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The influence of dietary energy source and dietary protein level on milk protein concentration from dairy cows

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Abstract

To investigate the effects of energy source and protein level of diets on milk protein content, 12 multiparous Holstein-Friesian cows were used in a 4 × 4 Latin square change-over experiment with 4-week periods. Four diets were offered, with ad libitum *silage as proportionately 0·40 of the diet, and the remaining 0·60 as one of four concentrates, two based on barley and two on molassed sugar-beet pulp. Two protein levels were achieved by altering the amounts of digestible undegraded protein in the concentrates, with all diets formulated to supply equal quantities of rumen degradable protein. There was no effect of diet on dry-matter intakes. Both starch and high dietary protein levels significantly increased milk protein concentration ($P < 0·05$), but had no effects on milk fat and lactose concentrations. Mean milk yields were significantly higher ($P < 0·05$) with increased dietary protein. Dietary protein significantly affected the yields of milk protein ($P < 0·01$) and lactose ($P < 0·05$) but not that of fat. Urinary allantoin excretion was significantly greater with both high protein ($P < 0·05$) and starch-based diets ($P < 0·05$). No significant interaction effects were found. It is concluded that dietary effects were due largely to differences in supply of rumen degradable protein; increases in milk protein concentration were therefore brought about by increasing the protein supply to the animal.*

Keywords: *allantoin, dairy cows, milk production, milk protein.*

Introduction

Recent changes in the uses of milk and in the consumption of milk products have meant that protein has become an increasingly important component of milk. Dietary energy intake and protein level have been identified as two major attributes of the dairy cow diet which exert an important influence on the concentration of protein in milk (Emery, 1978; Thomas and Martin, 1988; Spörndly, 1989; DePeters and Cant, 1992). In general, an increase in the intake of metabolizable energy (ME) yielding carbohydrates leads to an increase in the concentration of protein in milk, whereas an increase in intake of fat tends to reduce it (DePeters and Cant, 1992). Carbohydrate form can be important, with starchy concentrate supplements to forage-based diets tending to increase milk protein concentration in comparison with fibrous supplements (MacGregor *et al.*, 1983; Thomas *et al.*, 1986; Sloan *et al.*, 1987 and 1988; Lees *et al.*, 1990; de Visser *et al.*, 1990). This is not always so, with the reverse also reported (Castle *et al.*, 1981; Mayne and Gordon, 1984). Some of these effects may be explained through changes in rumen fermentation patterns, with the starchy diets which led to increased milk protein production tending to increase rumen propionate production (Grummer *et al.*, 1987; Lees *et al.*, 1990; de Visser *et al.*, 1992). Furthermore, Reynolds *et al.* (1988) estimated that proportionately 0·58 and 0·17 of the glucose produced by the liver in lactating Holstein dairy cows was derived from propionate and amino acids respectively. With the provision of more propionate from the rumen, the load on amino acid utilization for gluconeogenesis may be reduced, allowing more amino acids to be incorporated into milk protein.

The objective of this study was to investigate the effect on milk protein concentration of changing the sources of energy in the concentrate given to dairy

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cows, while altering the supply of digestible undegraded protein (DUP). Urinary purine derivative excretion was used as a non-invasive index of the supply of microbial protein from the rumen so that a better understanding of the nature of the responses to different diets might be gained.

Material and methods

Animals and management

Twelve multiparous Holstein-Friesian cows with a mean of 91 (s.d. 6·4) days in milk were used for the experiment. They were housed in individual stalls fitted with de Boer yokes and milked through a 20/20 herringbone milking parlour at approximately 08.00 h and 17.00 h. Animals were weighed every fortnight.

Diets

Four diets, consisting of grass silage offered *ad libitum* together with a concentrate ration, were mixed on farm. The concentrate diets were formulated to meet predicted ME requirements, and in accordance with draft recommendations of the current United Kingdom metabolizable protein system (Agricultural Research Council, 1980 and 1984), subsequently published in Agricultural and Food Research Council Technical Committee on Responses to Nutrients (AFRC TCORN, 1992).

