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Effects of dietary protein supply on caseins, whey proteins, proteolysis and renneting properties in milk from cows grazing clover or N fertilized grass

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SUMMARY. The objective of this work was to examine whether variation in the amino acid supply to cows could be a reason for the reduced casein content and poorer renneting properties of milk that often occur in late summer, or whether these effects are related to proteolysis in the raw milk. In a 2 × 2 × 2 factorial design, we investigated the effects of sward (clover *v.* rye-grass) and supplementary feed with a high or low level of rumen-soluble N or of rumen undegradable protein on milk protein composition during the grazing season. A total of 32 Danish Holstein cows were included in the experiment. Milk protein and casein contents and the ratios casein N:total N and casein:true protein were at a minimum in late summer, whereas the contents of urea, non-protein N and whey protein were higher during this period. These seasonal effects were unrelated to either the type of supplementary feed or the type of sward; neither were they clearly related to proteolysis, although casein:true protein was related to the proteose peptone content. The results indicated that whey proteins other than α-lactalbumin or β-lactoglobulin accounted for the higher proportion or concentration of whey protein in late summer. Based on a principal component analysis including variables such as citric acid, lactose and non-protein N, we suggest that the cows' energy supply during this period may be a critical factor in determining the milk protein composition, although our results were not conclusive. There was an interaction between the supplement of rumen undegradable protein and type of sward. When clover was grazed, a high supplement increased the concentrations of protein and casein in milk and the κ-casein:total casein ratio. When rye-grass was grazed, the opposite response was found, and overall milk protein yield was not affected. The very low N content of clover in early summer reduced milk protein and casein protein during this period.

The cheesemaking properties of milk are becoming ever more important in many countries as an increasing proportion of milk is used for cheese production. The caseins play an important role in cheesemaking and it therefore becomes essential to understand how and why variations in caseins arise, so that milk with good cheesemaking properties can be produced.

We have reported earlier that in mid summer, when cows are fed mainly by grazing, milk has a lower casein:total protein ratio and poorer renneting properties (Eskesen, 1994; Hermansen *et al.* 1994). Such a seasonal effect has also been reported in comprehensive surveys of bulk milk (Lacroix *et al.* 1994; Van den Berg, 1994), although Ng-Kwai-Hang *et al.* (1982) found no consistent seasonal effect on total milk protein content or casein number.

The reason for this seasonal variation in casein : total milk protein is not obvious. It may be related to the protein supply for the grazing cows resulting from a changed composition of the sward, since it has been demonstrated that the type of sward can affect the flow of non-ammonia N to the duodenum and milk protein yield and composition (Grandison *et al.* 1985; Thomson *et al.* 1985; Wilkins *et al.* 1994). Further, it has been shown that turning cows out to pasture can induce modifications in the content of individual caseins (Macheboeuf *et al.* 1993). However, an alternative explanation for the seasonal effect on caseins may involve proteolysis in raw milk (Phelan *et al.* 1982), which also can be affected by feeding (Yousef *et al.* 1970; O'Keeffe *et al.* 1982).

Therefore, we wished to investigate the influence of the quality of supplementary dietary protein, in terms of rumen degradability and expected amino acid supply for the cows, and in relation to type of sward, on the milk proteins, indicators of proteolysis and the renneting properties of milk throughout the grazing season.

MATERIALS AND METHODS

Cows, design and treatments

A total of 32 Danish Holstein cows were used, 16 of which were in their first lactation. All cows had calved before the grazing season, were 79 d in milk (SD 37) on average, and yielded an average of 30 kg of milk at the beginning of the grazing season. In the pregrazing period, all cows were fed on the same diet, consisting of a concentrate mixture in fixed and equal amounts, and grass silage *ad lib.* Immediately prior to the beginning of the grazing period, cows were randomly allocated, within number of lactation (heifers as well as others), to one of eight treatments in which they stayed until the end of the grazing period.

