTON 1992).

2.3.1.2. Microbial nitrogen contamination of feed residues in bag

Feed residues in the bag after incubation include contaminated microbes, and the degradation values obtained are lower than true values (VARVIKKO and LINDBERG 1985, VARVIKKO 1986). In Denmark a stomacher machine was used for washing bags to loosen microbes from the feed residue (MERRY and MCALLAN 1983, HVELPLUND and MØLLER 1987). In France, MICHALET-DOREAU and OULD-BAH (1989) developed a correction formula, whereby microbial nitrogen contamination is regressed against the nitrogen and NDF contents of the sample. On the other hand, after determining the nitrogen bound by NDF LINDBERG (1988) calculated that the EPD of grass is constant and independent of the growth stage or level of nitrogen fertilization.

2.3.1.3. Small particle loss from the bag

In finely ground samples the loss of nitrogen in small particles is a problem, even a severe one, especially severe in oats, ranging from 30% to 70% of the total protein content. WEISBJERG et al. (1990) designed a correction formula for degradation values adjusted for particle loss. Without any correction for the loss of N in particles from the bag, this part will have to be included into fraction a, which is protein degraded rapidly in the rumen. In such feeds the EPD value will be excessively high and the AAT value excessively low. For a 60% loss of oat particles the AAT value will be ca. 30% too low. For oil meals the problem is less severe, with particle loss seldom exceeding 15%.

Making corrections for particle loss is not devoid of problems, the underlying assumption being that protein in small particles is degraded at the same rate than the protein remaining in the bag, which may not always be true.

The pore size of the bag fabric cannot be reduced, because intrusion of bacteria into the bag would be reduced. According to MEYER and MACKIE (1986) the optimal pore size was 53 μm, and even then the concentration of microbes was only 60% to 80% of that outside bag in the rumen. With cellulolytic bacteria the effect was even greater. HUHTANEN and KHALILI (1992) reached the same conclusion after measuring enzyme activity in the bag and in the particles present in the rumen. Much higher carboxymethylcellulase and xylanase activity was measured in particles in the free rumen contents than in feed particles within the nylon bag.

In some feeds protein is fully soluble, and then the EPD value is 100% and UDP 0%. This need not be correct, because some of the peptides have been observed to flow the duodenum (CHEN et al. 1987).

2.3.1.4. Fractional outflow rate from the rumen

In the Nordic system the outflow rate of particles

from the rumen is constant, k = 0.08 (HVELPLUND and MADSEN 1990). In France the k-value is also constant, i.e. 0.06 for all feeds, but in Britain it varies from 0.02 to 0.08 depending on the feeding level (VÉRITÉ and PEYRAUD 1989, ARC 1984). The outflow rate for particles of concentrate has usually been reported to be higher than that of roughage particles (COLUCCI et al. 1982, COLUCCI et al. 1990). At maintenance level the outflow rate of particles has been measured as 3 to 4 per cent, rising to 6 to 9 per cent at higher feeding levels (GANEV et al. 1979, LINDBERG 1982, ELINAM and ØRSKOV 1984, COLUCCI et al. 1982, HADJIPANAYIOTOU et al. 1988, COLUCCI et al. 1990).

Markers, for instance straw or protein feed treated with neutral detergent and mortanded with chromium, were used in the studies quoted. Such treatment removes the soluble fraction of the marker carrier, and it behaves as indigestible feed particles. The outflow rate of indigestible NDF has been found to be much higher than that of digestible NDF (TAMMINGA et al. 1989, HUHTANEN and KHALILI 1991). This can be confirmed by means of the rumen evacuation technique, when the average rumen content can be accurately determined, and the ratio between daily faecal output of cell wall components and those contained by the rumen gives an indication of the average rate of passage of cell wall components from the rumen (ROBINSON et al. 1987, TAMMINGA et al. 1989).

The nitrogen flowing into the duodenum comprises ammonium nitrogen, with an outflow rate similar to that of the liquid phase, undegraded feed nitrogen with an outflow rate similar to that of NDF, and microbial nitrogen, which in the liquid phase is partly free and partly attached to feed particles. The outflow rate of nitrogen is between that of the liquid phase and that of the solid phase according to TAMMINGA et al. (1989). They reported an increase in the outflow rate of total-N from 0.038 to 0.060 when the feed intake of dairy cows increased from 6.0 to 24.0 kg per day. The average outflow rate of NDF, which is close to that of undegraded feed protein, varied from 0.019 to 0.025 in dairy cows in two trials. HUHTANEN and KHALILI (1992) reported outflow rates of NDF ranging from 0.016 to 0.017 in cattle on a diet of grass silage.

The *in sacco* method also gives too low degradation values for the potential degradable fraction in the bag (TAMMINGA et al. 1989), probably due to lower microbial enzyme activity in the bag than outside it (MEYER and MACKIE 1986, HUHTANEN and KHALILI 1992). This, together with the overestimated outflow rate for UDP, leads to underestimates of the effective degradation of feed protein in the rumen.

2.3.1.5. Efficiency of microbial protein synthesis

The most important factor for the new protein estimation systems is estimating the synthesizing efficiency of microbial protein in the rumen, as microbial protein accounts for 40% to 80% of the total amino acid nitrogen (AAN) entering the small intestine (SMITH 1975, SNIFFEN and ROBINSON 1987). All systems are based on constant factors related to the feed energy released in the rumen, but they differ in terms of how the energy released from the feed for use by microbes is estimated. The AAT-PBV system relates the energy released in the rumen to the total digestible carbohydrate content (DCHO), the British system relates it to the fermentable organic matter in the rumen (FOM), or total ME, whereas the French system relates it to the total digestible organic matter content less the sum of the ether extract, bag UDP and fermentation products.

The experimental data shows great variations in the efficiency of microbial protein synthesis between diets. For instance, the AAT-PBV system is based on a value of 20 grams of microbial amino-nitrogen per kilogram of DCHO. The figure was arrived at in an experiment where the microbial efficiency on a concentrate-rich diet was 60% lower than on a roughage-rich diet (20 g vs. 32 g AAN/kg DCHO) (HVELPLUND and MADSEN 1985). The flow of microbial nitrogen was measured from the difference between the total NAN content and the undegraded feed nitrogen, measured by the *in*

sacco method. The DAPA (2,6-diaminopimelic acid) system was also used to measure the flow of microbial nitrogen, and this gave approximately 10 per cent higher efficiency values for protein synthesis with concentrate-rich diets compared to the difference method (HVELPLUND and MADSEN 1985).

TAMMINGA (1981) measured an average efficiency value of microbial protein synthesis of 32 N grams per kilogram of fermentable carbohydrates in the rumen (FOM), with a variation of 25 to 38 grams. HAGEMEISTER et al. 1980 reported value of 35 g microbial N synthesis per kg FOM. In a review by ARC (1984) the microbial efficiency was 26.8 grams microbial N/kg fermented organic matter (FOM) with a grass silage diet, whereas the corresponding figure for silage diet with concentrate was 36 grams. The value of 32 grams was taken as a constant for this system.

Microbes in the rumen either exist free in liquid, or are attached to small feed particles, most of the microbial mass being of a particle-associated population and microorganisms associated with protozoa (OLUBOBOKUN et al. 1988). It is therefore important to know how the markers behave with microbes in different pools. Protozoa contain no diaminopimelic acid, and the microbes in particles contain less DAPA and ribonucleic acid (RNA) than the microbes in the liquid phase (MERRY and MCALLAN 1983, CRAIG et al. 1987). There is no difference in the purin content between particle and liquid associated microbes, but the ratio of purin over total nitrogen is lower in protozoa than in bacteria (FIRKINS et al. 1987a). The use of these markers will result in excessively low microbial protein flow rates. FIRKINS (1987b) observed a 10% lower nitrogen flow rate in duodenal bacteria using purins as markers, compared to ^{15}N.

Increasing the feeding level will also increase the efficiency of microbial protein synthesis. This effect is minimal in growing cattle (FIRKINS 1987b, GLENN et al. 1989, ROOKE et al. 1992), but in dairy cattle the effect of feeding level is really significant. Increasing the feed intake from 6.0 kg to 17.4 kg organic matter (OM) per day resulted in an increase in bacterial nitrogen yield at a growing rate 17.4-34.8 g bacterial N/kg OM apparently digested in the rumen (OMADR) (ROBINSON et al. 1985). The authors suggested that an increase in the quantity of fiber escaping the rumen, associated with increased feed intake, stimulates bacterial escape by washing out particles that are at an earlier stage of digestion with more bacteria attached. As the retention time of particles in the rumen decreases, the number of attached bacteria may increase rapidly. This may also explain the high efficiency values of 40 to 60 g microbial N/kg OMADR in dairy cows with a high feed intake (RODE and SATTER 1988, MCCARTHY et al. 1989, KLUSMEYER et al. 1990, CAMERON et al. 1991).

Fermented feeds, such as grass silage, contain fermentation products, that are not utilized as energy by microbes. Only about 25% of the energy derived from lactic acid is utilized by microbes (DEMEYER and VAN NEVEL 1979). On the other hand, CHAMBERLAIN (1987) observed that ca. 50% of the energy released by fermenting proteins is utilized by microbes. The new systems do not take into account these factors. NOUSIAINEN (1992) presented results on how this DCHO-correction affects the EPD and AAT values of silage. He also calculated the true indigestible duodenal protein as a function of the crude protein content of silage, and used a double pool model (ARC 1984). AAT-values, calculated with the system, increased from 71 to 83 g/kg silage DM, when crude fibre content increased from 26% to 32% of DM. The corrected AAT-values increased less, from 72 to 78 g/kg DM, respectively.

The results reported by JAAKKOLA et al. (1991) showed that microbial nitrogen yield decreased when the quantity of fermentation products increased. However, compared to silage, the microbial yield was lower with hay (JAAKKOLA and HUHTANEN 1990). There are inexplicable variations in the efficiency of microbial protein synthesis. For instance, JAAKKOLA et al. (1991) measured a mean efficiency of 39 g MN/kg OMADR in growing steers. JAAKKOLA and HUHTANEN (1992a) used growing steers again, obtaining a mean efficiency of only 15 g MN/kg OMADR. In both trials the

same methods and markers were used, and the feed was in both cases direct cut grass silage.

These results showed that many related factors affecting microbial synthesis in the rumen remain unexplained.

2.3.1.6. Amino acid content and digestibility of duodenal proteins

The proteins flowing through the duodenum consist of microbial, undegraded feed protein and endogenous protein. According to many published reports the proportion of amino acids in microbial protein is ca. 80% (ref. HVELPLUND and MADSEN 1990), and this value has been adopted by several systems (ARC 1980, NRC 1985, VÉRITÉ and PEYRAUD 1989). In the AAT-PBV system the proportion of amino acid in the microbial protein is lower, being 70%, based on the studies of HVELPLUND and MØLLER (1980). Later HVELPLUND (1986) estimated the proportion of amino acids to be between 0.62 and 0.72 in 49 isolated bacterial samples, and he still considers 70% to be the correct value. That value is also used in Germany (ROHR 1987).

The proportion of amino acids in undegraded protein is estimated as 100% by many systems (ARC 1980, NRC 1985, VÉRITÉ and PEYRAUD 1989). The AAT-PBV system uses estimates of 85% for concentrates and 65% for roughage. This is consistent with the data in eighteen published papers collected by HVELPLUND (1986).

The digestibility of the duodenal amino acids varies in different systems: 80% (NRC 1985), 85% (ARC 1984) and 90% (ROHR 1987). In the French system digestibility of microbial amino acid protein is 80%, and that of undegraded feed protein varies from 55% to 90% (VÉRITÉ and PEYRAUD 1989). In the AAT-PBV system the rate of true digestibility is 85% for amino acids of microbial origin and 82% for those of feed origin (MADSEN 1985). The true digestibility of undegraded feed protein can be determined by using the duodenal bag (or mobile bag) technique, whereby feed samples incubated in the rumen and abomasum are passed through the small intestine in small nylon bags (HVELPLUND 1985, RAE and SMITHARD 1985, VOIGT et al. 1985, VARVIKKO and VANHATALO 1988, VÉRITÉ and PEYRAUD 1989, HVELPLUND and MADSEN 1990). If TU means truly indigestible feed protein, the digestibility of UDP equals (UDP-TU)/UDP (VÉRITÉ and PEYRAUD 1989, HVELPLUND and MADSEN 1990). This method was adopted by the AAT-PBV system (HVELPLUND 1990). TU can be determined from an intact feed sample using the mobile bag technique for concentrates. Preincubation in the rumen is necessary for forage, as it increases digestion of feed protein in the intestines (HVELPLUND et al. 1992).

2.3.1.7. Utilization efficiency of amino acids for lactation in dairy cows

The utilization efficiency of amino acids is affected by the potential performance of the animal, the quantity of energy and AA supplied, and the balance of AA in the feed (RULQUIN and JOURNET 1987, OLDHAM 1987a). The theoretical maximum rate of 82% for efficiency would apply only when an ideal mix of AA is supplied, and the AA input places the first limit on performance (OLDHAM 1987a). The optimal values for efficiency obtained at normal feeding levels of energy and protein are, however, ca. 20% lower, (RULQUIN and JOURNET 1987).

The British protein evaluation system has adopted the value of 0.80 for the utilization efficiency of truly absorbed amino acids (ARC 1984), which may be too high for practical applications (OLDHAM 1987b). KAUFMANN (1977) estimated the utilization rate to be 70%, and PIATKOWSKI et al.(1990) suggested the same. The French system adopted the value of 64% for utilization efficiency of PDI in lactating cows, as values ranging from 0.58 to 0.69 were obtained in 24 trials (VÉRITÉ and PEYRAUD 1989). The AAT-PBV system adopted a variable rate of utilization efficiency ranging from 0.75 to 0.80 (MADSEN 1985). In a practical feeding trial a lower efficiency rate was obtained, and there have been great variations within a single year and on one site (KRISTENSEN et al. 1985, OLDHAM

1987a).

The protein requirement for milk production can be calculated by dividing the milk protein yield by the efficiency of protein utilization. In the French system the protein requirement is 48 g of protein digested in the intestine per kilogram of milk (31/0.64). The recommendation for AAT requirement in the Nordic system is 37-42 g AAT/kg ECM (HVELPLUND 1990) corresponding an efficiency of 0.74-0.84.

3. Objectives of the study

In Finland milk production is mainly based on forage and grain feeding. Unwilted grass silage usually accounts for one third of the total net energy intake (fattening feed units) in recorded herds. Grain is supplemented by protein concentrates, soybean meal (SBM) and rapeseed meal being the most important ones. Rapeseed (mainly of turnip rape, *Brassica campestris* L.) is the only important oilseed plant that thrives in Finland. The present study examined how milk yield and quality is affected by various quantities of rapeseed meal in the diet, and the different treatments of rapeseed.

The feed protein evaluation in dairy cattle, which in Finland is still based on digestible crude protein, is (or will be) based on the AAT-PBV system in neighbouring Scandinavia. The second objective of this study was to compare the DCP and AAT-PBV systems using data of Finnish milk production trials as material, from which various parameters describing the utilization of feed protein were calculated.

4. Material and methods

The experimental data of experiments of 1 to 5, including the materials and methods, is presented here. The data concerning experiments 6 to 9 has been reported elsewhere (See Table 12). The data of experiments 1 to 7 included feed intake and milk production with calculated DCP and AAT intakes, whereas the AAT intake was excluded in experiments 8 and 9 (RSM vs. control).

In all experiments unwilted grass silage was given *ad libitum* (in one experiment silage or hay cut at the same growth stage was given). The quantity of concentrate was fixed at 6-8.5 kg per day, except for two trials, in which the concentrate was given according to milk yield (Exp. 2 and 6).

Table 12. List of the experiments, of which the data was used in this study.

No. of Exp.	Research Station	Years	Number of Cows	Diets	Experimental design	Treatments	Source
1	Viikki[1)]	1983-84	24	4	Continuous, comparison period of 11 weeks	RSM levels, treament of RSM	
2	"	1984-85	24	2	Continuous, comparison period of 12 weeks	Treatment of RSM	1
3	"	1987-88	10	5	Two 5x5 Latin squares, 25-day's periods	RSM levels, treatment of RSM	
4a	Maaninka[4)]	1987-88	8	4	Two 4x4 Latin squares, 4-week's period	RSM-levels, silage	2,3
4b	Maaninka[4)]	1987-88	8	4	Two 4x4 Latin squares, 4-week's period	RSM-levels, hay	2,3
5	Viikki[1)]	1989-90	14	7	Cyclic change-over, 4 periods of 4 weeks	RSM-and SBM-levels and treatments	
6	Jokioinen[3)]	1986-87	30	6	Continuous, comparison period of 8 weeks	RSM-levels, silage growth stage	
4							
7	Viikki[1)]	1987-88	12	6	Cyclic change-over, 4 periods of 4 weeks	Treatment of RSM	5
8	Suitia[2)]	1987-88	20	4	4x4 Latin square, 4-week period	RSM-level	6
9	Viikki[1)]	1990-91	16	8	Cyclic change-over, 4 periods of 4 weeks	RSM-level	7

Research station:
[1)] University of Helsinki, Viikki Experimental Farm; [2)] University of Helsinki, Suitia Experimental Farm; [3)] Agricultural Research Center of Finland, Department of Animal Production; [4)] Agricultural Research Center of Finland, North Savo Experimental Station
Sources:
1) TUORI & SYRJÄLÄ-QVIST (1987); 2) TUORI & SYRJÄLÄ-QVIST (1988); 3) SUVITIE & RINNE (1988); 4) HEIKKILÄ et al. (1989); 5) HUHTANEN (1991): 2 experimental groups; 6) HUHTANEN et al. (1991): 2 experimental groups; 7) HUHTANEN (1992, unpublished)

4.1. Experiment 1

4.1.1. Animals and management

Twenty-four Finnish Ayrshire cows calving in the autumn were used in a continuous milk production trial. Sixteen cows were multiparous and eight primiparous. The cows were housed in a cowshed with individual stalls. Feed was given individually twice a day, and orts were collected and weighed once a day. The feed was accessible for seven hours daily. Unwilted grass silage was given in sufficient quantities to ensure orts averaging 5% to 10% of the mass of the silage, with intakes measured and recorded. Concentrate was offered at a fixed rate: 7 kg per day during lactation weeks 1 to 14, and 3.5 kg per day during weeks 15 to 28. The daily ration of hay was 2 kg. The cows were milked twice daily commencing at 6 a.m. and 4 p.m. The milk was weighed and the figures were recorded with a True-test milkometer five days a week. The cows were weighed every second week. This report includes feed intake and production data for lactation weeks 4 to 14 only.