Four concentrates were formulated, two based on barley (B), two on molassed sugar-beet pulp (S), representing starch and fibre sources respectively. For convenience during formulation, and in the absence of true figures, the ME and metabolizable protein levels of B and S were considered to be equal, i.e. it was assumed that the only significant difference was in the source of energy (Bhattacharya and Sleiman, 1971; Castle, 1972; Castle *et al.*, 1981). Mineral and vitamin supplements (McLellan Animal Health, Ayr) were incorporated in the rations at the rate 25 g/kg dry matter (DM); a general purpose in-feed supplement (230 g/kg calcium, 35 g/kg phosphorus) for the B diets, and a high-phosphorus (190 g/kg Ca, 105 g/kg P) supplement for the S diets.

The diets were formulated (including silage) to be isoenergetic in concentration. The two protein concentrations were designed to supply equal quantities of rumen degradable protein (RDP) but different concentrations of DUP for the high (H) and low (L) protein levels. The high protein concentrate was estimated to supply 1·32, and the low protein concentrate 0·87, of predicted requirement for DUP (AFRC TCORN, 1992). This was based on the group 7-day mean milk yield of 27 kg/day recorded immediately before the start of the experiment. High and low protein levels were achieved by the addition of soya and urea to the B or S concentrates, and represent the highest and lowest possible rates of soya-bean meal incorporation whilst keeping other factors constant according to the linear programming limits. The ingredients of the concentrate component of the diets are shown in Table 1.

Table 1 *Summary of the ingredient composition of high and low protein concentrates (g/kg fresh weight)*

	High protein	Low protein
Barley/sugar-beet pulp	836·9	959·8
Soya-bean meal	140·3	0·0
Urea	0·4	13·0
Mineral and vitamins	22·4	27·2

Cows were offered silage daily after the evening milking; this was topped up during the following day if necessary. Individual silage intakes were recorded daily by weighing silage offered and refused. The amounts of silage offered were proportionately 0·05 to 0·10 above *ad libitum* intakes, based on the previous day's intakes.

The concentrate ration for each cow was calculated daily according to the rolling average of silage intake recorded over the previous 3 days, keeping to a forage to concentrate (F : C) ratio of 40 : 60 on a DM basis, using analyses of food DM contents obtained before the start of the experiment. The concentrates were offered on top of the silage in three roughly equal portions, one each after evening and morning milkings, and one at mid day.

In addition to and regardless of the amounts of silage and concentrates given to the cows, a token amount of parlour concentrate (approx. 200 g) was offered at each milking via the automatic in-parlour feeders.

Data collection and analysis

Food intakes. Silage and concentrate samples were collected during the last 7 days of each experimental period. Samples of silage refusals were collected daily from each cow for DM analysis. Food samples were frozen immediately after collection and stored at –20°C until analysed.

Food DM content was determined by oven drying at 100°C, organic matter (OM) by difference after ashing at 500°C. Silage and concentrate OM digestibility was determined by a modified version of the Tilley and Terry (1963) *in vitro* method (Alexander, 1969) and was used to estimate ME from the digestible OM in the DM employing a factor of 0·016 (Thomas and Chamberlain, 1982). Food starch

content was determined by the method of Wainman, Dewey and Boyne (1981), and water-soluble carbohydrates of the concentrates by the Luff-Schoorl method (European Economic Community, 1971) and of silage by the Somogyi method of McDonald and Henderson (1964). Crude protein (CP) was determined by Kjeldahl (N × 6·25) using selenium dioxide as a catalyst, acid-detergent fibre by the method of Van Soest and Wine (1967), neutral-detergent fibre by the method of Van Soest *et al.* (1991), and calcium, phosphorus, magnesium, potassium and sodium by the method of Alexander *et al.* (1985). Silage fermentation characteristics were assessed by the electrometric titration method (Offer *et al.*, 1993).