The treatments were arranged in a $2 \times 2 \times 2$ factorial design. The factors investigated were (a) grazing of a mixture of clover and rye-grass with no N fertilization or rye-grass fertilized with 300 kg N/ha (6×50 kg during spring and summer (April–September)), (b) supplementary feed yielding a high or low level of N surplus in the rumen (PBV) and (c) supplementary feed yielding a high or low level of expected amino acid supply to the duodenum (AAT). The supplementary feeds were given twice a day and fixed for the entire experimental period of ~ 4 months during late spring and summer (May–September).

The pasture was two areas situated in the same field with irrigated perennial rye-grass or white clover mixed with perennial rye-grass. Cows grazed in pens (eight cows per pen) according to type of sward and level of PBV. Within each pen, half the cows were given supplementary feed yielding a high or low level of AAT. The size of the continuously grazed pens was varied during the year in order to compensate for different growth rates of the grass. In late spring–early summer (May–June) the size was regulated to match an average grass height of 70 mm measured by a plate meter (plate $0{\cdot}3 \times 0{\cdot}3$ m, $3{\cdot}8$ kg m^{-2}). During the summer (July–September) the grass allowance was regulated by the size of the less frequently grazed (i.e. fouled or rejected) area, allowing an increase varying from 0·12 in July to 0·22 of the total area in September. The average stocking rates were 3·9 and 5·3 cows/ha for clover and rye-grass respectively. Stocking rates in pens grazed by cows given a high or low level of PBV were equal.

Table 1. *Digestibility of organic matter, protein content and protein value in clover and rye-grass grazed by cows given supplementary feed containing a high or low calculated N surplus*

Pen		Date				
Pasture	N surplus	17/6	15/7	12/8	9/9	Mean
		Coefficient of organic matter digestibility				
Clover	High	86	79	80	79	81
	Low	85	79	79	79	81
Rye-grass	High	85	83	77	80	81
	Low	86	80	75	80	80
		Protein ($N \times 6{\cdot}25$), g/kg dry matter				
Clover	High	209	210	281	323	256
	Low	144	236	277	324	246
Rye-grass	High	216	221	244	249	233
	Low	215	179	223	244	215
		Intestinal amino acid absorption, g/kg dry matter†				
Clover	High	90	87	85	81	86
	Low	89	87	84	81	85
Rye-grass	High	85	85	90	88	87
	Low	85	83	87	87	86
		Protein balance in the rumen, g/kg dry matter†				
Clover	High	44	53	121	172	97
	Low	−20	71	125	176	88
Rye-grass	High	58	60	77	88	71
	Low	51	17	57	77	51

† According to the Nordic protein evaluation system (Madsen, 1985).

Feeds

The proportion of white clover in the clover sward increased from ~ 20% in May to ~ 50% in September, measured on a dry matter basis. Digestibility, protein content and protein value of the sward calculated according to the Nordic protein evaluation system (Madsen, 1985) are given in Table 1. No marked differences were found between pens in digestibility of organic matter. Overall, the N content was higher in clover than in grass and when the cows were given a high surplus of N. In terms of the expected amino acid supply to the duodenum and N surplus in the rumen per kg herbage dry matter, the most important difference was the different seasonal change in clover and grass. In clover PBV increased markedly and AAT decreased as the season progressed, whereas in the grass sward only small seasonal differences occurred.

The chemical composition of the supplementary feeds is given in Table 2. Important feeds to manipulate the AAT content of the feed mixture were rapeseed cakes, untreated or heat treated to reduce the rumen degradability of protein.

Milk sampling and chemical analysis of milk

Milk samples were collected once immediately prior to the grazing period, seven times over the summer (June–September) and once after the grazing season (October). The individual milk samples were collected during one evening and the following morning milkings with a Tru-Test HI on-line milk meter (Tru-Test Distributors Ltd, Auckland, New Zealand) calibrated to collect 22 ml/l milk. The

Table 2. *Chemical composition of the supplementary feeds and the supplementary nutrients used in this trial*