4.1.2. Experimental design and treatments

A randomized block design with four treatments was used. The cows were taken to the trial a few days after calving, being assigned to six blocks according to calving date and age, and then assigned within each block randomly to four treatments. The experiment covered lactation weeks 1 to 28, with the exception of the last blocks, which ended at about lactation week 20. One heifer died during the trial in her 19th week of lactation. The treatments consisted of diets including three different protein ratios in the concentrate and one group with treated protein. The control diet consisted of a concentrate including only a grain mixture, whereas the other diets consisted of a concentrate with 23% or 37% protein supplement of which 88% was rapeseed meal. The smaller protein supplement was either untreated or heat-treated.

4.1.3. Feeds

The feeds used in the experiment were direct cut silage, mainly of the first grass crop of the season, containing timothy, meadow fescue and red clover (ratio 60:20:20). The herbage was harvested with a precision harvester, and 5 litres of formic acid per tonne of silage was applied at harvesting. The silage was stored in concrete tower silos. The hay consisted mainly of timothy.

A pelleted protein supplement consisting of 88% either untreated turnip rapeseed meal (*Brassica campestris* L., var. "Span" and "Torch", both single low varieties), or dry heat-treated rapeseed meal (*Brassica napus* L.), 8% dried brewers grain and 4% molasses. The control group was given the same amount of molasses with grain as a supplement. The dry heat-treatment was performed by Öljynpuristamo Oy, Helsinki.

The total glucosinolate content of untreated RSM was 43 μmol/g of fat-free matter, and of treated RSM 8 μmol/g fat-free matter, measured by gas-liquid-gas chromatography (GLC). The grain mixture of oat and barley (ratio 1:1) was preserved with propionic acid and rolled before feeding. The cows were also given daily 250 grams of a mineral mixture containing 17.5% Ca, 7.8% P and 6.0% Mg.

4.1.4. Sampling and analytical methods

The silage samples taken weekly were dried at 103°C for 24 hours to determine their dry matter contents. The dry matter content was corrected for volatile losses by adding 41% of lactic acid, 89% of volatile fatty acids (VFA) and 100% of ammonia (PORTER et al. 1984). The samples taken for establishing the fermentation pattern of silage, and for the *in sacco* determination, were kept frozen until analysed. For analysis the samples were pooled to seven batches according to the date and origin of the silage. Samples were taken from every new batch of hay and grain brought into the barn. For proximate (Weende) analysis samples were dried in a vacuum at 50°C and then milled through a 1 mm screen. The

in vitro digestibility of silage and hay was measured (TILLEY and TERRY 1963), and standard procedures were followed in the proximate analysis. Samples of fresh silage were analysed for reducing sugars (SOMOGYI 1945, with modifications of SALO 1965), lactic acid (BARKER and SUMMERSON 1941), volatile fatty acids (HUIDA 1973), ammonia nitrogen (MCCULLOUGH 1967), and for total and water soluble Kjeldahl nitrogen. Degradation of protein was determined in a sheep *in sacco* (MEHREZ and ØRSKOV 1977, SETÄLÄ 1983). The incubation times of concentrates were 2, 5, 8 and 24 hours, and an additional 48 hours for silage and hay. The sheep was given a mixture of silage, hay and barley (ratio 45:45:10 on DM basis). The glucosinolate content of RSM was measured at the Department of General Chemistry of the University of Helsinki, using the GLC method described by HEANEY and FENWICK (1980), with minor modifications as per HASE et al. (1988). The digestibility values for concentrate feeds in calculating feed values were derived from feed tables (SALO et al. 1982). Energy corrected milk was calculated according to SJAUNJA et al. (1990).

Milk samples were taken once a week (evening and morning) and analysed at the laboratory of Valio Finnish Co-operative Dairies' Association for fat and protein with the infra red (IR) technique on a Milko-Scan 300 (Foss Electric, Denmark). Milk urea was measured using the enzymatic colorimetric method with a KONE CD analyzer (RAJAMÄKI and RAURAMAA 1984). Goitrin content (L-5-vinyloxazolidine-2-thione) was measured at the laboratory of Valio using high performance liquid chromatography (HPLC) (BENNS et al. 1979) and a reverse-phase column (RAURAMAA 1983).

4.1.5. Statistical analysis

A fixed model of least squares variance analysis was used to calculate the corrected means for intake and production data (HARVEY 1966):

$$y_{ijk} = \mu + diet_i + block_j + \varepsilon_{ijk}$$

The WSYS statistical software developed by VILVA (1989) was used in all calculations.

4.2. Experiment 2

4.2.1. Animals and management

Twenty-four autumn-calving Finnish Ayrshire cows, twelve primiparous and twelve multiparous, were used in a continuous milk production trial. A standardization period of 4 weeks preceded the test period of twelve weeks. At the start of the trial the time since calving averaged 38 days. Concentrate was offered at the rate of 0.3 kg per kilogram of fat-corrected milk. Unwilted grass silage *ad libitum* and about 2 kg hay were also available daily. The cows were housed as in experiment 1, except that milk yield was measured with a True-Test milkometer every day. They were weighed once a fortnight, and twice at the beginning and end of period.

4.2.2. Experimental design and treatments

A 2x2 factorial randomized block design was used, the factors being the treatment of RSM and bovine primiparity vs. multiparity. First the cows were assigned into five groups on the basis of calving date and age, after which they were randomly assigned to one of two treatments consisting of different diets. The control diet consisted of a grain mixture including 17% turnip rapeseed meal and the test diet of the same amount of heat-moisture treated turnip rapeseed meal.

4.2.3. Feeds

Direct cut silage was harvested partly with a flail and partly with a precision harvester into a tower silo, and 5 litres of formic acid per tonne was added to it. The grass was predominantly timothy, including some meadow fescue and red clover. A mixture of rolled barley and oat (ratio 1:1) was used as grain. The oat was dried, and the barley was preserved with propionic acid. The RSM was of a single zero variety (Emma). The heat treatment of RSM (™Öpex-treatment at the plant of Öljynpuristamo Oy) was carried out in a pressure chamber in elevated temperature and moisture. The total glucosino-

late content was 49 μmoles in untreated, and 15 μmoles in treated RSM, as measured with the GLC method by the Department of General Chemistry, University of Helsinki.

4.2.4. Sampling and analytical methods

Feed and milk samples were taken as described in experiment 1. For analysis the weekly silage and hay samples were pooled over four-week periods. Every two samples of concentrate were pooled for analysis. The feed was analysed, and the *in sacco* degradable protein was determined as in experiment 1. The *in sacco* determinations were carried out in a sheep feeding on silage, hay, barley and RSM (dry matter ratio 40:40:10:10).

4.2.5. Statistical analysis

The milk production data was analysed in terms of covariance, using the least squares variance analysis (HARVEY 1966):

$$y_{ijk} = \alpha + T_i + A_j + b_1 * P_{ijk} + \varepsilon_{ijk}$$

where T_i is treatment, A_j = primiparity vs. multiparity of the cow; and P_{ijk} is the production rate of the cow during the standardization period. The effects of the block and interaction between age and treatment were not significant, and they were excluded from the model. WSYS statistical software was used for the calculations (VILVA 1989). Feed intake was analysed without covariance. The least squares corrected means were calculated from production data (Appendix 3), but uncorrected data was used in comparing the protein systems.

4.3. Experiment 3

4.3.1. Experimental animals and management

Ten autumn-calving Ayrshire cows were used in a changeover trial of five periods, each lasting 25 days. Five of the cows were primiparous and five were multiparous. At the beginning of the experiment the average time since calving was 50 days (8 to 84 days). The cows were individually fed in a cowshed, having free access to silage at all times. Concentrate was fed at the fixed rate of 8 kg per day. In addition, 250 g of a mineral mixture (17.0% Ca, 8.0% P, 6.0% Mg, 6.0% Na) was given daily. No hay was offered. The cows were weighed on two consecutive days in the beginning, in the middle and at the end of each period, always before the afternoon feeding. The change in live weight was calculated by regression.

4.3.2. Experimental design and treatments

An experimental design of two balanced 5x5 Latin squares was used. Multiparous cows were assigned to one square and primiparous cows to another. The treatments consisted of different ratios or treatments of rape seed meal in the diet. The control treatment (A) consisted of a diet devoid of RSM, in which a mixture of barley and oat (ratio 1:1) was given as concentrate. The grain was partially replaced by varying quantities of heat-moisture treated (Öpex) double zero RSM (var. "Esko"), in treatment B by 12%, and in treatment C by 24%. Treatment D contained 24% untreated single zero RSM (var. "Emma"), and treatment E 24% untreated, glucosinolate-reduced double zero RSM treated with $FeSO_4$.

4.3.3. Feeds

The direct cut silage came from swards of first cut timothy and meadow fescue. It was harvested by precision harvester, treated with 5 litres of formic acid (80%) per tonne of grass, and stored in bunker silos.

The double zero RSM in treatments B and C was heat and moisture treated by the same method as in experiment 2 (Öpex treatment at the plant of Öljynpuristamo Oy). In treatment D the single zero RSM was not heat-treated, and in treatment E, instead of the heat and moisture treatment, the double zero RSM was treated with ferrosulphate to increase the breakdown of glucosinolates. The RSM of single zero variety "Emma" was not quite pure, however,

as some double zero RSM of variety "Kova" was mixed with it. The total glucosinolate content, measured by high performance liquid gas chromatography (HPLC), was 28, 13 and 7 μmol/g fat-free meal respectively for the single zero variety "Emma", heat treated double zero variety "Esko" and glucosinolate-reduced double zero "Esko".

4.3.4. Sampling and analytical methods

The weekly samples of silage were analysed for dry matter and pH, and then pooled over each experimental period. Samples of concentrates (grain, RSM) were taken in the last week of each test period and analysed separately. In addition to the analyses described under experiments 1 and 2, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to GOERING and VAN SOEST (1970). Acid insoluble ash was determined from feeds and faeces with 2-N HCL according to VAN KEULEN and YOUNG (1977). The glucosinolate contents of various rapeseed meals were measured by HPLC according to BJÖRKQVIST and HASE (1988) at the Department of Chemistry, Helsinki University of Technology.

Degradation of protein *in sacco* was determined using a cow, which was given 2 kg of hay, 6 kg of concentrate, and silage *ad libitum* daily. The concentrate contained oat, barley and RSM. The incubation times of concentrates were 3, 6, 12, 24 and 48 hours, with an additional 72 hours for silage. For all feeds the loss due to washing off, and for concentrates the loss of particles containing protein in the bag were determined. Particle loss was taken as the difference between washing loss and soluble loss. Soluble loss of crude protein was determined by taking a sample of ca. 1 g, incubating it in water for one hour at 39°C, centrifuging and washing it three times, after which the amount of nitrogen in the precipitate was determined (HUHTANEN 1991).

Milk samples were collected from four consecutive milkings during the last two weeks of each trial period. Milk fat, protein and lactose contents were determined by the Valio laboratory using the IR technique on a MilkoScan 605. The pooled samples of the last week of each trial period were analysed for urea (RAJAMÄKI and RAURAMAA 1984) by the Valio laboratory, and for goitrin (BENNS et al. 1979, RAURAMAA 1983) by the laboratory of Viljavuuspalvelu Oy. The goitrin content was measured from the samples of primiparous cows only.

The apparent digestibility of the diets was measured using acid-insoluble acid as marker (VAN KEULEN and YOUNG 1977). During the last five days of each trial period faeces samples were taken twice a day from the rectum of each cow. The individual samples were pooled into one sample per period, and kept frozen until analysed upon being dried at 100°C for 72 hours and milled through a 1.5 mm screen.

4.3.5. Statistical analysis

The production data was analysed in terms of least squares variance (HARVEY 1966) using WSYS statistical software (VILVA 1989) and following the model:

$$y_{ijklm} = \mu + S_i + C_j(S_i) + P_k + T_l + (S*P)_{ik} + (S*T)_{il} + \varepsilon_{ijklm}$$

where S_i, C_j, P_k and T_l are the effects of the square, the cows in the squares, the period and the treatment, respectively. The residual degree of freedom was 24. The effect of the treatment was further analysed using contrasts (SNEDECOR and COCHRAN 1980):

C1 linear effect of heat-moisture treated double zero variety RSM

C2 quadratic effect of heat-moisture treated double zero variety RSM

C3 effect of variety (zero variety diet D versus double zero variety diet E). The effect of $FeSO_4$ treatment was confounded.

C4 effect of heat treatment of RSM: heat treated RSM on diet C versus untreated RSM on diet E; the effect $FeSO_4$ treatment confounded.

4.4. Experiment 4

4.4.1. Animals and management

Sixteen autumn-calving Ayrshire cows were in a changeover trial covering four periods. The length of each period was 28 days. Eight of the cows were primiparous and the other eight multiparous. The average time lapse since calving to the beginning of the experiment was 89 days. The multiparous cows had calved 4.9 times on average. The cows were housed and individually fed in a cowshed with separate stalls, where they had free access to forage for 12 hours a day. They were given hay or silage at 6 a.m. and 1 p.m, and concentrates at 8 a.m. and 12 noon. Forage (hay or silage) was available *ad libitum,* whereas concentrates were rationed to 7.5 kg daily throughout the experiment. The daily diet was supplement with 250 grams of mineral mixture (See exp. 3 for composition). The consumption of feed was measured every day, and the milk yield was measured at every milking. The cows were weighed on two consecutive days at the beginning and at the end of each period.

4.4.2. Experimental design and treatments

The experimental design was four 4x4 Latin squares. Two squares, one with primiparous and another with multiparous cows, were feeding on silage, while the cows in the other two squares were feeding on hay. There were four treatments within each square: 0, 8, 16 or 24 per cent of RSM added to a concentrate containing mainly oat and barley.

4.4.3. Feeds

Direct cut silage and hay (a mixture of 20% timothy, 20% meadow fescue and 60% couch grass) were obtained from a sward cut for the first time in the season. In the spring the sward had been fertilized with 112 kg nitrogen, 22 kg phosphorus and 45 kg potassium per hectare. The grass was harvested with flail harvester, adding 4.9 litres of AIV-II-solution (80% formic acid) per tonne. The barn-dried hay was cut at the same time as the silage was harvested. The double zero variety "Esko" was used as RSM.

4.4.4. Sampling and analytical methods

Samples of silage and hay were taken weekly and the dry matter of both, in addition to the pH value of silage, were determined. Samples for feed analyses were taken during the last week of each period and analysed in the same manner as in experiment 3. Faeces samples were subjected to proximate and fiber analysis. Milk samples were taken once a week at morning and evening milkings and analysed at the laboratory of Lapinlahti Dairy for fat, protein and lactose, in addition to employing the NIR technique and Seralyzer reflection photometry for urea analysis. Milk samples taken during the last week of each period were analysed goitrin (BENNS et al. 1979, RAURAMAA 1983) by the Valio laboratory.

The energy content of feed, faeces and milk samples were measured with an adiabatic process on a Parr 1241 adiabatic bomb calorimeter. The energy values of milk samples were determined by soaking a filter paper in ca. 3.5 g of the sample and igniting it in the bomb.

Ruminal degradation of protein was determined *in sacco* using a cow. The cow was given grass silage, hay and concentrate. The incubation times of all feeds were 3, 6, 12, 24, 48 and 72 hours. Washing off and particle loss from the bag were also determined.

The apparent digestibility of the diets was measured using acid insoluble ash as a marker (VAN KEULEN and YOUNG 1977). Faeces were collected from the rectum twice a day during the last five days of each test period.

4.4.5. Statistical analysis

Production data was analysed for RSM levels by means of the least squares variance analysis (HARVEY 1966) with the general linear program of SAS (1989) following the model:

$$y_{ijklmn} = \mu + F_i + A_j + P_k + T_l + (F*A)_{ij} + (F*P)_{ik} + (F*T)_{il} + (A*P)_{jk} + (A*T)_{jl} + (F*A*P)_{ijk} + (F*A*T)_{ijl} + C_m(F_i) + C_m(A_j) + C_m(F*A_{ij}) + \varepsilon_{ijklmn}$$

where F_i, A_j, P_k, T_l and C_m signify the effects of forage (silage vs. hay), the age of the cow (primiparous vs. multiparous), period, treatment and the animal's position in the square, respectively. The residual degree of freedom was 24. The effect of the treatment was further partitioned into the effects of orthogonal contrasts (linear, quadratic and cubic).