Milk. Morning and evening milk samples were collected over 4 days at the end of each experimental period. These were preserved using Lactab milk preservative tablets (Thompson and Capper Ltd, Runcorn, Cheshire) and kept refrigerated until analysed for protein, fat and lactose concentrations using a Milko-Scan 203 analyser (Foss Electric, Denmark). Additional milk samples were collected during the same period at two consecutive milkings and bulked according to yield at the time of collection. They were frozen and stored at –20°C without the use of a preservative. These were later analysed for CP (N × 6·38; BS 1741 Section 5 : 2 1990 Modified), casein (FIL-IDF 29 : 1964), urea (Sigma test kit No. 640; Sigma Chemical Company Ltd, Poole) and total non-protein nitrogen by a Kjeldahl digestion after precipitation of protein-nitrogen by trichloroacetic acid.

Purine derivatives. Spot urine samples of approximately 100 ml were collected by vulval stimulation at about 10.30 h and 15.30 h for 7 days at the end of each experimental period. These were frozen and stored at –20°C until analysed by the high-performance liquid chromatography method of Balcells *et al.* (1992) for creatinine and the purine derivative (PD) allantoin using composite morning and afternoon samples. Additional analysis of the four most complete sets of individual samples was carried out to obtain information about the between- and within-day variation in PD excretion.

Whole tract diet apparent digestibilities. Approximately 100 ml of fresh uncontaminated samples of faeces were collected daily for 7 days from each cow for the determination of indigestible acid-detergent fibre (IADF; Penning and Johnson, 1983) as an estimate of whole tract OM apparent digestibility of the diets. Samples were immediately frozen and stored at –20°C before being dried at 60°C, milled, bulked by weight and stored at room temperature until analysed.

Statistical analysis

The experiment was designed as a 2 × 2 change-over, based on three 4 × 4 orthogonal Latin squares. Each experimental period consisted of a 3-week adjustment period followed by a 1-week collection period. The mean milk yields during the 7 days prior to the start of the experiment were used to allocate animals in yield blocks to Latin squares.

The experimental data were analysed statistically using analysis of variance with GENSTAT 5 (Lawes Agricultural Trust, 1990). The blocking structure was period × (square/cow) and treatment structure was diet energy source × protein level. Residual maximum likelihood (REML; Patterson and Thompson, 1971) was used to calculate variance components of urinary PD excretion (Box *et al.*, 1978), using dietary treatments (energy source × protein level) as fixed effects, and period/day/time/cow as random effects. REML was also used to test for carry-over effects from one diet to the next.

Table 2 *Composition of the concentrate portions of the diets (g/kg dry matter unless otherwise stated)*†

	BH	BL	SH	SL	PC
Dry matter (DM) (g/kg)	870	890	882	886	882
Organic matter (OM)	943	941	892	886	894
Crude protein	181	161	165	126	216
Effective rumen degradable protein‡	120	109	86	58	140
Digestible undegraded protein‡	82	13	32	37	31
Metabolizable energy (ME) (MJ/kg DM)	13·2	12·9	12·8	12·5	13·5
Fermentable ME§ (MJ/kg DM)	12·2	11·9	12·3	12·0	11·5
Neutral-detergent fibre	128	132	264	290	275
Acid-detergent fibre	68	68	153	168	158
Starch	477	517	0	0	151
Water-soluble carbohydrates	20	20	196	208	68
Acid hydrolysis ether extract	29	29	15	14	61
In vitro OM digestibility (g/kg OM)	893	858	897	858	751
Potassium	8·3	5·2	19·1	17·9	13·4
Calcium	8·2	9·8	13·5	15·4	14·3
Phosphorus	5·6	5·1	3·5	3·2	7·1
Magnesium	3·5	3·6	1·9	2·8	8·8
Sodium	2·4	2·8	5·9	6·7	4·9

† B = barley, S = sugar-beet pulp, H = high protein, L = low protein, PC = parlour concentrate.
‡ Estimated (Alderman and Cottrill, 1993).
§ Estimated, FME = ME –0·33 × AHEE.