N surplus in the rumen†	High		Low	
Intestinal amino acid supply†	High	Low	High	Low
Ingredients, g/kg				
Barley grain	72	71	131	579
Peas	159	275	—	—
Soyabean meal	231	141	—	—
Rapeseed cake, untreated	231	397	—	—
Rapeseed cake, heat-treated	159	—	168	—
Animal fat	—	—	29	49
Molasses	116	94	88	113
Dried beet pulp	—	—	558	223
Minerals	32	22	26	35
Chemical composition, g/kg dry matter				
Ash	91	79	87	73
Crude protein (N × 6·25)	321	303	149	120
Crude fat	61	61	67	68
Crude fibre	87	85	140	72
Digestibility of organic matter	0·86	0·86	0·87	0·87
Rumen degradability of protein	0·66	0·69	0·62	0·74
Daily supplement per cow				
Dry matter, kg	6·2	6·2	6·9	6·3
Metabolizable energy, MJ	100	100	106	97
N, g	318	305	164	120
Intestinal amino acid supply, g	781	719	718	567
N surplus in the rumen, g	794	756	−145	−195

† According to the Nordic protein evaluation system (Madsen, 1985).

samples were kept at 0 °C overnight and mixed with the morning milk to form one sample for each cow. Within 3 h, the samples were heated to ~ 40 °C and subdivided for different analyses. Total protein, fat and lactose were determined by i.r. analysis (Milko-Scan 104, Foss Electric, DK-3400 Hillerød, Denmark). For total protein, the calibration of the i.r. analyser was based on true protein. Non-casein N was determined as described in International Dairy Federation (1964) adjusted to the actual equipment (1015 DS-20 and Kjeltec 1002, Tecator AB, S-263 21 Höganäs, Sweden) and non-protein N after precipitation of protein with trichloroacetic acid (Rowland, 1938).

Samples for determination of whey proteins, caseins and urea were prepared in preperiod and post-period samplings and three times during the grazing period and then stored at −25 °C for later analyses. Caseins and whey proteins were analysed by HPLC using a strong anion-exchange column as described by Ostersen *et al.* (1997). Whey proteins were measured quantitatively in milk, and the distribution of different caseins was measured using the absorption values of Davies & Law (1987). Milk urea and citrate were determined by i.r. (Milko Scan 4000, Foss Electric). Proteose peptone content was measured in fresh milk and in milk after storage at 5 °C for 4 d as described by Waagner Nielsen & Edelsten (1992). Casein was precipitated at pH 4·6 with acetic acid (100 g/l)–1 M-sodium acetate buffer. After filtration, the whey was heated to 100 °C for 20 min and the N content was measured in the filtrate. The renneting properties were measured on milk samples without adjustment of pH or calcium using a Formagraph (Foss Electric) with standard rennet (Chr. Hansen Laboratorium, DK-2970 Hørsholm, Denmark) at 40 μl/100 kg milk and a renneting temperature of 30 °C. The renneting properties (renneting time, firmness after 30 min, rate of curd firming (the time from clotting until the amplitude

Table 3. *Overall milk yield and composition before, during and after the grazing season for cows grazing clover or rye-grass and given supplements containing a high or low calculated N surplus*