4.5. Experiment 5

4.5.1. Animals and management

A changeover experiment of four test periods using fourteen autumn-calving Ayrshire cows. The length of each period was 28 days. Seven of the cows were primiparous, and the other seven multiparous. The time since calving averaged 53 days at the beginning of trial. The cows were housed as in experiment 3. Grass silage was available *ad libitum,* the concentrate being rationed to 8 kg per day during the whole experiment. The diet was supplemented daily with 250 g of a mineral mixture containing 16.8% Ca, 7.7% P, 6.0% Mg and 6.9% Na. The feeds were freely accessible at all times. The cows were weighed on two consecutive days at the beginning, in the middle and at the end of the period. The change in weight was calculated by regression.

4.5.2. Experimental design and treatments

A cyclic changeover design was used in the experiment (DAVIS and HALL 1969), involving seven treatments, fourteen cows and four periods. The treatments consisted of diets including various proportions of SBM, treated SBM (TSBM), RSM, or treated RSM (TRSM) in the concentrate mixture (Table 13).

Table 13. Composition of concentrate mixtures in the experiment 5.

	Treatment						
	Control	RSM1	RSM2	TRSM	SBM1	SBM2	TSBM2
Barley	100.0	44.0	38.0	44.0	45.8	41.6	45.8
Oats	100.0	44.0	38.0	44.0	45.8	41.6	45.8
RSM		12.0	24.0				
TRSM				12.0			
SBM					8.4	16.8	
TSBM							8.4

RSM = rapeseed meal (00-var.), SBM = soybean meal, T = Öpex-treated meal

4.5.3. Feeds

Direct cut silage, containing predominantly timothy and meadow fescue, was harvested from swards for the first time in the season. The swards has been fertilized with 70 kg nitrogen, 30 kg phosphorus and 80 kg potassium per hectare. The silage was harvested with a fine chopper, and 4 to 5 litres of AIV-II solution (80% formic acid) was added to each tonne of silage as a preservative. SBM and RSM were extracted at Öljynpuristamo Oy. The soybeans were imported from USA, the rapeseed was of the Finnish double zero variety "Kova". Both SBM and RSM were either untreated after extraction, or heat-moisture treated (Öpex-method by Öljynpuristamo Oy). The total quantity of glucosinolates, determined by HPLC, was 31 µmoles in seeds, 13 µmoles in RSM, and 8 µmoles in the treated RSM per gram of fat-free matter.

4.5.4. Sampling and analytical methods

The feeds were sampled and analysed as in experiment 3. Silage and hay were analysed for each period, the samples of concentrates were pooled for periods 1 and 2 and again for 3 and 4. The glucosinolate contents were determined using HPLC (BJÖRKVIST and HASE 1988) at the laboratory of Öljynpuristamo Oy. Amino acid content of rapeseed and soybean meals was analyzed using a gas chromatographic method (NÄSI and HUIDA 1982).

Ruminal degradability of the feeds was measured *in sacco* using one heifer. The heifer was given grass silage, hay and 1.5 kg concentrate daily. The incubation times were 3, 6, 12, 24, 48 and 72 hours for concentrates, with an additional 96 hours for silage and hay. The loss through washing off the bag was determined for all feeds, as well as particle loss for concentrates.

Intestinal disappearance of protein was measured for intact rapeseed and soybean meals by the mobile nylon bag technique (HVELPLUND 1985, VARVIKKO and VANHATALO 1988). Approximately 1.1 g of feed was weighed in 3.5 x 5.0 cm heat sealed polyester bags. The pore size of the cloth was 16 μm and the open surface 5% of the area. The bags were introduced through the T-cannula into the proximal duodenum. Once excreted, the bags were machine washed for 50 minutes at 40°C. Initial incubations, averaging a batch of six bags each, were carried out in four heifers and one bull. After drying in 60°C all the residues of one batch (one incubation) were combined, milled through a 0.8 mm sieve and analyzed for nitrogen.

Milk samples were taken over 2 milkings and pooled to one sample in the third and fourth week of each test period. Milk fat, protein and lactose was determined by the IR-technique and urea was determined once in each period by the Valio laboratory.

The apparent digestibility of the diets was measured in multiparous cows, using acid insoluble ash (AIA) as a marker. The collection and treatment of faeces followed the method used in experiment 3.

4.5.5. Statistical analysis

The following formula for statistical analysis was used:

$$y_{ijklm} = \mu + B_i + C_j(S_i) + P_k + T_l + (B*P)_{ik} + (B*T)_{il} + \varepsilon_{ijklm}$$

where B_i, C_j, P_k and T_l are the effects of the block (viz. primiparity vs. multiparity), the cow in the block, the period and the treatment, respectively. The effect of the treatment was partitioned to the linear effect of protein level (C1), quadratic effect of protein level (C2), effect of SBM vs. RSM (C3) and effect of RSM treatment (C4).

4.6. Estimating the effect of RSM on the milk and protein yield

In each experiment there was a calculated deviation of the RSM group from the 0-RSM group for RSM intake and milk production. These deviations were used as measurements when calculating linear and non-linear regressions between RSM intake and production. Non-linear regression was calculated using the Gauss-Newton weighted least square technique (SAS 1989). Only RSM trials with varying proportions of RSM were included (Experiments 1, 3-6, 8-9).

The effect of treatment on protein protection of RSM was studied in experiments 1-3 and 5, in which a protected RSM group was included.

4.7. Comparison of AAT-PBV and DCP systems

Milk production experiments 1 to 8 were used as data for calculating the parameters, and their coefficient of variation, associated with the utilization of protein in milk production:

i. Linear regression between protein intake and production.
ii. Multiple regression, where protein production was explained in terms of protein intake and corrected ME-intake. The corrected ME was estimated by subtracting from the actual ME intake the intake adjusted for estimated regression, where ME intake was explained in terms of protein intake.
iii. Feed protein utilization in milk production: protein production by feed protein for production (g milk protein/g feed protein). Feed protein conversion in milk production: feed protein for production by milk production (g feed protein/kg ECM).

5. Results and discussion

5.1. Composition and nutritional value of feeds

5.1.1. Chemical composition of feeds

The chemical composition and energy and protein value of the feeds used in experiments 1 to 5 are is presented in Appendix 2, and Tables 14 and 15 contains a summary. The crude protein content of silage was fairly high in all experiments (mean 17.2%, variation 16.3% to 18.5% in DM), while the crude protein content of RSM ranged from 32.7 to 39.1 per cent. The fat content of RSM was higher in experiments 1 to 4, ca. 9% to 7% of DM, than in the fifth experiment, where it was 5% to 6% of DM.

The mean degradation parameters and AAT-PBV values of different feeds in experiments 1 to 5

Table 14. The average composition of the feeds (Exp. 1 - 5).

	No. of analysis	DM, %	In DM, % Ash	Crude protein	Ether extract	Crude fibre	NDF[1)]	ADF[1)]	ADL[1)]
Silage	28	22.9	7.1	17.2	5.6	28.1	50.8	29.2	1.9
Hay	11	85.1	6.8	8.8	2.2	34.7	70.4	36.9	2.7
Hay(early cut)	4	87.5	9.0	18.0	2.4	29.5	58.6	30.6	2.4
Barley	14	82.5	2.6	11.8	2.8	5.1	20.5	5.4	0.8
Oat	14	87.1	3.2	12.7	6.3	10.7	26.5	11.7	2.3
RSM	15	88.7	7.6	36.0	8.0	12.7	27.5	19.0	8.6
TRSM(Öpex)[2)]	13	88.1	7.8	34.9	8.3	13.0	27.1	18.5	7.9
SBM	2	87.9	7.4	49.5	3.0	7.6	13.8	8.0	0.8
TSBM(Öpex)[2)]	2	89.2	6.5	50.1	3.8	8.2	14.6	8.3	0.4

[1)] NDF, ADF and ADL are determined only in Exp. 3-5. [2)] heat-moisture treatment for protein protection.
Average fermentation quality of silage: pH 3.94; (percentage in DM): lactic acid 4.8, sugars 4.2, acetic acid 1.7, butyric acid 0.03; percentage of total N: soluble N 52.8; ammonium-N 4.1
Feeds fed in different experiments: Silage, barley, oats and RSM in all experiments; hay in Exp. 1,2 and 5; early cut hay in Exp. 4, RSM(Öpex) in Exp.1,2,3,4,5; SBM and SBM(Öpex) in Exp. 5.

Table 15. The average energy and protein values of the feeds (Exp. 1-5).

	Silage	Hay	Hay (early cut)	Barley	Oats	RSM Öpex	RSM	SBM Öpex	SBM
per kg DM:									
FFU	0.754	0.561	0.692	1.166	1.038	1.020	1.014	1.056	1.069
ME, MJ	10.64	9.18	10.14	13.62	12.34	12.12	12.07	12.46	12.58
NEL, MJ	5.92	5.19	5.90	7.99	7.20	7.26	7.23	7.48	7.56
DCP, g	124	53	128	88	102	299	290	445	451
AAT, g	76	75	80	103	72	141	157	183	242
PBV, g	40	-42	42	-49	9	149	115	223	146

FFU = fattening feed unit (0.7 kg starch), ME metabolizable energy according to MAFF (1975), NEL net energy in lactation according to VAN ES (1978), DCP digestible crude protein, AAT= absorbable amino acids in the duodenum, PBV = protein balance in the rumen.
Calculation of AAT/PBV-values: rumen outflow rate (k-value) 0.08, EPD-values calculated according to ØRSKOV and MCDONALD (1979) and values for roughages are corrected for microbial N contamination in bag residues (MICHALET-DOREAU & OULD-BAH 1989), true digestibility of UDP (TD.UDP) is constant 0.82.

are presented in Table 16. The mean values of degradation at different incubation times of feeds in experiments 3 to 5 are given in Appendix 2. The calculation methods of EPD and AAT-PBV values follow the system in general (See footnote in Table 15), except that there is no correction for degradation according to particle loss from the bag during incubation (HVELPLUND and MADSEN 1990).

The average degradability of the silage protein was low (Table 16). In the first two experiments the EPD of silage was only 59% to 68% (with microbial nitrogen adjustment), but rose to between 81% and 87% in the other experiments.

The effect of heat treatment on oilseed meals varied (Table 17). In the first experiment the EPD value of dry-heated RSM was only 11%, while that of the untreated RSM was 50%. In experiment 3 there was only a slight difference in EPD between the untreated and heat-treated RSM. In experiment 4 treated and untreated RSM were not compared, as only Öpex-treated RSM was used. That RSM was relatively highly degradable. In experiments 2 and

Table 16. The average values for protein degradation parameters and AAT-PBV values of the different feeds (Exp. 1-5).

	Degradation parameters % a	b	c	Micr.N corr.	EPD, %	AAT, MPS, g/kg DM	AAT, UDP, g/kg DM	AAT, total g/kg DM	PBV g/kg DM
Silage	43.9	45.7	0.088	8.6	74.9	53.3	22.5	75.8	40.2
Hay	21.5	48.9	0.098	14.3	61.5	57.4	17.8	75.2	-42.0
Hay,e	44.2	41.6	0.092	8.1	74.2	54.8	24.7	79.6	41.6
Barley	23.3	71.4	0.150		69.0	77.3	25.4	102.7	-48.5
Oat	67.7	25.6	0.218		86.3	60.0	11.9	71.9	8.7
RSM	19.5	75.7	0.086		57.8	35.4	105.2	140.6	149.4
RSM-Öpex	6.1	82.6	0.099		50.5	35.9	121.3	157.1	114.6
SBM	20.0	82.7	0.065		57.2	35.6	147.7	183.3	223.3
SBM-Öpex	9.3	100.4	0.037		40.8	35.0	206.9	241.9	145.7

Calculation of AAT/PBV-values: see footnote in Table 15.

Table 17. Protein degradation parameters and AAT-PBV values of RSM in the different experiments.

Exp. no.	Treatment	Degradation parameters % a	b	c	EPD, %	AAT, MPS, g/kg DM	AAT, UDP, g/kg DM	AAT, total g/kg DM	PBV g/kg DM
1 RSM-0	Untreated	19.8	78.1	0.050	49.8	34.8	123.8	158.5	117.5
RSM-00	Heated	4.3	71.2	0.008	10.4	32.3	253.1	285.4	-12.3
2 RSM-0	Untreated	22.5	72.4	0.080	58.7	36.3	104.0	140.3	151.2
RSM-0	Öpex	1.8	72.1	0.085	38.9	36.3	152.0	188.3	77.6
3 RSM-00	$FeSO_4$	4.6	88.8	0.115	56.9	34.6	100.1	134.7	131.4
RSM-0	Untreated	7.4	84.9	0.118	58.0	35.6	97.8	133.4	133.8
RSM-00	Öpex	3.2	95.6	0.103	50.7	35.5	114.2	149.8	108.9
4 RSM-00	Öpex	12.7	79.3	0.147	64.2	36.2	81.8	118.0	149.0
5 RSM-00	Untreated	29.8	65.4	0.095	65.3	35.4	94.4	129.8	195.8
RSM-00	Öpex	13.2	83.2	0.059	48.2	35.4	136.8	172.2	123.2

See footnotes in Tables 15 and 16

5 EPD of untreated RSM was 59% and 65%, and those of Öpex-treated RSM were 39% and 48% respectively.

5.1.2. Intestinal degradation of RSM and SBM as measured by mobile nylon bag technique

The disappearance in the intestines of intact samples of RSM and SBM in experiment 5 was measured using the mobile nylon bag technique (Table 18). Feeds were not treated with pepsin-HCl, as it has a negligible effect on the disappearance of feed crude protein (CP) in the intestine (VARVIKKO and VANHATALO 1991, VANHATALO and ARONEN 1991). Measured with this technique, the heat-moisture treatment had no effect on the intestinal degradation of dry matter and crude protein, a finding also reported by VANHATALO and ARONEN (1991).

The estimated true digestibility of UDP [TD.UDP = (UDP-TU)/UDP] was calculated using predetermined EPD values and the values for indigestible nitrogen of feeds determined in the present study. The true digestibility of UDP, as estimated with this technique, agreed with the results of VANHATALO and ARONEN (1991), when TD.UDP of SBM and RSM of the same varieties and treatments were measured with the bag technique after 10 hours' incubation in the rumen. Here estimates (based on the measured TU value) of true digestibility values of UDP were lower for RSM and higher for SBM than using 7% as the TU value, as proposed in the AAT-PBV system (HVELPLUND 1990). Microbial-N contamination of the mobile bag residues was not taken into account in this estimate, but it was lower for concentrate feeds than for fibrous feeds (VARVIKKO and VANHATALO 1990).

Table 18. Disappearance of DM and CP of intact RSM and SBM during intestinal incubation in mobile nylon bag (Exp. 5).

	EPD (%) in the rumen	Intestinal disappearance (%)		Calculated TD.UDP
		DM	CP	
RSM-untreated	65.3	67.6	89.1	68.6
RSM-Öpex	48.2	68.6	89.1	79.0
SBM-untreated	57.1	85.3	97.1	93.2
SBM-Öpex	40.8	83.4	97.4	95.6
S.E.1)1.971.17				

[1] 5 animals, 4 feeds, 19 observations, resid. d.f. 11 (1 missing obs.)

5.1.3. Amino acid content of RSM and SBM

The amino acid contents of RSM and SBM were measured in experiment 5 (Table 19). After heat and moisture treatment the amino acid content of both RSM and SBM was reduced, but more markedly in RSM than in SBM. Especially the lysine, histidine and arginine contents had decreased by more than 15% in RSM, the corresponding reduction being only 4% to 7% in SBM. Earlier experiences concerning these have been variable: NÄSI and SILJANDER-RASI (1991) found heat and moisture treatment to have a minor effect on lysine content, whereas NÄSI et al. (1985) found a noticeable reduction in lysine and the available lysine content of dry-heat treated RSM. RAE et al. (1983)

Table 19. Amino acid composition of the RSM and SBM (both untreated and Öpex-heat-moisture treated) in experiment 5.

	g/16g N			
	Rapeseed meal		Soybean meal	
	Normal	Öpex	Normal	Öpex
Lysine	5.7	4.8	6.7	6.2
Histidine	2.4	2.0	2.3	2.2
Arginine	6.2	5.3	7.6	7.1
Aspartic acid	7.9	7.4	11.4	11.0
Threonine	4.9	4.6	4.2	4.1
Serine	4.7	4.4	5.2	5.2
Glutamic acid	17.2	15.5	18.3	17.9
Proline	5.8	5.9	5.2	5.1
Glycine	5.5	4.8	4.1	4.5
Alanine	4.6	4.3	4.5	4.4
Valine	5.4	5.2	4.8	4.6
Isoleucine	4.7	4.6	4.8	4.7
Leucine	7.5	7.0	7.9	7.8
Tyrosine	3.4	3.2	4.0	3.9
Methionine	1.5	1.4	0.8	0.7
Phenylalanine	4.5	4.2	5.5	5.4
Total	91.6	84.0	96.9	94.3

reported that treating RSM with formaldehyde (1.2 g FA/100 g CP) reduced lysine content by 29%. Formaldehyde treatment with 0.4 to 0.8 g FA per 100 g CP decreased the lysine content only by 2% to 6% (SETÄLÄ and SYRJÄLÄ-QVIST 1984/85).

5.1.4. Glucosinolate content of RSM

A considerable reduction in glucosinolate content (Table 20) was observed between single-low (Experiments 1 and 2) and double-low varieties of rapeseed (Exp. 3, 4 and 5). The total quantity of glucosinolates was reduced from 40 - 50 μmoles per gram of fat-free material in experiments 1 and 2 to 14 μmoles per gram in experiment 5. However, even the largest quantity of glucosinolates, 49 μmoles in exp. 2, is quite moderate compared to values ranging from 100 to 205 μmoles/g for single-low canola meal (SHAHIDI 1990b).