Results

Samples were collected from all cows on all treatments except for one animal that was ill during period 2 of the experiment. The mean compositions of concentrates and silage given during the experiment are shown in Tables 2 and 3 respectively. Silage composition did not change greatly over the duration of the experiment, with, for example, 182, 174, 176 and 189 g CP per kg DM and 11·1, 11·3, 11·3 and 11·4 MJ ME per kg DM for periods 1 to 4 respectively.

The treatment mean values for daily intakes of dry matter (DMI) of silage and concentrate, crude protein (CPI) and metabolizable energy (MEI), with the contributions of ME from silage and concentrates assumed to be additive, are given in Table 4. There were no significant treatment effects on DMI. Whole tract OM apparent digestibilities (estimated using the IADF content of food and faeces; apparent digestibility = 1-(food IADF/faecal IADF)), and allantoin/creatinine ratios (A/C) are also given in Table 4. Whole-tract OM apparent digestibilities were similar for all diets, although both energy source and protein level significantly affected the urinary A/C ratios, with increased purine excretion on the barley and high protein diets. Rumen fermentable ME (FME) intakes were estimated from the ME and acid hydrolysis ether extract (AHEE) contents of the concentrates, using the equation for concentrate FME = ME –0·033 × AHEE, and from silage, using FME = 0·71 × ME (AFRC TCORN, 1992).

From Table 4 it can be seen that the initial specification of a dietary F : C ratio of 40 : 60 on a DM basis was met for all four diets. However, although the diets as offered contained similar concentrations of ME, as specified by the original formulation objectives, between-diet differences in intakes of silage meant that the intakes of ME differed by up to 13 MJ/day between treatments. Similarly, between treatment differences in intake

Table 3 *Composition of the silage offered (g/kg dry matter unless otherwise stated)*

Dry matter (DM)	207
Organic matter (OM)	925
Crude protein	180
Amino-acid nitrogen (g/kg fresh weight)	2·18
Total soluble nitrogen (g/kg fresh weight)	3·19
Amino-acid nitrogen/total soluble nitrogen	0·689
Metabolizable energy (ME) (MJ/kg DM)	11·3
Fermentable ME† (MJ/kg DM)	8·0
Lactic + formic acids (g/kg fresh weight)	36·8
Total volatile fatty acids (g/kg fresh weight)	6·16
Acid hydrolysis ether extract	62
Water-soluble carbohydrates	14
Residual sugars (g/kg fresh weight)	10·6
Neutral-detergent fibre	482
Acid-detergent fibre	324
Rumen degradable protein	154
Undegradable protein	26
In vitro OM digestibility (g/kg OM)	774
D-value	704
NH_4-N (g/kg total N)	128
pH	3·8
Neutralising value (meq per kg fresh weight)	284
Calcium	5·2
Phosphorus	3·3
Magnesium	2·3

† Estimated, FME = 0·71 × ME.

Table 4 *Effect of dietary treatment on dry-matter intake (DMI), crude-protein intake (CPI), metabolizable-energy intake (MEI), estimated intakes of effective rumen degradable protein (eRDPI), digestible undegraded protein (DUPI) and fermentable metabolizable energy (FMEI); whole tract apparent organic matter (OM) digestibilities; and allantoin/creatinine ratios*

	Diet†					Significance‡	
	BH	BL	SH	SL	s.e.d.	P	E
Silage DMI (kg/day)	6·8	6·4	6·5	6·8	0·31		
Concentrate DMI (kg/day)	10·5	10·0	10·2	10·6	0·46		
Total DMI (kg/day)	17·2	16·4	16·7	17·4	0·77		
CPI (kg/day)	3·1	2·8	2·9	2·6			
eRDPI (kg/day)	2·02	1·84	1·64	1·42			
DUPI (kg/day)	0·47	0·27	0·65	0·52			
MEI (MJ/day)	214	202	204	209			
FMEI (MJ/day)	182	170	182	186			
Whole tract apparent digestibility of OM (g/g)	0·81	0·83	0·83	0·84	0·010		
Allantoin/creatinine	2·00	1·90	1·90	1·79	0·059	*	*

† See Table 2 for diet codes.
‡ Significance of results: P = protein level effect, E = energy source effect, P × E interaction effects were not significant ($P > 0·05$).