	Date									
	Pre-experiment 28/4	23/6	21/7	4/8	18/8	1/9	15/9	Post experiment 25/10	SEM	Significance of date
Days in milk	75	131	159	173	187	200	215	254		
Milk yield, kg	30·2	27·8	26·2	25·0	22·8	22·6	21·4	18·3	0·8	***
Milk composition, /kg										
Fat, g	44·6	39·1	37·6	37·5	39·2	42·8	43·4	50·8	1·2	***
Lactose, g	50·4	50·3	49·4	48·8	48·5	47·8	47·4	46·3	0·4	***
True protein , g	30·5	31·4	31·1	31·1	31·1	31·9	32·9	36·0	0·5	***
Non-protein N, g	1·5	1·7	1·9	2·4	2·2	2·2	2·2	1·6	0·06	***
Whey protein, g	4·6	4·9	5·2	5·0	5·3	5·3	5·5	5·9	0·14	***
Casein, g	25·9	26·5	25·9	26·1	25·8	26·6	27·4	30·1	0·5	***
Urea, mg	227	260		570		430		240	22	***
Citrate, mg	165	130		134		146		138	5	***
Casein, g/kg total N	809	802	785	779	775	780	780	800	3	***
Casein, g/kg true protein	850	845	834	838	830	833	832	835	4	***
Whey proteins, g/kg milk										
α-Lactalbumin	1·17	1·19		1·19		1·02		1·08	0·02	***
β-Lactoglobulin	3·40	3·89		3·65		3·95		5·14	0·14	***
Proteose peptone, g/kg milk										
Day 1	1·07	1·22		1·36		1·34		1·74	0·08	***
Day 4	1·27	1·35		1·60		1·51		1·86	0·07	***
Caseins, g/kg total casein										
α_{s1}-Casein + α_{s2}-casein	506	492		499		502		515	3	***
β-Casein	398	393		403		404		375	4	***
κ-Casein	86	95		86		87		89	3	**
γ-Casein	11	21		11		8		21	6	***
Somatic cell count ($\times 10^{-3}$)/ml	—	62		114		98		143	20	***
Renneting properties										
Renneting time, min	20·0	20·7		21·3		13·7		18·9	1·6	***
Rate of curd firming, k_{20}, min	1·6	1·6		1·5		1·4		0·9	0·14	**
Curd firmness, mm	16·6	15·1		15·0		19·7		25·4	2·2	***
pH	6·693	6·690		6·669		6·657		6·702	0·014	***

$*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

reached 20 mm on the recording chart)) were monitored on Formagraph paper as described by Ostersen *et al.* (1997). Somatic cell count was measured with a Fossomatic (Foss Electric).

Statistical procedures

Measurements were evaluated statistically using mixed linear models that included both fixed and random effects and suggesting an autoregressive variance structure of repeated measurements on individual cows (SAS, 1992), i.e. a full model including a covariate of the actual milk component in the pre-experimental period, fixed effects of treatments including interactions, fixed effects of dates including interactions with treatments and a random effect of cows. Treatment differences were tested against the random effect of the cow. Effect of date and interaction between treatment and date were tested against the residual of the model. Interrelations between measurements were studied with principal component analysis using the procedure FACTOR (SAS, 1992) with a promax rotation, which allows some correlation between principal components. We included all components with an eigenvalue > 1. The input was standardized to zero mean and an SD of 1 prior to analysis.

RESULTS

The overall milk yield and composition and the average stage of lactation of the cows during the experiment are shown in Table 3. The average stage of lactation was ~ 10 weeks post partum in the pre-experimental period and ~ 36 weeks post partum at the end of the experiment. Thus the experiment covers a period of the lactation during which only limited and linear changes in milk composition would be expected.

All variables recorded were significantly affected by time during the experimental period. Milk yield and lactose concentration decreased steadily during the grazing season in line with the expected lactational changes. In contrast, the seasonal changes in fat and protein concentrations gave minima in late summer. The variation in protein content was most closely related to the content of casein, since the content of whey protein increased steadily during the season. There were also marked seasonal changes in the concentrations of non-protein N (NPN) and urea, which were increased by ~ 50 and ~ 100% respectively in late summer compared with pre-experimental and post-experimental values. As a result, casein expressed as a proportion of total milk N was reduced in late summer compared with pre-experimental and post-experimental values. When expressed as a proportion of true milk proteins, casein was lower in late summer compared with pre-experimental and early summer levels, but not compared with post-experimental values. The individual whey proteins α-lactalbumin and β-lactoglobulin were lower in late summer than might have been expected from the lactational effect. The seasonal variation in the distribution of individual caseins was small. Renneting properties were almost unchanged until late summer, when renneting times fell and curd firmness increased. The content of proteose peptone measured in fresh milk and after 4 d storage increased slightly as season and lactation progressed, as did somatic cell counts.

In Tables 4 and 5 the main treatment effects on milk composition are given, together with the levels of significance of these effects and of time. However, the significance of interactions is given only for those cases in which statistical analysis indicated a significant interaction for one or more of the variables. Least square means of the treatment combination 'pasture × AAT' are given.