In 1987 (Exp. 3) the single-low RSM was found to contain less glucosinolates than the canola standard of 30 μg alkenylglucosinolates per one gram of defatted meal. That was due to the contamination of the single-low rapeseed with double-low varieties. The rapeseed meal produced in 1989 (Exp. 5) fulfilled the EEC standard of 20 μmoles per gram of seed (included the indolylglucosinolates 4-hydroxyglucobrassicin and glucobrassicin) (Table 21). Since the cultivation of the new turnip rapeseed variety, "Kulta", began in Finland, the glucosinolate content has dropped to below 10 μmoles per gram of seed (VILKKI 1991).

The main glucosinolates of the double-low turnip rapeseed are progoitrin, glucobrassicanapin, gluconapin and 4-hydroxyglucobrassicin. The proportion of the last one has increased, while total quantity of glucosinolates has decreased. SANG and SALISBURY (1988) measured doubled proportions of that glucosinolate in double-low rapeseed compared to the single zero varieties (Table 10).

Having been crushed and extracted, the rapeseed meal contained about half the quantity of glucosinolates found in intact seeds, as calculated from defatted dry matter. The heat and moisture treatment of RSM further reduced the quantity to a half (Tables 20 and 21). Especially the proportion of 4-hydroxyglucobrassicin had decreased during the processing, whereas the proportion of progoitrin had increased (Table 21). The analytical methods used do not, however, include measurements of the degraded products of glucosinolates. The presence of moisture during heating was found to be an essential factor in reducing the quantity of glucosinolates (REYNOLDS and YOUNG 1964, APPELQVIST and JOSEFSSON 1967, BELZILE et al. 1963, EAPEN et al. 1968, SHAHIDI and NACZK 1990).

Table 20. Glucosinolate content (μmoles/g defatted meal) of the rapeseed meal in different experiments.

Exp. no.	Origin	GNA	GBN	PRO	NAP	Others	Total
1	RSM0 (1983, B. camp.)	10	21	10	2		43
	TRSM (1983, B. napus)	3	2	3	0		8
2	RSM0 (1984, B. camp.)	14	11	21	3		49
	TRSM0 (Öpex) (1984, B. camp.)	3	4	7	1		15
3	RSM0 (1987, B. camp.)	8	8	10	2		28
	RSM00(Öpex) (1987, B. camp.)	4	3	4	1	1	13
	RSM00($FeSO_4$) (1987, B. camp.)	2	1.5	2	1.5	1	7
5	RSM00 (1989, B. camp.)	1.4	3.4	5.6	0.8	2.3	13.5
	RSM00(Öpex) (1989, B. camp.)	1.5	1.6	3.2	0.4	0.8	7.5

TRSM (1983, B.napus) was of Danish origin, all others were of Finnish origin.
Treatments: 1983: dry-heated, 1984-1989 heat-moisture treated in a pressurized chamber; $FeSO_4$ = reduced glucosinolates by $FeSO_4$;
GNA = gluconapin, GBN = glucobrassicanapin, PRO= progoitrin, NAP = napoleiferin

Table 21. Glucosinolate content of RSM in details from experiment 5.

	µmoles/g DM			µmoles/g defatted matter		
	Seed	RSM	TRSM	Seed	RSM	TRSM
Gluconapin	3.0	1.4	1.5	5.0	1.5	1.6
Glucobrassicanapin	3.7	3.4	1.6	6.2	3.6	1.7
Progoitrin	5.6	5.6	3.2	9.3	5.9	3.4
Napoleiferin	0.9	0.8	0.4	1.5	0.8	0.4
Gluconasturtiin	0.2	0.2	0.1	0.3	0.2	0.1
Glucobrassicin	0.1	0.05	0	0.2	0.05	0
Neoglucobrassicin	0.03	0	0	0.05	0	0
4-hydroxyglucobrassicin	3.0	0.5	0.06	5.0	0.5	0.06
S1[1)]	0.8	0.7	0.4	1.3	0.7	0.4
S2	1.0	0.4	0	1.7	0.4	0
S3	0.2	0.2	0.1	0.3	0.2	0.1
Others	0.3	0.2	0.1	0.5	0.2	0.1
Total	18.83	13.45	7.46	31.35	14.05	7.86
-indolyl glucosinolates	3.13	0.55	0.06	5.22	0.58	0.06

[1)] S1-S3 are glucosinolates with S-containing R-moiety

Table 22. The effect of RSM on the digestibility of the diet.

	RSM-level in the concentrate (%)					S.E.	Significance of contrasts		
	0	8	12	16	24		Lin.	Quadr.	Cubic
Experiment 3									
OM	76.6	-	77.2	-	74.7	1.21	0.73	0.22	
CP	72.4	-	74.5	-	74.4	1.13	<0.001	0.024	
EE	61.9	-	67.8	-	67.8	4.41	0.007	0.10	
CF	67.7	-	68.4	-	68.4	1.95	0.49	0.61	
NFE	82.1	-	82.2	-	81.7	1.05	0.49	0.45	
NDF	65.7	-	67.2	-	67.3	2.01	0.081	0.38	
ADF	67.0	-	67.8	-	67.6	2.01	0.54	0.52	
HMC	64.2	-	66.5	-	67.0	2.13	0.006	0.29	
CEL	73.9	-	75.2	-	76.0	1.72	0.010	0.67	
Experiment 4									
OM	78.6	79.2	-	79.2	79.4	1.52	0.16	0.62	0.64
CP	76.5	77.6	-	78.4	79.0	1.66	<0.001	0.48	0.91
CF	68.0	68.1	-	69.4	70.1	2.46	0.010	0.61	0.55
NFE	82.4	83.0	-	82.7	82.7	1.35	0.68	0.48	0.43
NDF	67.6	68.3	-	69.4	70.1	2.29	0.002	0.96	0.81
ADF	66.8	67.5	-	68.4	68.7	2.80	0.041	0.79	0.85
HMC	68.3	69.0	-	70.3	71.5	2.84	0.002	0.68	0.81
CEL	72.4	74.0	-	75.4	76.3	2.76	<0.001	0.63	0.90
Experiment 5									
OM	74.7	-	75.4	-	74.6	0.94	0.88	0.21	
CP	69.1	-	71.8	-	72.2	1.80	0.046	0.37	
EE	64.1	-	65.5	-	64.4	1.77	0.776	0.32	
CF	66.3	-	66.6	-	65.4	1.85	0.51	0.56	
NFE	80.2	-	80.7	-	79.7	0.81	0.47	0.19	
NDF	67.6	-	68.9	-	67.6	1.35	0.99	0.18	
ADF	68.7	-	68.8	-	66.9	1.52	0.13	0.34	
HMC	66.5	-	69.0	-	68.4	1.29	0.077	0.096	
CEL	72.7	-	74.6	-	73.5	1.60	0.54	0.21	

HMC = NDF-ADF (hemicellulose)

5.2. Effect of protein supplement on the digestibility of the diet

The digestibility of the diet was measured in three experiments. In all three experiments the digestibility of crude protein increased significantly (Table 22). This has been reported in most experiments comparing different proportions of protein in feeds. The increase in the apparent digestibility of CP is largely attributable to the declining proportion of metabolic faecal N in total faecal N (GORDON 1980, HOLTER et al. 1982, RAE et al. 1983, MAYNE and GORDON 1984, MURPHY et al. 1985, PEOPLES and GORDON 1989, CODY et al. 1990). There are only few other reports of increased digestibility of fiber (Exp. 4.) (PEOPLES and GORDON 1989). RSM had no effect on the digestibility of OM in the diet, but soybean meal had a positive linear effect (P=0.03) on the digestibility of OM. The same was observed by GORDON (1980) and HOLTER et al. (1982).

5.3. Effect of proportion of RSM on milk production

5.3.1. Feed intake

Details of the results of the experiments 1, 3, 4 and 5 (Experiments with different RSM levels) are given in Appendix 3. In all experiments a part of the grain was replaced by RSM, so that the total concentrate allowance was equal and forage was given *ad libitum*.

A significant increase in the intake of grass silage of 6% to 10%, attributable to RSM, was observed in one experiment only (Exp. 3). In experiments 4 and 5 the forage intake was higher in the control group, which had plain grain as a concentrate. In this case, however, the intake of concentrate by the control groups was lower, because they had more leftovers than the groups given RSM.

Table 24 shows the responses to increased intake of RSM or increased crude protein content. The data is based on experiments 1, 3, 4 to 6, 8 and 9, (excluding the group with the high RSM level in experiment 1, due to reduced feed intake; a total of 24 observations). The observations are deviations between RSM and control group. The responses were positive, but with great variations, as reported by CHAMBERLAIN et al. (1989) and noted in the review (Table 23) of data collected on *ad libitum* silage feeding and in, most cases, fixed quantity of concentrate. SMALL and GORDON (1990) calculated the response to the total DM intake of 0.10 kg per 100 g increase in supplementary protein intake (SCPI). In the present study the response was greater, 0.14 kg DM/SCPI.

5.3.2. Milk yield

In experiments 3, 4 and 5 (Appendix 3) a significant positive linear effect of RSM level on the milk yield was observed. In Experiment 1 milk yield increased at 0-RSM level 1 (20% RSM in the concentrate), but decreased at level 2 (33% 0-RSM in the concentrate). The ECM (energy-corrected milk) value decreased by ca. 4%, the main reason being the reduced concentrate intake of that group. The high glucosinolate content of these meals is likely to have affected the concentrate intake. Furthermore, a reduction in the intake of concentrate by the group given 20% dry-heat treated rapeseed meal was observed. The protein degradation rate of that RSM was only 11% (Table 17), and the lysine content was reduced. The heat treatment had probably been too severe, affecting the palatability, and thus the intake, of the concentrate.

The RSM supplement had no significant effect on the milk protein content. In experiment 4 the milk fat content was significantly decreased by RSM, but there was no similar effect in the other experiments. Fat content of RSM in experiment 4 was 9.4% and proportion of rapeseed fat in the total diet was higher than in other experiments, which may have reduced fat content of milk as reported TESFA et al. (1991) and TESFA (1992). After calculating the deviations between the RSM groups and the control group (the same data was used to calculate feed intake) in milk yield and protein composition and RSM or ME (metabolisable energy) intake, linear regressions between the intake and

Table 23. The effect of protein supplements on the performance of lactating cows on *ad lib.* silage feeding (a review).

Silage CP (% in DM)	Protein supplem.	Variation in the CP-% of concentr. DM	Response per 10 g/kg increase of concentrate CP				Source
			Milk yield (kg/d)	Protein yield (g/d)	Protein content (g/kg)	Silage intake (kg DM/d)	
14.2	SBM	10.9-15.8	0.28	12.0	0.18	0.03	GORDON 1979
	"	10.9-20.0	0.27	11.5	0.16	0.05	
	"	10.9-24.0	0.28	10.2	0.08	0.03	
16.7	SBM	11.8-15.5	0.06	6.2	0.24		GORDON & McMURRAY 1979
	"	11.8-19.8	0.19	10.1	0.25		
	"	11.8-24.0	0.16	9.6	0.25		
	"	11.8-28.9	0.17	7.1	0.11		
	"	11.8-34.4	0.08	4.5	0.11		
12.4	GNC	15.8-20.2	0.48	16.8	0.11	0.19	LAIRD et al.1979
12.3	SBM	15.5-20.1	0.20		0.0		THOMAS et al.1984
12.8	"	15.5-20.1	0.13		-0.04		
18.2	"	15.5-20.1	0.35		0.22		
11.9	SBM	10.0-17.0	0.43	19.7	0.29	0.31[1)]	BURGESS & NICHOLSON 1984
	"	10.0-24.6	0.27	9.6	0.04	0.10[1)]	
11.3	SBM	14.2-17.3	0.06	6.8	0.29	-0.03	MAYNE & GORDON 1985
	"	14.2-20.7	0.09	5.7	0.17	0.00	
	"	14.2-24.1	0.10	5.8	0.16	0.03	
12.7	SBM	12.7-20.7	0.13	8.5	0.24	0.07	MURPHY et. al. 1985
	RSM	12.7-20.4	0.12	7.3	0.19	0.06	
12.8	SBM	19.3-25.0	0.05	4.2	0.02		GORDON & UNSWORTH 1986
15.2	SBM	18.4-25.1	0.10	4.2	0.06	0.03	PEOPLES & GORDON 1989
20.0	RSM	13.5-16.0	0.24	14.8	0.32	0.00	HEIKKILÄ et al. 1989
	"	13.5-18.0	0.11	9.6	0.27	-0.09	
16.0	"	13.5-16.0	0.28	14.0	0.24	0.40	
	"	13.5-18.0	0.13	9.1	0.22	0.22	
15.7	SBM	12.2-21.0	0.19	8.1	0.14	0.07	CODY et al.1990
18.7	SBM,FM	17.5-22.9	0.13	7.4	0.13	0.08	SMALL & GORDON 1990
14.2	RSM	12.7-16.2	0.23	15.0	0.32	0.06	HUHTANEN et al. 1991
15.5	RSM	13.3-17.1	0.52	17.8	0.03	-0.10	HUHTANEN 1992 (unpublished)
	"	14.2-17.8	0.37	22.4	0.22	0.08	
	"	15.8-19.6	0.35	14.7	0.10	0.23	
	"	16.5-20.2	0.36	15.1	0.14	0.10	

FM = fish meal, GNC = groundnut cubes, RSM = rapeseed meal, SBM = soybean meal;
[1)] total DM intake

Table 24. Responses of intake when increasing rapeseed meal or crude protein content in the concentrate or diet (n=24).

	Mean	s.d.	Min.	Max.
per kg increase of RSM DM				
Forage	0.15	0.43	-0.58	1.45
Total intake	0.34	0.40	-0.35	1.59
per 10 g increase of concentrate CP (in DM)				
Forage	0.05	0.13	-0.18	0.40
Total intake	0.11	0.12	-0.111	0.44
per 10 g increase of diet CP (in DM)				
Forage	0.11	0.32	-0.45	0.96
Total intake	0.27	0.30	-0.27	1.06

production parameters were calculated (Table 25). The non-linear regressions are shown in Table 26 and Figures 1-4. The determination coefficients for nonlinear equations were calculated from the regressions between the actual and regressed variable Y's (Table 26).

The effect of RSM on the milk and protein yield deviate only slightly from the linearity, as can be seen from the determination of coefficients in Tables 25 and 26. This is due to the maximum quantities of RSM having been reasonable, the largest being only 1.7 kg DM per day. The reactions in terms of milk yield to the intake of increased quantities of RSM can be calculated from the exponen-

Table 25. Linear regressions estimating the response in milk and protein yield and content: $Y = a + b_1X_1$ (X_1 = change in intake or content of the diet).

Dependent variable Y	Independent variable X	a	b	R^2	S.E.	Significance (P-value)
Δmilk yield (kg/d)	ΔRSM (kg DM/d)	0.168	0.77	22.4	0.56	0.020
ΔECM (kg/d)	ΔRSM "	0.235	0.70	23.5	0.49	0.016
Δprotein yield (g/d)	ΔRSM "	6.75	29.2	31.1	17.1	0.005
Δmilk yield (kg/d)	ΔCP_c (g/kg DM)	-0.01	0.28	33.3	0.52	0.003
ΔECM yield (kg/d)	ΔCP_c "	0.32	0.19	19.7	0.51	0.030
Δprotein yield (g/d)	ΔCP_c "	11.3	7.9	24.5	17.9	0.014
Δmilk yield (kg/d)	ΔCP_d "	-0.06	0.74	33.7	0.52	0.003
ΔECM yield (kg/d)	ΔCP_d "	0.33	0.48	18.1	0.51	0.038
Δprotein yield (g/d)	ΔCP_d "	11.9	19.3	22.0	18.2	0.021
Δprotein yield (g/d)	ΔCP_d (g CP/d)	7.0	0.108	41.0	15.8	<0.001
Δprotein content (g/kg)	ΔME_d (MJ/d)	-0.084	0.071	24.9	0.59	0.013

ΔCP_c = change in concentrate CP, ΔCP_d = change in diet CP, ECM = energy corrected milk yield

Table 26. Non-linear regressions between change in RSM or ME intake and response of milk or protein yield or protein content: $Y=a*(1-e^{-bx})$.