Table 5 *Effect of dietary treatment on milk yield and composition*

	Diet†					Significance‡	
	BH	BL	SH	SL	s.e.d.	P	E
Milk yield (kg/day)	18·2	17·6	18·5	17·2	0·57	*	
Protein (g/kg)	36·3	35·1	35·1	34·9	0·40	*	*
Fat (g/kg)	40·4	41·6	42·7	42·0	1·09		
Lactose (g/kg)	45·7	45·6	45·2	45·8	0·27		
Protein yield (g/day)	656	617	647	595	21·7	**	
Fat yield (g/day)	737	737	774	720	22·0		
Lactose yield (g/day)	837	805	840	786	27·0	*	

† See Table 2 for diet codes.
‡ Significance of results: P = protein level effect, E = energy source effect, P × E interaction effects were not significant ($P > 0·05$).

Table 6 *Effects of dietary treatment on major milk nitrogenous (N) constituents*

	Diet†					Significance‡	
	BH	BL	SH	SL	s.e.d.	P	E
Crude protein (g/kg)	35·6	34·4	34·2	34·1	0·51		*
True protein (g/kg)	33·8	32·4	32·5	32·5	0·55		
Casein (g/kg)	27·1	25·6	26·1	26·2	0·61		
Whey (g/kg)	6·68	6·81	6·29	6·34	0·176		*
Non-urea non-protein N (g/kg)	0·132	0·139	0·120	0·101	0·0144		*
Urea (g/kg)	0·348	0·341	0·333	0·345	0·0247		
Crude protein yield (g/day)	645	605	630	583	22·9	*	
True protein yield (g/day)	611	572	604	556	22·1	*	
Casein yield (g/day)	489	453	481	448	19·3	*	
Whey yield (g/day)	122	119	117	109	4·7		*
Non-urea non-protein N yield (g/day)	2·49	2·49	2·30	1·81	0·270		*
Urea yield (g/day)	6·19	5·89	5·95	5·14	0·444		

† See Table 2 for diet codes.
‡ Significance of results: P = protein level effect, E = energy source effect, P × E interaction effects were not significant ($P > 0·05$).

coupled with unformulated between diet differences in composition meant that estimated intakes of effective rumen degradable protein (eRDP) and DUP (Alderman and Cottrill, 1993) were not as originally predicted. Estimated intakes of DUP were greater with diets formulated to be relatively high in DUP content within concentrates containing the same energy source; however, estimated intakes of DUP were greater on the sugar-beet-based diets than on the barley-based diets. Estimated intakes of eRDP followed the opposite trend. The combination of differences in estimated intakes of eRDP and FME resulted in differences in eRDP/FME ratios between diets. These were 11·2, 10·8, 9·0 and 7·6 g eRDP/MJ FME for diets BH, BL, SH and SL respectively.

A summary of the mean milk yield and composition obtained by Milko-Scan analysis from each of the four diets, and the treatment means of the major nitrogenous constituents in the milk are given in Tables 5 and 6 respectively. Milk yields were far lower than predicted by the diet formulation software; nevertheless differences were seen between the four experimental diets. Milk yield was significantly increased by the high protein diets ($P < 0·05$), but was unaffected by source of energy. Milk protein concentration, like the urinary A/C ratio, was significantly increased on the barley and high protein diets ($P < 0·05$). There was a trend ($P = 0·082$) for milk fat concentrations to be increased by the inclusion of sugar-beet pulp. Milk casein concentrations were not significantly affected by dietary treatment, and differences in true protein concentration were brought about by changes in whey protein concentration. Thus, there was no significant effect of diet on the proportion of milk true protein that was casein.