Table 4. *Effects of pasture, ruminal N surplus and intestinal amino acid supply on milk yield and composition, least square means and statistical significance of effects for cows grazing clover or rye-grass and given supplements containing a high or low calculated N surplus*

		Milk composition							
	Milk yield, kg	Fat, g/kg	Lactose, g/kg	True protein, g/kg	Whey protein, g/kg	Casein, g/kg	Non-protein N × 6·38, g/kg	Casein, g/kg protein	Urea, mg/kg
Pasture									
Clover	25·0	40·2	49·0	31·9	5·3	26·6	2·1	833	400
Grass	24·3	39·5	48·8	31·4	5·0	26·4	2·1	841	430
Significance of difference	NS	NS	NS	NS	*	NS	NS	NS	NS
Ruminal N surplus (PBV)									
High	24·4	41·5	48·6	32·2	5·2	27·0	2·4	838	540
Low	24·9	38·2	49·2	31·1	5·1	26·0	1·8	836	300
Significance of difference	NS	*	NS	NS	NS	NS	***	NS	**
Amino acid supply (AAT)									
High	24·3	40·5	48·5	31·5	5·0	26·5	2·1	841	430
Low	24·9	39·2	49·2	31·8	5·3	26·5	2·0	833	400
Significance of difference	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pasture × amino acid supply									
Clover–high AAT	23·8	42·5	48·6	32·7	5·3	27·4	2·1	837	430
Clover–low AAT	26·2	37·8	49·3	31·2	5·4	25·8	2·1	829	380
Grass–high AAT	24·8	38·3	48·4	30·4	4·7	25·7	2·2	845	440
Grass–low AAT	23·7	40·6	49·1	32·4	5·3	27·1	2·0	838	430
Significance of difference	NS	*	NS	**	NS	**	NS	NS	NS
Significance of other treatment interactions†									
AAT × PBV	NS	*	NS	NS	NS	NS	NS	NS	NS
Pasture × AAT × PBV	NS	NS	NS	NS	*	NS	NS	NS	NS
Significance of date	***	***	***	***	***	***	***	***	***
Significance of interaction with date†									
Pasture	*	NS	NS	NS	NS	NS	**	NS	**
PBV	*	NS	NS	NS	N	NS	**	NS	NS
Pasture × PBV	*	NS	NS	*	NS	NS	NS	NS	NS
Pasture × PBV × AAT	NS	NS	NS	NS	NS	*	NS	*	NS

AAT, amino acid supply to the duodenum; PBV, nitrogen surplus in the rumen.

† Only interactions where one or more variables interacted significantly are given.

NS not significant, $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

Table 5. *Effects of pasture, ruminal N surplus and intestinal amino acid supply on milk casein and whey protein composition, least square means and statistical significance of effects on composition of milk protein fraction for cows grazing clover or rye-grass and given supplements containing a high or low calculated N surplus*

	Whey proteins, g/kg milk		Caseins, g/kg total casein			
	α-Lactalbumin	β-Lactoglobulin	α	β	κ	γ
Pasture						
Clover	1·12	3·85	496	399	89	16
Grass	1·14	3·82	499	400	90	11
Significance of difference	NS	NS	NS	NS	NS	NS
Ruminal N surplus (PBV)						
High	1·15	3·90	497	401	90	14
Low	1·11	3·77	498	399	89	13
Significance of difference	NS	NS	NS	NS	NS	NS
Amino acid supply (AAT)						
High	1·14	3·75	499	400	88	13
Low	1·13	3·91	496	399	90	14
Significance of difference	NS	NS	NS	NS	NS	NS
Pasture–AAT interaction						
Clover–high AAT	1·14	3·95	498	397	93	13
Clover–low AAT	1·11	3·74	493	402	85	18
Grass–high AAT	1·13	3·56	499	404	84	13
Grass–low AAT	1·15	4·08	499	397	96	9
Significance of difference	NS	**	NS	NS	*	NS
Significance of other treatment interactions						
AAT × PBV	NS	NS	*	NS	NS	NS
Pasture × AAT × PBV	NS	*	NS	NS	NS	NS
Significance of date	***	***	*	**	*	***
Significance of interaction with date						
AAT × PBV	NS	*	NS	NS	NS	NS