Dependent variable Y	Independent variable X	a	b	R2	S.E.	Significance (P-value)
Δmilk yield (kg/d)	ΔRSM (kg/d)	2.41	0.523	23.9	0.55	0.019
ΔECM (kg/d)	ΔRSM "	2.37	0.520	23.6	0.49	0.016
Δprotein yield (g/d)	ΔRSM "	84.21	0.602	34.3	16.7	0.004
Δprotein content (g/d)	ΔME (MJ/d)	0.61	0.412	25.7	0.59	0.012

tial equation. The responses in terms of milk yield to changes in the quantity of RSM were:

Change in RSM supplement kg/DM/day	0-0.5	0-1.0	0-1.5	0-2.0
Increase in milk yield, kg	0.55	0.98	1.31	1.56

When the response in terms of milk production was calculated against the increase in crude protein content of the concentrate, the response averaged 0.28 kg milk for each change in the crude protein content of 10 g in one kg concentrate (Table 25). Compared to corresponding responses reported in other studies, where the protein supplement has usually been soybean meal (Table 23, mean 0.22), the responses to RSM were slightly greater in the present study. THOMAS and RAE (1988) reported responses of 0 to 0.51 kg milk per a 10 g increase in CP in the concentrate, with soybean meal used as

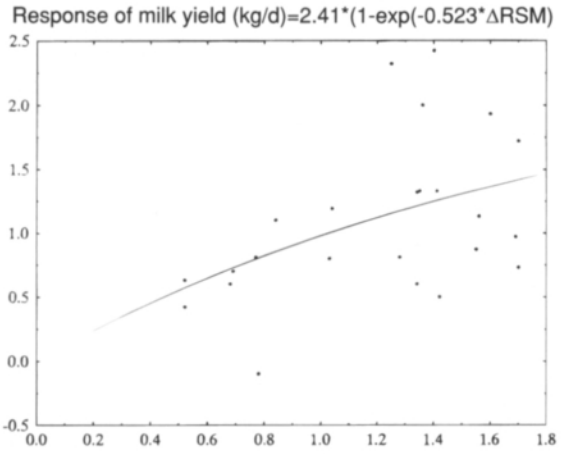


Fig. 1. Response of milk yield on the increase in RSM intake (kg milk per kg RSM DM)

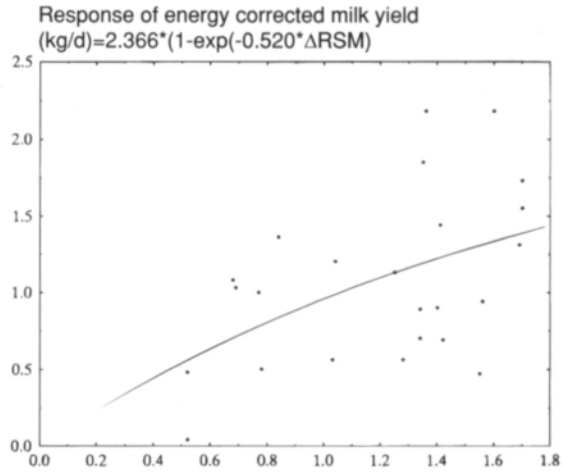


Fig. 2. Response of energy corrected milk yield (ECM) on the increase in RSM intake (kg ECM per kg RSM DM)

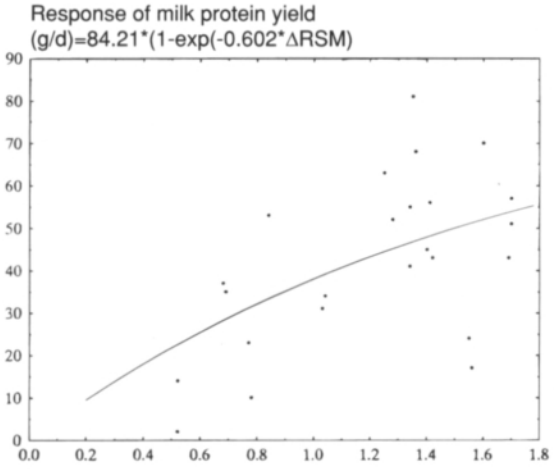


Fig. 3. Response of protein yield on the increase in RSM intake (g protein per kg RSM DM)

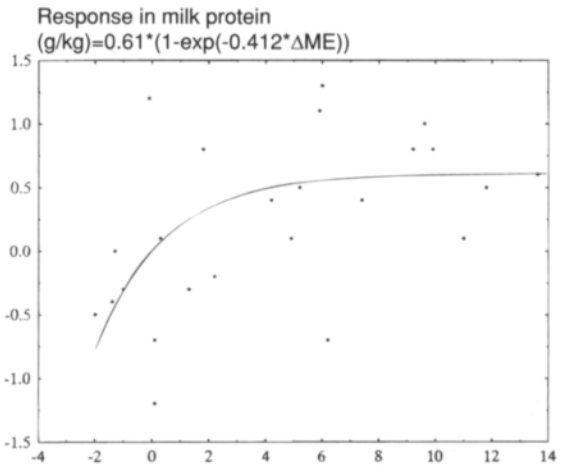


Fig. 4. Response of protein content on the increase in ME intake (g/kg per MJ ME)

the protein supplement.

A change in CP content of the concentrate or of the whole diet (Table 25) gave the highest determination coefficient for a change in milk yield. For both ECM and protein yield the best models were obtained with a change in RSM intake. The change in protein content of milk was explained by a change in the total metabolisable energy (ME) (Table 25), or by changes in the intake of RSM and ME from other feeds (Table 27).

The change in milk protein content was 0.07 g/kg per 1 MJ ME. A similar positive effect has been found in many studies (ETTALA 1976, EMERY 1978, SPÖRNDLY 1986, 1989). In the present study the effect was, however, markedly greater than that reported by EMERY (1978) and SPÖRNDLY (1989), who calculated responses of 0.036 and 0.03 g/kg for each additional MJ of ME. In terms of protein content the corresponding reaction to the increase in energy concentration of the diet (MJ ME/kg DM) was 5.3 g/kg (R^2 = 23.1%, P=0.02). This increase was much greater than that of 1.0 reported by SPÖRNDLY (1989).

The ME concentration of the diet had either a slightly negative effect, or none at all, on the milk or protein yield. In contrast, SPÖRNDLY (1989) reported a highly significant positive effect of energy concentration on milk yield. The negative or zero effect in the present study is attributable to the experimental design, which was based on giving the cows a fixed quantity of concentrate: using RSM instead of grain decreased the ME-concentration of the concentrate mixture, and with increased intake of silage the calculated energy concentration was also diluted. SPÖRNDLY's data was based on feeding trials where concentrate rations were based on milk yield, and hay and straw, in addition to silage, were the sources of roughage. Thus the higher concentrate intake was based on higher milk production, with the result that the energy concentration of the diet increased.

In the present study the effect of forage intake on milk production was not statistically significant, although a slight positive effect was observed. ETTALA (1976) and SPÖRNDLY (1989) reported significant negative effects of the proportion of dietary roughage on milk yield. That, again, can be explained in terms of the data. Feeding concentrate at a constant rate, with high quality silage as almost the only forage, is not comparable to feeding concentrate according to yield, when the intake of roughage will be reduced as that of concentrate increases.

The effect of feed protein supplements on milk yield is partly attributable to increased energy intake (due to increased feed intake and digestibility, or only the latter) and partly to the (additional) protein supply itself. This ''protein factor'' may increase protein synthesis in the rumen, as well as increase the flow of amino acid to the duodenum, or improve the quality of the amino acid mixture absorbed. Rumen microbes can benefit from amino acids introduced to the rumen (BEN-GHEDALIA et al. 1978), and the inclusion of mixtures of branched-

Table 27. Linear multiple regressions estimating response in milk yield and protein content: $Y=a + b_1X_1 + b_2X_2$ (X_1 = change in RSM intake; X_2 = change in ME_{TOTAL}-ME_{RSM}).

Dependent variable Y	a	b_1	b_2	R^2	S.E.	Significance (P-value) X_1	X_2
Δmilk yield (kg/d)	0.17	0.74	-0.003	13.9	0.57	0.08	0.91
ΔECM yield (kg/d)	0.20	1.01	0.03	34.4	0.48	0.007	0.16
Δprotein yield (g/d)	5.3	41.0	1.23	42.0	16.5	0.002	0.12
Δprotein content (g/kg)	-0.14	0.89	0.07	32.0	0.62	0.05	0.03

chain volatile fatty acids (derivatives of leucine valine, isoleucine and proline) has increased the milk production of dairy cows (PAPAS et al. 1984, PEIRCE-SANDNER et al. 1985).

In the present data of 24 observations, the increase in energy-corrected milk (ECM) yield averaged 1.07 kg/day, when the increase in ME intake averaged 4.37 MJ/day. Assuming 5.3 MJ ME/kg ECM, the increased energy intake covered 77% of the growing energy requirement for increased production, when the daily RSM ration was increased from zero to 1.2 kg DM. Under these circumstances the protein factor covers 23% of the increase in milk production. Calculated in a similar way, the proportion of protein in the increased milk yield was 19% according to SMALL and GORDON (1990) and 66% according to MAYNE and GORDON with high concentrates (10 kg/day). With low concentrates (7 kg/day) the supplementary protein had no positive effect. On the contrary, MAYNE and GORDON (1985) reported a stronger reaction to the supplementary protein in the low and medium concentrate diets, but this applies perhaps only to cases where the total food intake is restricted. The above mentioned ME requirement for milk production, 5.3 MJ or 0.19 kg milk/ MJ ME, is theoretical. In practical feeding situations rates of 0.09 to 0.11 kg milk per MJ ME increase in the diet have been reported (GORDON 1984). The protein supplementation of the diet is likely to have affected the partitioning of food energy in the body, increasing energy mobilisation from tissue.

5.4. Effect of protein protection on milk yield and protein content

The effect of protein protection was compared in experiment 1-3 and 5 (Appendix 3). The effect of treatment on the degradation of protein and AAT values is presented in Tables 16 and 17. In trial 2 the treatment of RSM (Öpex heat-moisture treatment) had a significant positive effect on milk yield. In experiment 1 and 3 no such effect was observed, and in experiment 5 the treatment of RSM decreased milk yield, although not statistically significantly.

Table 28 contains a review of some experiments with silage-based feeding having been supplemented by treated protein. In two experiments the milk yield increased significantly after the supplement had been treated with formaldehyde (FA) or heat, viz. REES and ROWLINSON (1983) treated SBM with FA, and BERTILSSON (1991) used heat-treated RSM. In nine other experiments, however, no significant effect has been demonstrated. Although the protection of protein for high yielding ruminants is accepted in theory, only few experiments have produced data to support the procedure, e.g. VÉRITÉ and JOURNET (1977) supplemented a restricted maize diet with an SBM-RSM mixture treated with FA, and FALDET and SATTER (1991) used heat-treated full-fat soybeans. In the last mentioned experiment heating reduced the amount of antinutritional agents in SBM (e.g. trypsin inhibitors), and this was confounded by reduced degradability. KAUFMANN and LÜPPING (1979) demonstrated that SBM treated with FA had an effect on milk and protein yield, but in their experiment the confounding factor was the protein level. Crude protein content of the whole diet varied in the control group from 14.5% to 15.9%, and in the test group from 18.6% to 19.2%.

The objective of protein protection was to prevent the degradation of amino acids in the feed by microbes in the rumen, thereby increasing the flow of amino acids to the duodenum. For several reasons the desired effect is not always achieved (THOMAS and RAE 1988). There is evidence to suggest that microbes in the rumen require preformed amino acids. Overprotection of feed protein may result in a shortage of degradable nitrogen and a shortage of amino acids for microbes in the rumen. PIATKOWSKI and VOIGT (1990) reported that raising the proportion of UDP in the feed protein decreases the efficiency of microbial synthesis per energy unit, which partly compensates the increased flow of amino acids to the duodenum. THOMAS and RAE (1988) concluded that dietary amino acid supplements should be rumen degradable rather than protected.

Table 28. Effect of degradability of protein supplements on the performance in dairy cows on silage/hay based feeding (a review).

Source and treatment of conc. mixt.	CP, % in DM		Intake, kg DM/d		Milk yield (g/kg)	Milk fat (g/kg)	Milk protein	Live weight change
	Concen-trate	Forage	Concen-trate	Forage (kg/d)				
SYRJÄLÄ et al. (1978)								
9.6 % SMP	16.4	14.9	8.1	5.8	18.6	47.5	35.6	
8.2 % FA-SMP	15.9	14.9	6.6	5.9	17.6	43.5	32.9	
RAE et al. (1983)								
9.1 % RSM			9.4	9.4	32.4	3.93	2.97	
9.1 % FA-RSM			9.5	9.5	31.2	4.06	2.94	
REES & ROWLINSON, (1983)								
16 % SBM			10.0	7.5	26.9a	38.6	34.4a	
16 % FA-SBM			9.7	7.4	28.5b	38.1	32.0b	
CASTLE & WATSON, (1984)								
16.7 % SBM	17.4	16.3	6.4	8.8	23.9	37.5	31.0	-0.14
16.7 % FA-SBM	17.4	16.3	6.4	9.3	23.8	37.7	30.9	-0.02
MORGAN (1985)								
8.2 % SBM	15.2	14.0	7.8	9.1	18.8	36.9	33.2	+0.26
8.2 % FA-SBM	15.0	14.0	7.8	9.1	19.6	38.0	32.3	+0.17
24.5 % SBM	21.6	14.0	7.8	8.9	20.3	37.5	32.6	+0.31
12 % FA-SBM + 4.3 % SBM	19.6	14.0	7.8	9.1	20.7	37.3	31.6	+0.31
GORDON (1987)								
23.3 % SBM	21.4	15.6	7.2	26.6	36.9	30.6	-0.39	
10 % FA-SBM + 14.6 % SBM	21.9	15.6	7.2	27.3	37.1	30.6	-0.31	
ROBINSON & KENNELLY (1988)								
24.7 % RSM	18.9	14.6	7.8	10.5	19.6	40.1	34.4	
11.7 % CGM	19.6	14.6	7.9	10.8	20.2	40.7	34.3	
SLOAN et al. (1988)								
15 % SBM	16.7	12.2	9.2	8.7	26.1	39.7	34.5	
10 % FM	16.7	12.2	9.3	8.6	26.3	36.4	34.7	
GARNSWORTHY (1989)								
7.5 % SBM+ 1.3 % urea	18.0	8.5	9.1	4.9	22.5	40.4a	28.7	-0.10
5.5 % FM+ 9 % SBM	17.6	8.5	9.1	4.9	23.9	32.4b	28.0	-0.03
SMALL & GORDON (1990)								
23.7 % SBM	20.6	18.7	6.8	9.4	26.2	40.0	30.2	
23.9 % FA-SBM	20.5	18.7	6.8	9.7	26.7	37.6	30.0	
CODY et al. (1990)								
23 % SBM	21.0	15.7	7.0	8.4	23.0	36.0	30.0	
8 % FM+ 12 % SBM	21.8	15.7	7.0	8.7	23.6	34.5	31.3	
BERTILSSON (1990)								
8 % RSM	14.3	14.1	8.2	10.4	24.9[a]	47.3[a]	32.8	-0.07
8 % HT-RSM	14.3	14.1	8.0	10.5	27.1[b]	45.1[b]	31.7	-0.10
25 % RSM	18.8	14.1	8.0	10.4	27.1	45.3	32.1	-0.09
25 % HT-RSM	19.0	14.1	8.6	10.5	27.9	43.7	32.3	-0.06
HUHTANEN (1991)								
17.6 % RSM	17.5	18.2	7.1	10.0	24.5	43.3	32.2	+0.04
17.6 % HT-RSM	17.4	18.2	7.0	10.1	24.7	42.9	32.3	+0.05
HUHTANEN et al. (1991)								
17.6 % DDS	20.2	14.2	7.1	10.6	24.0	39.7	29.1	+0.13
17.6 % FA-DDS	20.9	14.2	7.0	10.7	24.5	40.0	29.3	+0.23

CGM = corn gluten meal; DDS = dried distiller's grain; FA = formaldehyde treatment, FM = fish meal; HT = heat treatment; RSM = rapeseed meal, SBM = soybean meal; SMP = skim milk powder

[a,b]($P<0.05$)

Chemical or heat treatment of feed protein can affect the availability of amino acids, although no impairment in digestibility is observed. Lysine, tyrosine and cystine are most sensitive to formaldehyde (ASHES et al. 1984).

Cows with negative energy balances may respond more strongly to protected protein (VÉRITÉ and JOURNET 1977, AHRAR and SCHINGOETHE 1979, ØRSKOV et al. 1981, KAIM et al. 1987). In BERTILSSON's (1991) experiment, where heat treatment of RSM increased milk yield, restricted feeding resulted in weight loss by the cows. With high quality grass silage feeding *ad libitum* there has been no advantage in decreasing the degradability of the CP in the supplementary concentrate (CASTLE and WATSON 1984, GORDON and UNSWORTH 1986, GORDON 1987, SMALL and GORDON 1990).

In the present study experiments 1 and 2 were continuous trials, the others being changeover trials by design, in which the length of the period was either 25 or 28 days, and the results of the last two weeks were calculated. Perhaps the apparent lack of response to treated RSM was due to the changeover design of the experiment. CASTLE and WATSON (1984) raised the same question, when FA-treated SBM failed to elicit any response in dairy cows in a changeover trial. However, in an earlier trial of the same design and protein supplements (SBM, groundnut cake and single cell protein) significant differences were noticed by CASTLE and WATSON (1976). They concluded that at an early stage of lactation, when diets were evaluated with a high degree of precision, and small groups of experimental animals were used, the changeover design can play a vital part in providing consistently reliable results.

Now it appears that the changes in glucosinolate content during different experiments may explain the results of the present study. The total glucosinolate content in the untreated RSM was the highest in experiment 2 (49 µmoles), followed by 28 µmoles (Exp. 3) and 13.5 µmoles (Exp. 5). The Öpex treatment did have an effect on milk yield in experiment 2, but not in the other experiments. The difference in protein degradability between untreated and Öpex-treated RSM was minor in experiment 3, being greater in experiment 5. The most likely effect of Öpex treatment was the reduction in glucosinolate content. As the RSM used by BERTILSSON (personal communication 1992) did not differ in glucosinolate content, his results must have been influenced by other factors.

5.5. Goitrin content of milk

The goitrin (5-vinyl-oxazolidine-2-thione) content of milk in experiments 1 to 3 is presented in Table 29. The heat treatment of RSM, or the removal of glucosinolates by ferrous sulphate had the most noticeable effect on the goitrin content of milk. The

Table 29. Goitrin content in milk in the experiments 1-3.