Urinary PD excretion was found to differ with time of sampling, with higher A/C ratios obtained in the

morning than in the afternoon (1·94 *v.* 1·86; s.e.d. 0·034). No effect of an interaction between time of sampling and diets was seen. Furthermore, significant differences in the diurnal variation of the A/C ratios due to experimental period were seen, with diurnal variation assessed as the ratio of the a.m. value divided by the p.m. value. The a.m./p.m. values were 1·00, 1·10, 1·05 and 1·02 (s.e.d. 0·047) for periods 1, 2, 3 and 4 respectively. The value for period 2 was significantly different ($P < 0·05$) to that for period 1. Analysis of variance components by REML (Table 7) showed that the greatest proportion of variance was associated with animal effects, and the smallest with sampling time. No carry-over effects of diet between experimental periods were identified.

The mean treatment effects on the concentrations and daily yields of sodium (Na), potassium (K), and chloride (Cl) ions are presented in Table 8. The ratio of K/Na in the milk tended to be higher ($P = 0·099$) on the B diets, due to the significant increase in K on the B diets, despite small and non-significant increases in Na concentration on the S diets. Similarly, there was a tendency ($P = 0·056$) for the lactose/Cl ratio to be higher on the low protein diets. Overall, however, there was no difference in the overall ratios of these compounds (i.e. K/Na/lactose/Cl), which are the main factors influencing milk osmolarity.

Ratios of the three main milk constituents, fat, protein and lactose, to one another, are presented in Table 9. Significant effects were found on the ratio of protein to fat due to energy source ($P < 0·01$), as a result of increased rates of protein production and reduced rates of fat production, and on the ratio of protein to lactose due to protein level ($P < 0·01$), as a result of increased rates of protein production and reduced rates of lactose production on those diets. Relative protein efficiencies for each diet (i.e. milk protein output/CPI) are shown in Table 10. The only significant effect on these was that of a protein × energy interaction effect on non-urea non-protein nitrogen.

Table 7 *Estimated components of variance in urinary allantoin/creatinine excretion*

Component due to:	Variance component
Experimental period	0·0088
Sampling day within each period	0·0044
Time of sampling within each day	0·000016
Cow within each treatment	0·1574

Table 8 *Effect of dietary treatment on milk sodium (Na), potassium (K), and chloride (Cl) ion concentrations and yields in milk*

	Diet†					Significance‡	
	BH	BL	SH	SL	s.e.d.	P	E
Na (g/kg)	0·398	0·389	0·416	0·419	0·0316		
K (g/kg)	1·369	1·348	1·352	1·309	0·0170	*	*
Cl (g/kg)	1·031	1·004	1·013	1·002	0·0170		
Na (g/day)	7·46	6·79	8·05	7·52	0·679		
K (g/day)	25·91	24·65	25·92	23·49	0·886	*	
Cl (g/day)	18·76	17·46	18·73	17·01	0·609	**	

† See Table 2 for diet codes.
‡ Significance of results: P = protein level effect, E = energy source effect, P × E interaction effects were not significant ($P > 0·05$).

Table 9 *Effect of dietary treatment on ratios of milk protein to fat and lactose, and of fat to lactose*

	Diet†					Significance‡	
	BH	BL	SH	SL	s.e.d.	P	E
Protein/fat	0·911	0·856	0·838	0·839	0·0209		**
Protein/lactose	0·794	0·770	0·777	0·763	0·0082	**	
Fat/lactose	0·883	0·911	0·946	0·920	0·0247		

† See Table 2 for diet codes.
‡ Significance of results: P = protein level effect, E = energy source effect, P × E interaction effects were not significant ($P > 0·05$).