NS not significant, * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

Type of sward significantly affected only the concentration of whey protein in milk ($P < 0·05$), but the difference was too small to affect the casein : true protein ratio significantly (Table 4). The lower whey protein content for the grass regimen was entirely related to the low value (4·4 g whey protein/kg) produced by a combination of high AAT and high PBV levels in the supplementary feed. It was related specifically to the lower content of β-lactoglobulin (3·4 g/kg), indicated by the significant pasture × AAT × PBV interaction for β-lactoglobulin (Table 5). The PBV supply to the cow significantly affected the contents of fat, NPN and urea in milk. The lower fat content for the low level of PBV was, however, entirely caused by the low fat content (36 g/kg) when both AAT and PBV in the supplementary feed were low, i.e. for the diet in which barley was the main constituent (Table 2). Further, the variation in NPN content during the grazing period was dependent on pasture and the PBV in feed ($P < 0·01$). When clover was grazed, the NPN content was relatively low in June and at the beginning of July compared with grass, whereas in the autumn the NPN content was highest when clover was grazed. Giving a high N surplus in the supplementary feed particularly increased the NPN content of milk in late July and August compared with June and September.

The AAT level in the supplementary feed had no main effect on any milk component, but in combination with type of sward it affected fat, protein, casein and β-lactoglobulin contents and the proportion of κ-casein in casein. Concentrations

Table 6. *Interrelationships between milk components and renneting properties for milk from cows grazing clover or rye-grass and given supplements containing a high or low calculated N surplus expressed as loadings (multiplied by 100) of common rotated principal components*†

Variable	Principal component no. 1	2	3	4	5	6	Proportion of variance explained
Days in lactation	78		−27				86
Milk yield, kg	−67		35				81
Concentration in milk, g/kg							
Protein	84						83
Fat	55		−62				92
Lactose	−71			40			87
Citrate			−81				66
Non-casein N	48	62					95
True whey protein	60			−62			92
Non-protein N		96		−54			95
Urea		90					88
Proteose peptone, day 1	64	−30		−30		−28	69
Proteose peptone, day 4	45	−34		−35			48
β-Lactoglobulin	75						84
α-Lactalbumin			45	44	46		70
Protein:fat ratio			85				81
Casein in total N		−76		55			97
Casein in true protein				87			87
Somatic cell count, ($\times 10^{-3}$)/ml	43						23
Proportions of caseins							
α-Casein	90	26					70
β-Casein	−57					−70	89
κ-Casein	−39					92	87
γ-Casein		−37			39	39	64
Renneting time, min					92		85
Time for aggregation, min	−68			−34	34		65
Curd firmness, mm	66						84
pH		−32			70		63
Accumulative proportion of total variance explained	34·1	50·4	61·7	67·8	73·6	78·2	

† Loadings > 25 are given.

were increased when clover was supplemented with a high level of AAT, whereas the opposite was the case when rye-grass was supplemented with a high level of AAT. The effects on protein and casein contents ($P < 0{\cdot}01$) were pronounced, but varied in the opposite direction to milk yield, thus counterbalancing variations in total milk protein yield. Indeed, milk protein and casein yields were not significantly affected.

In addition to the general effect of the AAT × pasture combination, the casein content in milk and the casein in true protein were significantly influenced by an interaction between date and treatment (Table 4). This was brought about as a result of a markedly lower casein content in milk from cows grazing clover and supplemented with low AAT and low PBV in June and at the beginning of July (−0·30 and −0·25% respectively) compared with all other treatments. The whey protein content of the milk from this group of cows was similar to or slightly higher than that with the other treatments, resulting in a lower proportion of casein in true milk protein, 820 g/kg, compared with 850 g/kg for all other treatments during this period.

In addition, milk yield and protein content were influenced by an interaction between date and treatment. The nature of these interactions could be attributed largely to the different pattern of response in milk yield and protein content in the group of cows grazing clover and supplemented with a low level of rumen degradable protein, and to an enhanced response in casein content and casein : total milk protein ratio in the subgroup of cows given a supplement with a low level of rumen undegradable protein. This may indicate that the amino acid supply for the cow was implicated in the different responses.