Exp. no.	RSM	Intake of RSM (kg/d) Period1	Period2	Goitrin in milk (µg/l) Period1	Period2	Goitrin output/ progoitrin intake, % Period1	Period2
1	0-RSM	1.21	0.59	39.4	9.0	0.064	0.026
	0-RSM	1.93	0.93	55.6	18.2	0.052	0.030
	00-RSM-Heated	1.20	0.57	4.6	1.0	0.027	0.008
2	0-RSM	1.12	1.04	21.6	12.7	0.016	0.009
	0-RSM-Öpex	1.14	1.16	13.5	9.8	0.031	0.020
3	00-RSM-Öpex	0.84		7.9		0.040	
	00-RSM-Öpex	1.69		22.8		0.059	
	0-RSM	1.70		75.3		0.080	
	00-RSM-$FeSO_4$	1.71		9.0		0.046	

effect of variety could not be compared, because in experiment 3 untreated single-low RSM was used in contrast to treated double-low RSM. Treating RSM reduced the goitrin content of milk significantly in experiments 1 and 2 ($P<0.05$), and in experiment 3 there was a significant difference between untreated 0-RSM and all other RSM supplements.

In experiment 2 a significant difference in goitrin content between the samples taken six weeks apart was also observed. The intake of RSM changed very little during these periods. It is possible that the cows adapted to some extent to the progoitrin in the feed. In experiment 1 the amount of RSM was reduced during period 2, which accounts for the lower goitrin content. However, the ratio between the output of goitrinous milk and the intake of goitrin in the form of progoitrin in the feed was significantly lower during the latter period in both experiments. This ratio varied between 0.009% and 0.064%, being lower than that reported by VIRTANEN et al. (0.05%)(1959), and by BACHMANN et al. (1985), the latter having reported a value of 0.1%. The observations of ARSTILA et al. (1969) do not support the adaptation theory, as the goitrin content of milk actually increased at fixed feeding levels. On the other hand, their observation period was five days only. Hence their results may be explained in terms of the relatively long biological half-life of goitrin in the body.

In the present study the goitrin level of milk was still high in experiment 3 with a diet of untreated single-low RSM, although the glucosinolate content of RSM was lower than in experiments 1 and 2. ARSTILA et al. (1969) reported similar goitrin contents. RAURAMAA (1983) measured goitrin contents of 2-31 µg per litre of milk supplied by 37 dairies throughout Finland, but detected goitrin in only 19 of a total of 224 samples. BACHMANN et al. (1985) measured goitrin contents as high as 700 µg per litre of milk when 1 kg/d RSM was given in the feed. The intake of goitrin was 46.2 µM/day. With an RSM supplement of 0.5 kg/day the goitrin content of milk was 163 µg/l, and with 0.1 kg of RSM, the goitrin content of milk was 38 µg/l. The total glucosinolate content of RSM was about 80 µmoles/g (assuming the progoitrin content was 60%). In the present study the highest goitrin content, 75.3 µg/l in experiment 3, was measured with 2.2 g of progoitrin (5.1 µM/day) in RSM. The variations in the goitrin content of milk are partly attributable to the amount of progoitrin in the feeds and partly to the increased efficiency of goitrin transfer from feed to milk.

Goitrin inhibits the synthesis of the thyroid hormone even when the daily doses do not affect the relative radioiodine uptake of the thyroid (ARSTILA et al. 1969), the rate of 10 µg/l being high enough to produce thyroid enlargement in rats. The quantity needed by man is unknown (ARSTILA et al. 1969). In England it is estimated that the intake of progoitrin from vegetables varies from 5 to 26 µmoles/d, and that 5 per cent of the population consumes more than 117 µmoles/d during the winter. Yet there is no record in the United Kingdom of any diet-related (*Brassica*) thyroid dysfunction in the population (HEANEY and FENWICK 1985).

In Finland the new double zero varieties of RSM contain less than 10 µmoles of glucosinolates per gram of fat-free matter. A 16% mixture of such RSM in a concentrate (1.3-1.6 kg/d) resulted in goitrin contents of 3.5 to 6.4 µg/l in the milk of six cows (TUORI and SYRJÄLÄ-QVIST 1992, unpublished). As the daily milk consumption in Finland averages less than 1 l per person, goitrin intake through milk products cannot constitute any serious risk of thyroid dysfunction in the Finnish population.

5.6. DCP and AAT protein evaluation systems in milk production

5.6.1. Comparison of DCP and AAT systems

The DCP and AAT-PBV systems were compared by calculating different parameters for the utilization of protein in milk production. The data was based on seven milk production trials (Table 12), in which the cows were fed various protein supplements in different quantities and degrees of degradability. A total of 34 observations, covering the

feeding of groups of 4 to 8 cows, provided the mean data.

Table 30 contains the figures for average protein intake andutilisation in different experiments calculated in terms of DCP and AAT. The standard error illustrates the variations between feeding groups. The variation coefficient (s.e./mean) for the utilization of DCP was 8.0% and that for AAT was 4.2%. Measured this way the AAT value of the feeds provided a better basis for estimating the utilization of protein in different experiments. When milk protein yield was explained in terms of protein and ME intake (the effect of protein intake was eliminated from ME intake), the standard error and determination coefficient were 18.5 g and 82.8% using AAT, or 16.4 g and 91.6% using DCP as the protein measurement (Table 32).

THUEN and VIK-MO (1985) and VIK-MO (1985) regressed milk yield against protein intake, which had been calculated with different methods. There, again, the coefficient of variation was higher using DCP than AAT or the French PDI method.

In practical diet formulation protein can be saved by using new protein evaluation methods, whereby the degraded protein in the rumen can be of lower quality than the undegraded protein. The importance of degradation may have been overestimated, especially when feeding was based on high quality grass silage, and the expectations of increased milk production due to new systems may be too high. In a Norwegian experiment two kinds of comparisons were made: on the one hand a constant DCP level with two varying AAT levels (fish meal at two stages of degradation), and on the other hand a constant AAT level with two different DCP levels (less degraded fish meal versus more degraded fish meal plus SBM). The results did not fully support the new system (VOLDEN et al. 1992).

5.6.2. Effect of corrections of AAT-PBV values of RSM and other feeds

The AAT-values above were calculated without correction for particle loss. Further, the unavailable energy of fermentation acids in silage, or the available energy in lactic acid (CHAMBERLAIN 1987), or the rumen degradable feed proteins available to microbes (DEMEYER and VAN NEVEL 1979) were

Table 30. The average intake of feed protein in different experiments and utilization of AAT[1)] and DCP in milk production.

	Experiment								
	1	2	3	4a	4b	5	6	7	S.E.
n	4	2	5	4	4	7	6	2	
Intake (g/d)									
CP	2377	2530	2830	2155	2312	3056	3044	3056	199.8
DCP	1728	1853	2164	1614	1725	2399	2348	2284	175.8
AAT	1456	1357	1273	1095	1159	1449	1335	1361	58.8
PBV	-67	213	715	325	337	643	802	799	147.8
EPD (%)	57.6	67.5	79.8	79.6	75.7	78.0	84.1	79.2	2.28
Protein yield (g/d)	747	724	754	584	598	787	785	787	28.7
Utilization[2)]									
AAT	0.68	0.71	0.84	0.79	0.74	0.74	0.82	0.79	0.032
DCP	0.53	0.46	0.41	0.45	0.42	0.38	0.39	0.40	0.035
g protein/kg ECM									
AAT	43.7	41.8	36.4	38.9	42.8	42.1	37.2	39.8	1.80
DCP	56.3	64.0	74.2	68.4	75.2	81.5	78.0	78.4	5.62

[1)] Calculation of AAT/PBV-values: see footnote in Table 15

[2)] Utilization: milk protein/(protein intake-protein for maintenance)

S.E. is standard error of the estimate from the model: $y_{ij} = \mu + \text{experiment}_i + e_{ij}$; n = 34

not taken into account.

Table 31 shows the effect of corrections of the protein values of feeds in the experiment 5. Loss of nitrogen, with particles escaping the nylon bag is fairly high for oat ca. 60%. Correction (WEISBJERG et al. 1989) decreased the EPD value by 20 percentage points, increasing the AAT-value of oat by 19 g (27%). Particle loss for RSM varied from 16% to 19% between Öpex-treated and untreated RSM. The correction decreased the EPD value by c. 10%, increasing the AAT-value by 27 g and 25 g (16% and 19% respectively). In the earlier experiments the rate of particle loss with RSM was lower, only a few percentage points. The factors that may have an effect on particle loss include the rapeseed variety, the oil extraction processes and milling of the sample, and the pore size of the nylon bag.

Correcting the EPD value according particle loss is based on the assumption that the nitrogen in the small particles escaping from the bag is degraded at the same rate as the protein remaining in the bag. The degradation of protein in different soluble and insoluble fractions should be determined, for instance with the so-called Cornell system (CHALUPA 1992).

Decreasing the outflow rate from the rumen from 0.08 to 0.03 increased EPD value from 38 % to 62 % on treated RSM and from 56 % to 74 % on

Table 31. Effect of some corrections on the EPD and AAT-PBV values of the feeds in the experiment 5.

	Hay	Silage	Barley	Oat	RSM	TRSM	SBM	TSBM
Micr.N correct. of EPD	14.1	8.2						
Water soluble N			2.6	4.0	11.6	4.6	13.4	8.7
Particle loss of N			18.7	59.6	18.9	15.7	11.1	11.3
EPD[1] (k = 0.08)	73.2	82.8	60.3	88.1	65.3	48.2	57.1	40.8
EPD[1][2] (k = 0.08)	73.2	82.8	50.8	68.6	55.9	38.0	50.8	32.5
EPD[1][2] (k = 0.03)	84.5	92.4	73.2	81.4	73.9	61.6	73.2	59.5
AAT1 (system; k=0.08)	73	72	111	71	130	172	183	242
PBV1	-34	55	-47	23	196	123	223	146
AAT3 (k=0.08)	73	72	120	90	155	199	205	271
PBV3	-34	55	-60	-4	159	84	192	104
AAT7 (k=0.08)	76	73	123	95	167	207	219	279
PBV7	-40	52	-66	-13	140	72	169	90
AAT12 (k=0.03)	72	65	104	83	122	149	147	192
PBV12	-31	68	-39	4	204	153	270	213
AAT17 (k=0.03)	103	94	145	116	147	173	175	218
PBV17	-85	20	-106	-52	161	113	224	170
AAT18 (k=0.03)	74	85	103	93	138	162	169	209
PBV18	-28	43	-29	-4	183	139	243	193

[1] EPD with microbial-N correction for roughage (MICHLET-DOREAU & OULD-BAH 1989)
[2] Particle loss correction (WEISBJERG et al. 1990)
Explanations for the AAT values (see also Table 32):
- Efficiency of microbial protein synthesis = 20 g AAN/kg DCHO (AAT1, AAT3, AAT7, AAT12), 30 g/kg (AAT17), (185-1.31*UDP%)*DOM (AAT18);
- Particle loss correction for concentrates (AAT3, AAT7, AAT12, AAT17, AAT18)
- DCHO correction (AAT7, AAT12, AAT17)

untreated RSM. AAT value decreased by 28 % (Table 31). Higher values for outflow rate were measured by using mortanded straw marker or protein supplements, which produce more rumen indigestible particles. Lower values were measured for NDF, which presented the outflow of protein better (TAMMINGA et al.1989).

Using corrected digestible carbohydrate values obtained by calculating the microbial protein synthesis had only minor effect on the AAT-values of the feeds. In silage the AAT-value remained unchanged, when increased energy for microbes from rumen degradable protein was balanced with the loss of energy in fermentation acids. Table 31 also contains AAT-values calculated with a higher value for the efficiency of microbial protein synthesis (30 g AA-N/kg DCHO), or calculated with the formula devised by VOIGT and PIATKOWSKI (1991).

5.6.3. Effect of corrections of AAT values on the utilization of feed protein

Average protein utilization and its coefficient of variation was calculated after the above mentioned corrections or adjustments to AAT-values were made (Tables 32 and 34). In Table 33 milk protein yield has been regressed against the protein and energy intake. Energy intake (ME) is corrected by eliminating the effect of protein intake.

The correction for microbial-N contamination with the k-value of 0.08 gave roughage similar AAT values to those obtained with the k-value of 0.03 without microbial-N correction. The variation in protein utilization also stayed at the same level (Table 32).

Both particle loss correction and that of DCHO reduced the variation in utilization parameters. The

Table 32. Mean values and variation coefficients for utilization of feed protein using different correction for AAT ($yij = \mu + experiment_i + e_{ij}$; n=34).

Feed protein	Assumptions for calculating the AAT					Diet EPD-%	Utilization of feed protein		Protein conversion rate g/kg ECM	
	k-value of roughage[2]	TD.UDP	Micr.N corr.	Particle loss	DCHO-corr. corr.		Mean	CV[4]	Mean	CV
DCP							0.432	8.03	72.0	7.80
AAT1[1]	0.08	0.82	+	-	-	75.2	0.763	4.15	40.4	4.45
AAT2	0.03	"	-	-	-	75.7	0.767	4.24	40.1	4.54
AAT3	0.08	"	+	+	-	70.1	0.693	3.57	44.4	3.87
AAT4	0.03	"	-	+	-	70.5	0.697	3.67	44.1	3.95
AAT5	0.08	"	+	-	+	75.2	0.716	3.91	43.0	4.24
AAT6	0.03	"	-	-	+	75.7	0.719	3.35	42.7	4.27
AAT7	0.08	"	+	+	+	70.2	0.659	3.32	46.7	3.72
AAT8	0.03	"	-	+	+	70.5	0.661	3.57	46.6	4.03
AAT9	0.08	TU=.07[3]	+	-	-	75.2	0.809	5.10	38.2	5.30
AAT10	0.08	"	+	+	+	70.2	0.678	4.14	45.5	4.59

[1] AAT1 is assumed to be according to the AAT-PBV system; [2] for concentrate feeds k = 0.08; [3] TD.UDP = (UDP - TU)/UDP; [4] CV = coefficient of variation (%)

Corrections for AAT: Microbial-N correction for roughage (MICHALET-DOREAU & OULD-BAH 1989); particle loss correction for concentrate feeds (WEISBJERG et al. 1990); DCHO-corr. for all feeds: DCHO + 0.50 * RDP - 0.75 * lactic acid - VFA, (NOUSIAINEN 1992).

Table 33. Standard error and coefficient of variation of milk protein yield:
$(\text{protein yield})_{ij} = \alpha + \text{experiment}_i + b_1*(\text{protein intake})_{ij} + b_2*(\text{corrected ME intake})_{ij} + e_{ij}$.

Protein intake	R^2	S.E. of estimate	CV
DCP	91.6	16.4	2.24
AAT1	82.8	18.5	2.54
AAT2	81.8	18.8	2.58
AAT3	84.9	18.3	2.51
AAT4	84.3	18.6	2.55
AAT5	85.1	16.8	2.35
AAT6	84.1	17.0	2.33
AAT7	87.4	16.4	2.25
AAT8	86.5	16.9	2.32
AAT9	82.2	19.4	2.65
AAT10	86.7	17.7	2.43

effect of changing the value of TD.UDP was unexpected. The constant value of 0.82 resulted in lower variations than with the estimated value of the equation TD.UDP = (UDP-TU)/UDP, where TU had the fixed value of 0.07 (Table 32). The value of TU should probably be determined for different feeds as HVELPLUND et al. (1992) have proposed, otherwise a constant value for TD.UDP is preferable.

The variation was further reduced as the value of protein synthesis efficiency increased, in addition to all the corrections (Table 34). The rumen outflow rate of 0.03 was used for all feeds. This rate is higher or close to the outflow rate of NDF or roughage particles reported in many studies (MÄKELÄ 1956, SETÄLÄ 1983, TAMMINGA et al. 1989, HUHTANEN and KHALILI 1991).

The efficiency of microbial protein synthesis per energy unit varies greatly (HVELPLUND and MADSEN 1985), especially by rising at a direct ratio with increased feeding levels (ROBINSON et al. 1985, SNIFFEN et al. 1987). The production level in the present data was moderate (22.5 kg milk/day, intake 16.1 kg DM/day). The proportion of AAT, calculated from the microbial mass, increased from 79% to 83% of total AAT intake when the value of efficiency was changed from 20 to 30 g microbial amino-N per kg DCHO (with k = 0.03). This may be too high a value for protein of microbial origin, although in some studies involving dairy cows with high milk yields, the proportion has been 70 to 80 per cent (KLUSMEYER et al. 1990, FERLAY et al. 1992). All the same, the variation in protein utilization decreased.

VOIGT and PIATKOWSKI (1990, 1991) found a

Table 34. Mean values and variation coefficients for utilization of feed protein using different efficiencies of microbial protein synthesis
$(y_{ij} = \mu + \text{experiment}_i + e_{ij}; n=34)$.

Feed protein	Efficiency of micr. protein synthesis, g amino-N/kg DCHO	k-value (all feeds)	AAT intake (g/d)	Utilization of feed protein Mean	CV	g protein/kg ECM Mean	CV
AAT7	20	0.08	1311	0.659	3.32	46.7	3.72
AAT11	18	0.03	1190	0.869	3.29	35.4	4.07
AAT12	20	0.03	1289	0.776	2.94	39.7	3.62
AAT13	22	0.03	1388	0.701	2.70	43.9	3.29
AAT14	24	0.03	1487	0.639	2.51	48.1	3.03
AAT15	26	0.03	1585	0.588	2.38	52.3	2.84
AAT16	28	0.03	1684	0.544	2.28	56.6	2.69
AAT17	30	0.03	1783	0.506	2.22	60.8	2.58
AAT18[1)]		0.03	1448	0.663	2.05	46.3	2.43

Corrections: microbial-N for roughage, particle loss for concentrate feeds, DCHO for all feeds
[1)] Efficiency of microbial protein synthesis: (185-1.31*UDP%)*DOM (VOIGT and PIATKOWSKI 1991)

negative correlation between the proportion of UDP and the efficiency of microbial protein synthesis in the rumen. This may be caused by a decrease in the energy released in the rumen, or a decrease in the availability of amino acids and peptides to microbes. In the present study the dietary intake of AAT was calculated using the formula for microbial protein synthetized (MPS) of VOIGT and PIATKOWSKI (1991):

MPS = (185 - 1.31 * UDP%) * DOM

where DOM refers to digestible organic matter (kg). The proportion of amino acid nitrogen was taken as 0.75 and digestibility as 0.85. For UPD the respective values were taken from the Nordic system. Variation coefficients for protein (AAT18) utilization are shown in Table 34.