Table 10 *Relative protein efficiencies of milk nitrogenous constituents (milk protein output/crude protein intake, g/kg)*

	Diet					
	BH	BL	SH	SL	s.e.d.	Significance† of P × E
Crude protein	205	218	220	226	12·1	
True protein	194	206	208	216	12·0	
Casein	156	163	168	174	9·9	
Whey	38·6	43·1	40·5	42·2	2·51	
Non-urea non-protein nitrogen	1·00	1·11	1·04	0·96	0·005	*
Urea	1·98	2·15	2·13	1·99	0·19	

† Significance of P × E = protein × energy interaction. Effects of protein level and energy level were not significant ($P > 0·05$).

Table 11 *Yields of milk protein fractions relative to urinary purine derivative excretion (constituent yield/allantoin/creatinine)*

	Diet					
	BH	BL	SH	SL	s.e.d.	Significance† of E
Crude protein	335	327	345	326	18·2	
True protein	318	309	324	311	17·8	
Casein	255	245	263	250	14·7	
Whey	63	64	63	60	3·6	
Non-urea non-protein nitrogen	2·8	2·8	2·8	2·4	0·16	*
Urea	3·1	3·2	3·3	3·0	0·29	

† Significance of results: P = protein level effect was not significant ($P > 0·05$), E = energy source effect, P × E interaction effects were not significant ($P > 0·05$).

The A/C ratio (Table 4) gives an indication of the supply of microbial protein to the animal. In order to take this into account, production variables were divided by the A/C ratio. When this was done (Table 11), the effects of dietary protein level were lost for all variables.

There was no change in mean live weights of the animals over any of the experimental periods, nor over the course of the whole experiment (grand mean 593 (s.d. 41·5) kg).

Discussion

Two factors which are generally acknowledged to have an important influence on milk protein concentration are diet F : C ratio and MEI. In this experiment, the F : C ratio was kept constant throughout on all four diets. Mean silage intakes were slightly higher on the BH and SL diets than on the BL and SH diets, but not significantly so, and silage DM accounted proportionately for only 0·4 of the total DMI. It is assumed, therefore, that any effects observed were the results of differences in the composition of the concentrate portion of the diets, and these in turn were the causes of large differences in the intake of protein and the form in which dietary energy was consumed.

Increasing dietary protein level caused increases in both milk CP concentration and yield. An increase in dietary protein also caused significantly higher milk yields. The barley-based diets, as opposed to sugar-beet pulp diets, caused significantly increased milk protein concentrations, but not yields. However, yields of milk from all animals on this experiment were low compared with the yields of the same animals which formed the basis of formulation parameters for the experimental diets. This discrepancy between predicted and observed milk yields must either have been a function of the diet formulation software or of the production of experimental diets, or a combination of the two.

One of the original specifications of the diet formulation was to increase the level of protein in the diet from a deficit of DUP to an excess whilst keeping the eRDP supply constant. A second objective was that of keeping the diets isoenergetic, and whilst this was essentially achieved in terms of diet energy concentration, unpredicted differences in

food intake meant that energy intake differed between treatments, and differences in estimated eRDP content of the diets meant that estimated eRDP supply was even further from the original objective. To avoid the need to estimate the effects of diet on microbial protein supply from theoretical values, *in vivo* estimates of microbial protein supply were obtained from purine derivative excretion (see below). Based on the original formulation specifications of a 577-kg cow yielding 28 kg milk per day, theoretical daily requirements (based on the recommendations of AFRC TCORN, 1992) for ME, eRDP and DUP were estimated to be 205 MJ ME, 1·82 kg eRDP and 0·52 kg DUP. For the same animal yielding 20 kg milk per day the same daily requirements were estimated at 164 MJ ME, 1·43 kg eRDP and 0·41 kg DUP. The lower theoretical requirements for eRDP were therefore met or exceeded by the estimated intakes on all diets by animals yielding even less milk; estimated DUP intake on the SL diet was actually higher than predicted requirements, and milk protein concentrations were lowest from this treatment. Since the observed yields of milk protein were relatively low compared with predicted values, the model used to predict nutrient requirements must have been inaccurate leading to an underestimation of actual requirements.