In order to facilitate the interpretation of the pattern of changes in milk characteristics through the season, the results of a principal component analysis are given in Table 6. As the principal component analysis was carried out on the residuals after removing the cow effect by the statistical analysis, the principal components illustrate the interrelationships between the milk components not affected by the planned dietary treatments. Principal component 1 extracts the variation related to stage of lactation, i.e. the increasing contents of protein, fat and the nitrogenous fractions of β-lactoglobulin and proteose peptone, and the proportion of α-casein as lactation progresses. Principal component 2 represents the variation in NPN and its effect on casein as a proportion of total milk protein. This component also includes an inverse relationship between NPN or urea and indicators of proteolysis (proteose peptone and γ-casein). Principal component 3 may represent the variation in the cows' energy supply: a relatively high energy supply decreases the concentration of citrate in milk and increases milk yield and the content of α-lactalbumin. Principal component 4 includes the variation in casein as a proportion of true protein, which is related positively to content of lactose and negatively to proteose peptone. Principal component 5 includes the well known relation between renneting time and pH, and principal component 6 includes the interrelations between the casein fractions that depend on degree of proteolysis, when β-casein is degraded.

DISCUSSION

Our hypothesis was that the supplementary amino acid supply for the cows or the type of sward could modulate the seasonal change in the casein content of milk or casein as a proportion of total protein. However, it seemed that this was not the case, for we found neither a general effect of these factors nor a specific date × pasture or date × AAT effect. We did see a three sided interaction (pasture × AAT × PBV) with date on casein content and casein as a proportion of true protein brought about by the group of cows grazing clover and supplemented with a low level of PBV. Probably the total N supply in early summer for the cows in this group was too low to secure an optimal microbial growth and protein synthesis in the rumen. This is indicated by the milk urea, which in this group was as low as 1·2 mM compared with ~ 4·0 mM in the other groups. This is below the optimum level as assumed by Bang & Strudsholm (1993) and Carlsson & Pehrson (1994). This may in turn have impaired the balance of absorbed amino acids important for milk protein synthesis. Clark *et al.* (1992) estimate a lysine content of ~ 80 g/kg total amino acids in microbial protein. This is beyond the 70 g/kg critical for milk protein and milk casein content (Rulquin *et al.* 1993). Therefore, if microbial protein synthesis is impaired, the lysine supply may be limiting for milk protein synthesis. The effects were most pronounced in the subgroup for which the AAT supply was also low, which supports the idea of a limited lysine supply.

We also found that the sward affected the whey protein content of milk, the content being higher when clover was grazed instead of fertilized grass. This is in agreement with our earlier results (Hermansen *et al.* 1994) but disagrees with the results of Grandison *et al.* (1985), who reported a higher content of casein (mainly α-casein and β-casein) when clover was grazed instead of rye-grass. In their investigation, however, the clover grazing regimen was related to a markedly higher feed intake (Thomson *et al.* 1985), which was probably not the case in the present experiment.

Thus our results indicate that the diet can also affect milk protein composition and the proportion of casein in milk protein during grazing, but do not support the hypothesis that the cows' amino acid supply is a principal reason for the reduced casein in milk proteins in mid summer.

Alternatively, the variations in casein could be related to proteolysis. Indicators of proteolysis are a reduced proportion of β-casein, increased levels of proteose peptone or an increased proportion of γ-casein in total caseins. However, these effects did not appear during the experimental period and no convincing single factor including these effects was found in the factor analysis. Hence our results do not support the alternative hypothesis that proteolysis was an important cause of the variation observed in casein in milk protein.