The residual variance of the utilization of protein was significantly different ($P<0.01$) between AAT1 (Nordic system) and AAT18 (Rostock). However, when AAT intake was calculated using individual AAT18 values for feeds, the variations in the Nordic and Rostock systems differed less. This may indicate that the protein values of the feeds are not quite additive, and a more correct protein intake value can be calculated from the DOM and UDP values of the total diet. This would be a disadvantage to practical diet formulation.

The Rostock method is less sensitive to variations of feed protein degradation. The results of some experiments in the present study agree with that finding, e.g., when reducing the protein degradation of RSM had, on average, no effect on milk or protein yield. With the Nordic method the AAT value of hay was equal or even higher than that of grass silage, regardless of the higher digestibility of organic matter and the crude protein content of silage. The Rostock method gave higher protein values for silage than for hay. The values of protein feeds were also higher than those obtained with the Nordic method (Table 31).

The AAT system gave carbohydrate concentrates very high values compared to DCP values. The AAT value of barley was 40 to 50 per cent higher than that of good quality grass silage, and the value of hay was higher than that of silage cut earlier. JAAKKOLA and HUHTANEN (1992) have showed that the flow of non-ammonium nitrogen (NAN) remained almost equal when the proportion of concentrate (barley plus RSM) increased from 25 to 75% in the diet in terms of dry matter. In that study forage consisted either of direct cut silage or hay cut at the same maturity. Calculated on the basis of AAT, the AAT increase should have been 37% according to the concentrate. When the microbial AAT flow was calculated according to VOIGT and PIATKOWSKI (1991), the increase was almost the same as measured, i.e. 3 to 4 per cent with an increased proportion of the concentrate.

The results of the present study (experiment 4), where silage and hay cut at same maturity were compared, no difference between the two kinds of roughage in terms of milk yield was observed, and the calculated AAT consumption per kg ECM was 3 grams higher on a diet of hay than on silage. A shortage of amino acids was evident, as replacing some of the concentrate with RSM increased the milk production on both diets.

The fermentation characteristics of silage affect microbial protein synthesis. Good fermentation properties increase microbial protein synthesis, compensating for the greater amounts of bypass protein of dried forage (JAAKKOLA et al. 1991).

In experiment 6 (HEIKKILÄ et al., unpublished) silage cut at two different growth stages were compared. Calculated AAT values were equal for both lots of silage (EPD values were determined), yet milk production was 7.7% higher, and ME intake was 5.5% higher with the earlier lot of silage added to the diet than with the later one. Milk yield correlated much higher with CP or DCP concentrations than AAT concentrations in the diet.

There are considerable advantages in using the new protein systems compared to DCP in that the former can be developed far easier. Although these present studies show that on the diets commonly used in Finland, i.e. high quality grass silage and hay with grain-based concentrate, there are some problems associated with the AAT-system, but they can be overcome applying new knowledge.

However, more research is needed to develop a more precise method of estimating the microbial protein synthesis in the rumen, which appears to be the most important protein source for dairy cows.

6. Conclusions

6.1. Glucosinolate content

In the course of the study the types of RSM were changed from single zero to double zero rapeseed varieties, whereby their glucosinolate contents were reduced from 40-50 to 15 μmoles per gram of defatted meal. Heat-moisture treatment further reduced the glucosinolate content by half. The glucosinolate content of the latest Finnish turnip rape varieties is less than 10 μmoles per gram of defatted meal.

6.2. Effect of rapeseed meal on milk yield

The inclusion of RSM in the concentrate to supplement the staple diet of grass silage of dairy cows (silage *ad libitum)* increased the average milk yield by 0.7 kg ECM per kg RSM dry matter. The average protein yield increased by 27 grams per kg RSM dry matter. These increases were statistically significant. Milk yield increased when the ratio of RSM in the concentrate was raised to between 12% and 16 %. Increasing the ratio of RSM further to 24% had a minor effect on milk yield. Although the additional RSM seemed to reduce the fat content significantly in one trial, a similar effect was not observed in the other trials. The effect on protein content of the milk was not significant. The effect RSM had on milk yield and quality was attributed to the increase in energy supply and the specific protein effects. RSM and SBM were of equal value when 12% or 24% of RSM in the concentrate were substituted with SBM with comparable protein contents.

6.3. Protein protection

Protecting the RSM protein by heat-moisture treatment reduced the effective rumen degradation (EPD) by 6 to 20 %-units. In experiment 2, where EPD of RSM was reduced 20 %-units by the treatment, milk yield was improved significantly by heat-moisture treatment (21.9 vs. 23.9 kg milk/d). In two other experiments, where EPD of RSM was reduced 6 or 17 %-units, there was no effect on milk yield. In experiment 1 EPD of heated RSM was 39%-units lower than that of untreated with no positive effect on milk yield. In that experiment heat treatment seemed to be too severe, judging by the reduced quantity of available lysine. One explanation for the different effect of treatment of RSM could be the level of glucosinolates. In experiment 2 the original level of glucosinolates in RSM was high, and was reduced by treatment. In the experiments 3 and 5 the glucosinolate content in RSM was lower, and presumably further reduction by the heat treatment did not give any advantage.

6.4. Goitrin content of milk

Changing from single zero to double zero varieties of RSM reduced the goitrin content of milk. Heat-moisture treating the RSM resulted in a further notable reduction in its goitrin content. For cows fed on heat-moisture treated RSM made of the "Kulta" variety, with a glucosinolate content of 2.5 μmoles per gram defatted meal, the goitrin content was only 3.5 to 6.4 μgrams per litre. Such a low goitrin content of milk should not cause any risk of thyroid problems in people.

6.5. DCP and AAT

The applicable parameters for the utilization DCP and AAT were calculated from the average data of the feeding trials involving 34 groups of cows. The observations related to trials where a staple diet of silage was supplemented with concentrate of varying protein contents. In relating protein yield to the energy and protein supply, DCP was better than uncorrected AAT, whereas the protein utilization varied less with the AAT system than with DCP. The variation in protein utilization was further reduced when AAT was corrected in such a way

that the rumen-degradability of protein increased, or the microbial synthesis became more efficient, or both. It would indicate that as far as cows with high milk yield are concerned, the AAT system exaggerates the role of rumen undegradable feed protein in the supply of absorbable protein. As a result, hay was given a better protein value than silage, and the calculated utilization of AAT was poorer with a diet of hay than with one of silage. As a consequence, the current calculation method leads to AAT variable requirements of AAT according to dietary variations. Using the method of VOIGT and PIATKOWSKI (1991) in estimating MPS, which gave the lowest coefficient of variation, the AAT values of silage, oat and rapeseed and soybean meals were increased, whereas AAT value of barley was reduced compared to AAT values of the system. These changes are supported by the present results.

The present feeding trial data is limited to predominantly silage-based feeding. The results of making corrections to AAT systems indicate trends only, and more basic study is needed especially conserning the efficiency of microbial protein synthesis with high producing dairy cows.

The AAT-PBV system, like the other new systems, has many advantages compared to DCP, which is at the end of its development. With the new protein systems there is more opportunity to fullfill the requirements of diminish nitrogen output into the environment, and it is possible to derive new knowledge concerning the protein metabolism in the ruminant.

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SELOSTUS

Rypsirouhe lypsylehmien valkuaisrehuna säilörehuvaltaisella ruokinnalla

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Lypsylehmien väkirehun täydentämistä rypsirouheella tutkittiin viidessä ruokintakokeessa tuoresäilörehuun perustuvalla ruokinnalla. Rypsirouheen osuus väkirehuseoksessa vaihteli 0-33 % loppuosan ollessa ohran ja kauran seosta. Yhdessä kokeessa oli mukana samat koejäsenet sekä rypsirouheesta että soijarouheesta, ja toisessa kokeessa säilörehu tai samalla kasvuasteella korjattu latokuivattu heinä. Lisäksi tehtiin laskelmia rypsirouheen vaikutuksesta maitotuotokseen aineistosta, johon oli kerätty Suomessa viime vuosina tehdyt rypsivertailukokeet. Valkuaisen hyväksikäyttöä tarkasteltiin pohjoismaisen AAT-PBV -valkuaisjärjestelmän mukaan.

Tutkimusaikana rypsilajikkeet muuttuivat runsaasti glukosinolaatteja (40-50 µmoolia per g rasvatonta rouhetta) sisältävistä 0-lajikkeista 00-lajikkeisiin, joiden glukosinolaattipitoisuus aleni noin kolmannekseen. Rypsirouheen lämpökäsittely Öpex-menetelmällä alensi rouheen glukosinolaattipitoisuuden edelleen noin puoleen.

Kun viljaa korvattiin rypsirouheella (maks. 1.7 kg KA/d) säilörehun ollessa *ad libitum*, säilörehun syönti lisääntyi keskimäärin 0.43 kg KA per kg rypsirouheen kuiva-aineen lisäystä (n.s.). Vaikutus maitotuotokseen oli keskimäärin 0.77 kg ($P<0.02$) ja energiakorjattuun maitotuotokseen (ECM) 0.70 kg per kg rypsirouheen kuiva-aineen lisäystä ($P<0.02$). Rypsirouheen sisällyttäminen väkirehuun 12-16 % lisäsi tuotosta, mutta osuuden noustessa edelleen 24 %:iin vaikutus oli vähäinen. Maidon valkuaispitoisuuteen vaikutti merkitsevästi muuntokelpoisen energian saannin lisäys, valkuaispitoisuus nousi 0.07 g/kg per MJ ME:n lisäystä ($P<0.02$).

Rypsirouheen Öpex-käsittely (kostea kuumennus) lisäsi maitotuotosta merkitsevästi yhdessä kokeessa (21.9 vs. 23.9 kg maitoa tai 23.4 vs. 25.2 kg ECM/d), ($P<0.03$). Kahdessa muussa kokeessa käsittelyllä ei ollut vaikutusta maitotuotokseen. Soijarouheella ja rypsirouheella ei ollut eroa vaikutuksessa maitotuotokseen, kun rouheita syötettiin sama määrä raakavalkuaisena mitattuna.

Maidon goitriinipitoisuus aleni rypsilajikkeiden glukosinolaattipitoisuuden ja rypsirouheen määrän alentuessa. Käytettäessä Öpex-käsiteltyjä 00-rypsirouheita maidon goitriinipitoisuus oli enää alle 10 µg/l (analyysin herkkyysraja 2 µg/l).

AAT:n hyväksikäyttöä maidontuotannossa estimoitiin käyttämällä erilaisia vakioita AAT-PBV -arvojen laskemisessa. Hyväksikäytön vaihtelukerroin aleni, kun laskennallisesti lisättiin mikrobivalkuaisen osuutta AAT:stä. Tähän suuntaan vaikuttavat mikrobi-N kontaminaation huomioiminen *in sacco* määrityksessä, pötsin virtausvakion alentaminen 0.08:sta 0.03:een ja mikrobivalkuaissynteesin tehokkuuden lisääminen. Paras malli saatiin laskemalla mikrobivalkuaissynteesin tehokkuus VOIGT ja PIATKOWSKIN (1991) mukaan.

AAT-PBV feed protein evaluation system, calculation of feed values and requirements

AAT = MCP * aam * daam + UDP * aaf * daaf
MCP = 179 * DCHO (or MCP = 20 g microbial amino-N per kg DCHO)
UDP = CP * EPD
PBV = RDP - MCP

where
AAT = absorbable amino acid protein of the feed
PBV = ruminal protein balance of the feed
MCP = microbial crude protein synthetized in the rumen
CP = crude protein content of the feed
UDP = rumen undegraded crude protein of the feed
EPD = effective degradation of feed crude protein in the rumen
aa = proportion of amino acid protein in the microbial protein (aam) or in the undegraded feed protein (aaf)
daaf = digestibility of undegraded feed amino acids
daam = digestibility of microbial amino acids
DCHO = digestible carbohydrates of feed (dig. crude fibre + dig. nitrogen free extracts)
RDP = EPD * CP (= rumen degraded dietary crude protein)

Constants:

Proportion of amino acid protein in absorbable crude protein:
- 0.70 in microbial crude protein
- 0.85 in undegraded concentrate crude protein
- 0.65 in undegraded roughage crude protein

Digestibility of amino acids (AA):
-0.85 for AA from microbial crude protein
-0.82 for AA from undegraded feed crude protein (UDP)
Digestibility of UDP can be estimated by using the equation of HVELPLUND & MADSEN (1990):

TD = (UDP-TU)/UDP

where TD = true digestibility of UDP in the small intestine
TU = fraction of true indigestible crude protein of the feed

TU should be determined by using the mobile nylon bag technique, and if not determined, value of 0.05-0.07 can be used.

Calculation of EPD value for feeds according to ØRSKOV & MCDONALD (1979):

$p = a + b * (1 - e^{-ct})$
$EPD = a + b * c/(c + k)$

where p = measured bag degradation of protein at time t
a = rapid degraded fraction of protein
b = slow degraded fraction
c = degradation rate of fraction b
k = fractional outflow rate
EDP = effective degradation of protein

Recommendations of AAT values for dairy cattle:

Requirements of AAT are from MADSEN (1985) and later revised by Nordic protein group (HVELPLUND 1990). Requirement for maintenance is 3.25 g AAT * $W^{0.75}$. For the milk production the recommendation was 45 g/kg ECM (MADSEN 1985), now 40 g/ECM (energy corrected milk yield, SJAUNJA et al. 1990), variation 37-42 g. At production levels below 25 kg ECM per day a decrease of 3-4 g AAT per kg ECM may be justified ((HVELPLUND 1990).

In Denmark the AAT-concentration of the diet can be expressed as 97 g AAT/feed unit for high producing, decreasing to about 90 g AAT/FU for low producing cows. In Norway AAT-recommendation (VOLDEN et al. 1992) is:

$$\text{AAT, g/kg ECM} = (40 * \text{ECM} + 0.2 * \text{ECM}^2)/\text{ECM}$$
$$= 40 + 0.2*\text{ECM}$$

Additional requirement for pregnancy is 60, 100 and 172 g AAT/d at the 7th, 8th and 9th month of pregnancy.

The ideal PBV value should be 0, but minimum value of -200 g/d for high producing and -300 g/d for low producing cows can be accepted. As maximum PBV-value 900 g/d is accepted in Denmark ((HVELPLUND 1990) but there is no recommendation in Norway (VOLDEN et al. 1992).

Composition of feeds in experiments 1 and 2

	DM (%)	In dry matter (%) Ash	Crude protein	Ether extract	Crude fibre
Experiment 1					
Silage	24.9	7.4	16.3	5.6	28.3
Hay	83.8	6.3	7.7	2.2	35.4
Barley	75.6	2.5	10.1	2.3	5.8
Oat	82.4	3.5	11.7	5.4	12.2
RSM	88.6	7.4	35.4	9.3	12.8
RSM-heated	86.3	7.5	40.5	4.5	14.6
Experiment 2					
Silage	22.5	6.6	16.8	6.4	30.3
Hay	83.5	6.5	9.6	2.1	34.3
Barley	75.1	2.3	11.4	2.3	5.2
Oat	87.4	3.1	13.3	5.5	10.8
RSM0	88.0	8.0	36.2	6.7	12.5
RSM0-Öpex	88.0	8.0	35.7	6.9	13.0

RSM = turnip rapeseed meal (0-var.), RSM-heated = dry heated rapeseed meal; RSM-Öpex = heat-moisture treated turnip rapeseed meal (0-var.)

Composition of feeds in experiment 3

	DM (%)	In dry matter (%) Ash	Crude protein	Ether extract	Crude fibre	NDF	ADF	ADL
Silage	20.9	6.8	18.5	5.4	27.3	49.7	29.4	1.7
Barley	87.3	3.0	12.7	3.7	5.2	20.9	5.5	0.4
Oat	88.6	3.2	11.5	7.0	10.8	27.3	12.4	2.2
RSM00-Öpex	88.2	8.0	33.3	10.7	12.1	27.2	17.4	7.4
RSM0	88.5	7.7	33.4	10.2	12.9	28.2	18.9	8.6
RSM00-$FeSO_4$	88.8	8.1	33.3	11.5	12.1	25.7	16.5	6.9

RSM0 = turnip rapeseed meal (0-var.); RSM00-Öpex = heat-moisture treated turnip rapeseed meal (00-var.); RSM00-FeSO4 = turnip rapeseed meal (00-var.), glucosinolate content is reduced by ferrosulphate treatment.

Composition of feeds in experiment 4.