The principal component analysis showed that casein in true protein was positively related to the contents of lactose and α-lactalbumin and negatively related to proteose peptone and total true whey protein, but not related to β-lactoglobulin. This indicates that other whey proteins were responsible for the relatively high whey protein content in autumn. It has been shown that a reduced energy intake may result in a higher level of total whey protein (Kefford *et al.* 1995). Furthermore, Gray & Mackenzie (1987) showed that a restricted plane of nutrition, whereby milk yield and protein content were reduced, increased the content of blood serum albumin and reduced the contents of α-lactalbumin and β-lactoglobulin in the milk. They also found that milk β-lactoglobulin was the most sensitive to reductions in the plane of nutrition. There was an immediate marked fall in its concentration, whereas the reduction in α-lactalbumin followed the slower fall in milk yield. This pattern is in agreement with our seasonal effect (Table 3). β-Lactoglobulin, which would be expected to increase steadily during this period of lactation (Ostersen *et al.* 1997), actually fell in early August whereas milk yield was not markedly reduced. In September, milk yield had fallen and the concentration of α-lactalbumin, which would be expected to fall steadily during lactation, suddenly fell to resume a steady decline afterwards.

Thus it can be argued that the variation in casein in true milk protein is related to the energy status of the cows. Principal component analysis appears to separate the effect of an excessive nutrient supply compared with the requirements for milk production (component 3) and a suboptimal energy balance (component 4). The citrate content (component 3) is an indicator of the extent of *de novo* fat synthesis in the mammary gland (Banks *et al.* 1984), where a reduced concentration indicates a good supply of nutrients for *de novo* fat synthesis (e.g. acetic acid) originating from the carbohydrate in the cows diet. It is known that lack of dietary energy results in a fall in the lactose concentration (Friggens *et al.* 1995) and increases in NPN and urea (Carlsson & Pehrson, 1994). These are main factors in principal component 4, which also includes casein in true protein. Thus it seems that a lack of energy in the cows' diet in mid and late summer can enhance the contents of whey proteins other than α-lactalbumin and β-lactoglobulin and hence be responsible for the lower proportion of casein in milk protein. However, this needs to be addressed more directly before a definite conclusion can be reached.

With regard to renneting properties, only small lactational effects would be expected during the period of lactation studied in the present experiment (Coulon *et al.* 1991; Ostersen *et al.* 1997), so that we should expect no systematic change in renneting characteristics in this study. We have reported two conditions that could impair renneting properties (Hermansen *et al.* 1994). Firstly, a high intake of grass was related to an increased renneting time, probably mediated through a higher natural pH of the milk, which was also increased by a high grass intake. This is in line with the findings of Coulon (1995) and Macheboeuf *et al.* (1993). Secondly, we found an increased renneting time in late summer, obviously not related to a higher natural pH of the milk. Guinott-Thomas (1992) found that adding urea to cheesemilk had a negative effect on renneting time, and because milk urea is often high in late summer it could be suggested that this may affect renneting properties. However, in the present investigation we found no effect on renneting properties of giving a diet that increased milk urea from ~ 300 to 540 mg/kg (Table 4). Neither did we observe any increased renneting time in mid summer when the average milk urea was as high as 570 mg/kg. Thus it is not likely that the poorer renneting properties often observed in mid and late summer are related to milk urea.

Overall, the renneting properties were relatively stable during the summer period in our investigation. Lucey & Fox (1992) found an interaction between plane of nutrition and poorer renneting properties in late lactation and late summer milk, indicating that a good nutritional status could prevent impairment of renneting properties. In our investigation, all cows were given the same amount of supplemental feed during the season despite the natural decline in milk yield as the stage of lactation progressed. This probably ensured a good general energy supply and may be the reason why we found only a small seasonal effect on renneting properties. Therefore, it seems that keeping the cows in a good nutritional status may be a major component in ensuring milk with good cheesemaking properties.

In conclusion, we found significant changes in milk protein composition during the grazing season. In late summer, casein as a proportion of total milk N and casein as a proportion of total milk true protein were reduced compared with the pregrazing period and early summer. In addition, κ-casein as a proportion of total casein tended to be lower in late summer, whereas β-casein was higher. These seasonal changes were not significantly influenced by type of sward or supplementary feed. It therefore seems unlikely that variation in the cows' amino acid supply is the main reason for the lower proportion of casein in milk protein often observed in late summer. Our results indicate that the variation in milk casein is more likely to be related to the energy status of the cows.

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