	DM (%)	In dry matter (%) Ash	Crude protein	Ether extract	Crude fibre	NDF	ADF	ADL
Silage	21.9	6.6	16.6	5.3	31.0	56.2	33.0	2.2
Hay	87.5	9.0	18.0	2.4	29.5	58.6	30.6	2.4
Barley	87.0	2.4	11.7	2.6	4.9	19.9	5.2	0.8
Oat	88.7	2.7	12.9	7.0	9.3	23.5	10.3	1.9
RSM00-Öpex	87.2	8.0	32.7	9.4	13.7	25.6	17.9	7.0

RSM-Öpex = heat-moisture treated turnip rapeseed meal (00-variety); hay is cut at the same maturity as silage

Composition of feeds in experiment 5

	DM (%)	In dry matter (%) Ash	Crude protein	Ether extract	Crude fibre	NDF	ADF	ADL
Silage	24.4	7.9	17.8	5.3	23.5	46.5	25.2	2.0
Hay	87.9	7.5	9.1	2.4	34.3	70.4	36.9	2.7
Barley	87.5	2.2	13.0	3.3	4.6	20.9	5.5	1.3
Oat	88.3	3.4	13.9	6.6	10.2	28.7	12.5	2.8
RSM00	89.7	7.3	39.1	5.1	13.2	28.0	20.3	9.5
RSM00-Öpex	88.9	7.3	37.9	6.4	13.0	28.5	20.2	9.5
SBM	87.9	7.4	49.5	3.0	7.6	13.8	8.0	0.8
SBM-Öpex	89.2	6.5	50.1	3.8	8.2	14.6	8.3	0.4

RSM = turnip rapeseed meal (00-var.); RSM-Öpex = heat-moisture treated turnip rapeseed meal (00-var.); SBM = soybean meal

Energy values of the feeds in experiments 1-5

	Exp. 1			Exp. 2			Exp. 3			Exp. 4b			Exp. 5		
	FFU	ME	NEL	FFU	ME	NEL	FFU	ME	NEL	FFU	ME	NEL	FFU	ME	NEL
Silage	0.730	10.30	5.67	0.742	10.47	5.79	0.735	10.39	5.78	0.771	10.88	6.04	0.791	11.18	6.32
Hay	0.519	8.77	4.94	0.554	9.07	5.12				0.692	10.14	5.90	0.611	9.69	5.51
Barley	1.166	13.61	7.97	1.163	13.59	7.96	1.172	13.69	8.06	1.173	13.70	8.04	1.158	13.53	7.92
Oat	0.950	11.53	6.61	1.036	12.32	7.17	1.080	12.59	7.38	1.072	12.78	7.51	1.053	12.54	7.35
RSM	1.088	12.96	7.57	0.983	11.82	7.03	1.063	12.40	7.48				0.960	11.59	6.84
RMS-Öpex[1)]	1.065	12.56	7.61	0.984	11.82	7.03	1.072	12.51	7.57	1.019	12.15	7.29	0.981	11.81	7.01
RSM-$FeSO_4$							1.082	12.63	7.67						
SBM													1.056	12.46	7.48
SBM-Öpex													1.069	12.58	7.56

[1)] In experiment 1 dry heat-treated RSM

Average rumen degradability of the feed protein in experiments 3-5.

	No of analysis	Water soluble	Washing loss	Incubation time, hrs 3	6	12	24	48	72
Silage	13		60.6	63.8	70.1	81.1	86.2	90.0	91.3
Hay	1		47.3	48.3	50.4	62.0	79.1	80.9	85.8
Hay(e)	4		56.3	54.8	59.8	73.5	79.8	85.2	85.8
Barley	9	15.3	41.9	56.0	66.4	88.3	91.9	95.4	96.3
Oat	9	14.7	80.0	84.3	88.1	93.6	94.6	94.9	95.0
RSM	12	11.2	18.5	36.0	47.3	74.0	88.4	92.2	92.8
RSMtr	11	9.7	14.7	31.8	46.2	70.7	84.7	91.3	92.4
SBM	2	13.4	24.5	40.8	40.2	60.3	93.2	99.1	99.3
SBMtr	2	8.7	20.0	23.0	26.3	43.8	66.6	97.9	99.2

Feed intake and milk production in trial 1 (means of least squares of the lactation weeks of 4-14)

	Treatments					Significance
	Control	RSM1	RSM2	TRSM1	S.E.	(P-value)
DM intake (kg/d)						
Forage	9.92	10.15	9.90	10.21	0.97	0.92
Grain	5.73	4.62	3.49	4.30	0.22	
RSM		1.40	2.10	1.25	0.11	
Total	15.87	16.39	15.70	15.98	1.12	0.78
Milk production						
Milk (kg/d)	24.36	26.78	23.89	26.68	2.98	0.24
ECM (kg/d)	26.06	26.96	24.91	27.18	2.57	0.43
Fat (g/d)	1176	1173	1102	1182	126	0.66
Protein (g/d)	752	797	736	815	66	0.18
Milk composition						
Fat (g/kg)	48.4	44.3	46.0	44.2	4.0	0.27
Protein (g/kg)	30.9	30.2	30.9	30.6	1.8	0.92
Urea (mg/100 ml)	24.3	32.1	34.4	27.8	2.9	<0.001
Live weight (kg)	523.7	511.9	522.0	537.8	58.0	0.94
Live weight change (kg/d)	-0.15	-0.02	0.08	-0.06	0.31	0.65
CP intake (g/d)	2204	2606	2635	2538	177	0.003
Energy intake						
ME (MJ/d)	173.7	179.9	170.8	172.8	11.8	0.66
NEL (MJ/d)	98.0	101.8	96.9	97.5	6.6	0.66
FFU/d	13.14	13.65	12.91	13.00	0.85	0.55
Feed conversion						
FFU/kg ECM	0.33	0.35	0.34	0.31	0.037	0.54
FFU/kg ECM (with LWC)	0.34	0.34	0.33	0.31	0.021	0.18
Utilization of ME						
kl	0.68	0.66	0.67	0.72	0.059	0.47
kl (with LWC)	0.65	0.68	0.69	0.73	0.052	0.20

RSM = turnip rapeseed meal (0-var.); TRSM = heat-treated rapeseed meal (00-var.); ECM = energy corrected milk (SJAUNJA et al. 1990); ME = metabolizable energy (MAFF 1975), NEL = net energy in lactation (VAN ES 1978); CP = crude protein; FFU = fattening feed unit (0.7 kg starch); kl = utilization of ME in lactation (ARC 1980), LWC = live weight change (kg/d)

Feed intake and milk production in trial 2 (means of least squares)

	Treatments		Significance	
	RSM	TRSM	S.E.	(P-value)
DM intake (kg/d)				
Forage	9.32	8.89	0.69	0.14
Grain	4.95	5.28	0.87	0.37
RSM	1.08	1.15	0.18	0.36
Total	15.58	15.55	1.27	0.97
Milk production				
Milk (kg/d)	21.91	23.90	1.96	0.021
ECM (kg/d)	23.35	25.16	1.81	0.023
Fat (g/d)	1036	1113	93	0.055
Protein (g/d)	697	750	53	0.024
Milk composition				
Fat (g/kg)	47.4	47.3	2.8	0.88
Protein (g/kg)	31.9	31.6	1.2	0.49
Urea (mg/100 ml)	35.3	36.7	4.8	0.48
Live weight (kg)	502.3	489.6	51.0	0.55
Live weight change (kg/d)	0.12	0.09	0.17	0.65
CP intake, (g/d)	2528	2532	237	0.97
Energy intake				
ME (MJ/d)	171.8	172.7	15.3	0.89
NEL (MJ/d)	97.4	98.0	8.8	0.86
FFU/d	13.14	13.29	1.25	0.77
Feed conversion				
FFU/kg ECM	0.39	0.37	0.029	0.069
FFU/kg ECM (with LWC)	0.38	0.36	0.023	0.11
Utilization of ME				
kl	0.61	0.65	0.05	0.055
kl (with LWC)	0.64	0.68	0.05	0.10

TRSM = Öpex-treated RSM (heat-moisture treatment); both Öpex-treated and untreated RSM were 0-varieties

Feed intake and milk production in trial 3

	A Control	B TRSM1200-var.	C TRSM2400-var.	D RSM240-var.	E RSM24 $FeSO_4$	S.E.	Significance (P-value) Between diets	Contrast C1	C2	C3	C4
DM intake (kg/d)											
Forage	8.91	9.43	9.79	9.64	9.22	0.51	0.006	<0.001	0.68	0.08	0.02
Grain	6.76	6.19	5.35	5.34	5.31	0.18	<0.001	<0.001	0.07	0.79	0.68
RSM	0.00	0.84	1.69	1.70	1.70	0.02	<0.001	<0.001	0.73	0.89	0.47
Total	15.91	16.71	17.08	16.92	16.47	0.51	<0.001	<0.001	0.28	0.06	0.01
Milk production											
Milk (kg/d)	24.01	25.11	24.98	25.73	24.74	0.90	0.005	0.02	0.09	0.02	0.55
ECM (kg/d)	23.53	24.88	24.83	25.07	25.26	0.89	0.002	0.003	0.05	0.64	0.29
Fat (g/d)	1008	1060	1064	1064	1096	57	0.034	0.04	0.29	0.23	0.23
Protein (g/d)	713	766	756	764	770	26	<0.001	0.001	0.004	0.61	0.22
Lactose (g/d)	1062	1120	1115	1148	1102	48	0.008	0.02	0.10	0.04	0.54
Milk composition											
Fat (g/kg)	41.6	41.8	42.0	41.1	43.7	2.2	0.15	0.69	1.00	0.17	0.11
Protein (g/kg)	29.8	30.6	30.3	29.9	31.1	1.1	0.08	0.35	0.20	0.02	0.10
Lactose (g/kg)	44.3	44.8	44.7	44.6	44.6	0.6	0.55	0.17	0.32	1.00	0.81
Urea mg/100 ml)	22.4	26.8	30.1	29.6	27.7	2.5	<0.001	<0.001	0.52	0.12	0.049
Live weight (kg)	548.6	551.5	551.1	551.3	556.3	9.9	0.54	0.58	0.68	0.27	0.25
Live weight change (kg/d)	0.13	0.20	0.27	0.25	-0.03	0.38	0.41	0.41	1.00	0.11	0.08
CP intake (g/d)	2476	2781	2994	2987	2911	100	<0.001	<0.001	0.25	0.10	0.08
Energy intake											
ME (MJ/d)	178.3	188.2	190.1	189.3	184.3	7.1	0.005	0.001	0.15	0.13	0.08
NEL (MJ/d)	108.1	112.7	114.2	113.3	111.3	3.2	0.003	<0.001	0.23	0.17	0.05
FFU/d	14.64	15.21	15.40	15.28	15.00	0.41	0.003	<0.001	0.24	0.15	0.04
Feed conversion											
FFU/kg ECM	0.42	0.42	0.42	0.42	0.43	0.034	0.68	0.68	1.00	0.52	0.75
FFU/kg ECM (with LWC)	0.43	0.42	0.43	0.42	0.43	0.033	0.68	0.75	0.79	0.50	0.74
Utilization of ME											
kl	0.61	0.59	0.58	0.59	0.64	0.05	0.16	0.29	0.99	0.01	0.002
kl (with LWC)	0.64	0.63	0.64	0.66	0.63	0.13	0.07	0.99	0.99	0.94	0.99

TRSM = Öpex-treated turnip rapeseed meal (heat-moisture treatment); Contrasts: C1 = linear effect of RSM, C2 = quadratic effect of RSM, C3 = effect of rapeseed variety (D vs. E), C4 = the effect of Öpex-treatment (C vs. E). In contrasts C3 and C4 the effect of $FeSO_4$-treatment is confounded)

Feed intake and milk production in trial 4

	A	B	C	D	Silage	Hay	S.E.	Significance between contrasts (P-value)		
	Control	TRSM 8 %	TRSM 16 %	TRSM 24 %				linear	qudratic	cubic
DM intake (kg/d)										
Forage	7.41	7.20	7.35	7.33	7.22	7.42	0.44	0.83	0.40	0.31
Grain	6.35	5.96	5.44	4.93	5.69	5.64	0.05			
RSM	0.00	0.52	1.03	1.56	0.78	0.77	0.02			
Total	13.96	13.89	14.03	14.02	13.90	14.05	0.44	0.51	0.77	0.48
Milk production										
Milk (kg/d)	17.04	17.34	17.96	18.04	17.85	17.34	0.90	0.001	0.62	0.39
ECM (kg/d)	18.59	18.64	19.37	19.35	19.06	18.92	0.62	<0.001	0.81	0.047
Fat (g/d)	808	805	834	832	829	811	30.6	0.007	0.99	0.084
Protein (g/d)	574	576	606	599	581	596	21.9	<0.001	0.42	0.012
Lactose (g/d)	799	812	839	850	829	821	47.7	0.002	0.94	0.60
Milk composition										
Fat (g/kg)	47.8	46.8	46.6	46.5	46.9	47.0	47.7	0.002	0.94	0.60
Protein (g/kg)	34.0	33.4	33.9	33.4	32.8	34.5	2.6	0.17	0.50	0.81
Lactose (g/kg)	46.9	46.7	46.8	47.0	46.3	47.4	0.7	0.63	0.21	0.95
Urea (mg/100 ml)	26.5	29.0	31.0	33.1	28.0	31.9	1.3	<0.001	0.56	0.66
Live weight (kg)	516.3	516.6	517.6	516.5	518.8	514.7	10.0	0.89	0.77	0.81
Live weight change (kg/d)	0.14	0.19	0.21	0.14	0.17	0.17	0.24	1.00	0.32	0.82
CP intake (g/d)	2078	2162	2296	2397	2154	2312	79	<0.001	0.66	0.36
Energy intake										
ME (MJ/d)	161.3	160.4	161.3	160.7	163.2	158.7	4.6	0.86	0.88	0.50
NEL (MJ/d)	92.8	92.3	93.0	92.7	92.8	92.6	2.6	1.00	0.88	0.52
FFU/d	12.50	12.44	12.49	12.44	12.71	12.23	0.32	0.74	1.00	0.56
Feed conversion										
FFU/kg ECM	0.46	0.45	0.44	0.44	0.46	0.43	0.022	0.005	0.65	0.27
FFU/kg ECM	0.43	0.42	0.41	0.42	0.43	0.41	0.034	0.093	0.23	0.61
Utilization of ME										
kl	0.52	0.53	0.55	0.55	0.53	0.55	0.029	0.014	0.55	0.69
kl (with LWC)	0.57	0.59	0.60	0.59	0.58	0.60	0.056	0.19	0.24	0.90

TRSM = Öpex-treated rapeseed meal (00-variety)

Feed intake and milk production in trial 5

	A Control	B RSM12	C RSM24	D TRSM12	E SBM8	F SBM16	G TSBM8	S.E.	Significance between contrasts (P-value) C1	C2	C3	C4
DM intake (kg/d)												
Forage	11.68	11.49	11.56	11.53	11.79	11.52	10.92	0.76	0.79	0.73	0.93	0.66
Grain	5.90	5.54	4.95	5.64	5.66	5.31	5.55	0.26				
RSM/SBM	0.00	0.77	1.60	0.78	0.52	1.07	0.53	0.04				
Total	17.81	18.06	18.38	18.21	18.24	18.17	17.26	0.75	0.17	0.92	0.71	0.21
Milk production												
Milk (kg/d)	23.29	24.10	25.22	23.19	24.26	24.21	22.93	1.41	0.019	0.82	0.25	0.45
ECM (kg/d)	24.27	25.27	26.44	24.75	26.02	25.79	24.22	1.61	0.020	0.90	0.57	0.80
Fat (g/d)	1040	1093	1134	1081	1134	1115	1055	87.5	0.060	0.89	0.80	0.97
Protein (g/d)	759	782	829	769	812	806	750	45.7	0.009	0.59	0.59	0.81
Lactose (g/d)	1062	1094	1152	1046	1098	1107	1030	64.6	0.017	0.67	0.18	0.40
Milk composition												
Fat (g/kg)	45.3	45.5	45.1	46.8	46.5	46.1	46.2	2.4	0.88	0.76	0.33	0.60
Protein (g/kg)	32.8	32.6	32.9	33.2	33.5	33.3	33.0	0.75	0.77	0.48	0.16	0.14
Lactose (g/kg)	45.5	45.3	45.7	45.2	45.2	45.7	44.9	0.73	0.52	0.43	0.72	0.64
Urea (mg/100 ml)	21.7	26.8	29.6	25.4	27.6	30.5	27.0	2.4	<0.001	0.32	0.31	0.20
Live weight (kg)	574.4	577.5	578.9	580.0	574.4	574.6	578.5	6.6	0.22	0.79	0.49	0.20
Live weight change (kg/d)	0.46	0.20	0.54	0.40	0.04	0.55	0.00	0.39	0.71	0.11	0.35	0.19
CP intake (g/d)	2817	3058	3232	2902	3035	3293	3056	125	<0.001	0.75	0.77	0.17
Energy intake												
ME (MJ/d)	206.3	208.5	211.2	210.5	211.2	210.3	200.1	8.3	0.29	0.95	0.66	0.33
NEL (MJ/d)	118.2	119.6	121.2	120.8	121.1	120.8	114.8	4.8	0.26	0.96	0.64	0.33
FFU/d	15.62	15.82	16.02	15.99	16.02	15.98	15.21	0.60	0.23	0.99	0.61	0.34
Feed conversion												
FFU/kg ECM	0.46	0.46	0.44	0.47	0.46	0.45	0.45	0.023	0.04	0.60	0.31	0.92
FFU/kg ECM (with LWC)	0.42	0.43	0.39	0.43	0.45	0.40	0.45	0.042	0.19	0.10	0.84	0.24
Utilization of ME												
kl	0.51	0.52	0.54	0.51	0.53	0.53	0.53	0.03	0.052	0.91	0.33	0.71
kl (with LWC)	0.60	0.56	0.67	0.59	0.54	0.64	0.55	0.10	0.089	0.06	0.55	0.28

Rapeseed meal is 00-variety; T means heat-moisture treatment (Öpex) for RSM and SBM
Contrasts: C1 = linear effect of RSM, C2 = quadratic effect of RSM, C3 = effect of heat treatment of RSM, C4 RSM vs. SBM