The excretion of allantoin in relation to that of creatinine (A/C) was used as an index of microbial protein yield with the simple assumption that an increased A/C ratio indicates an increased supply of microbial protein. This is because exogenous purines originate mainly from rumen micro-organisms (McAllan and Smith, 1973) and PDs appearing in the urine result largely from the degradation and absorption of microbial nucleic acids (McAllan, 1982). Allantoin excretion was expressed in relation to urinary creatinine concentration assuming a constant rate of excretion of creatinine (de Groot and Aafjes, 1960; Albin and Clanton, 1966) so that spot urine samples could be taken. The analysis of the components of variance of urinary A/C over a 7-day period (Table 7) indicated that the largest source of variation is that attributable to individual animals; variation which is efficiently taken into account with a change-over design experiment as used in the present study.

Microbial protein production relies on the supplies of rumen degradable nitrogen and potentially FME. Total daily intakes of FME were similar for all diets (grand mean 180 (s.d. 6·9) MJ/day), with the exception of diet BL, which supplied an average of 10 MJ FME per day (some 0·05) less than the other diets. Estimated supplies of eRDP were more variable (grand mean 1·73 (s.d. 0·258) kg/day) and were therefore largely responsible for the between diet differences in eRDP/FME ratios. Relative differences in available rumen degradable nitrogen are, therefore, more likely to have been a major factor in the production of microbial protein than the availability of FME. Estimating the effect of dietary eRDP/FME ratio on microbial protein production can be misleading if the ratio is lower than considered necessary by an oversupply of FME. On the other hand, the lactic + formic acid content of the silage was relatively high (Offer *et al.*, 1993), indicating that the factor of 0·71 used to estimate silage FME may have caused an overestimation of the contribution of FME from silage. If this was the case, then the eRDP/FME ratios would have been higher than indicated. Figure 1 shows the relationship between urinary A/C and estimated eRDP intake, and Figure 2 shows the relationship between urinary A/C and FME intake. These two figures suggest that PD excretion, and hence microbial protein synthesis, increased with an

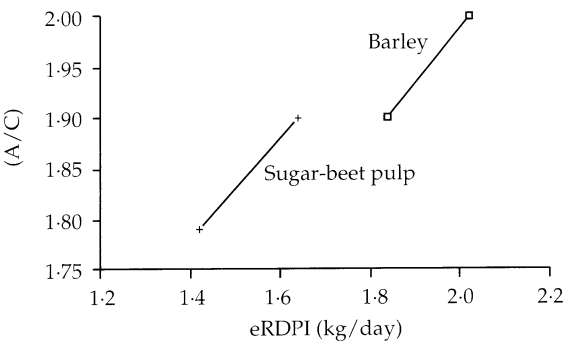

Figure 1 Response of urinary allantoin/creatinine (A/C) ratio to estimated effective rumen degradable protein intake (eRDPI) on barley- and sugar-beet pulp-based diets.

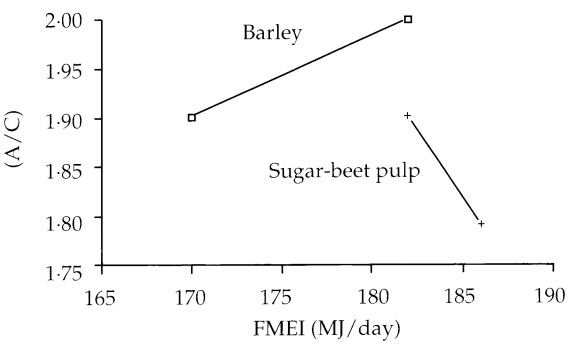

Figure 2 Response of urinary allantoin/creatinine (A/C) ratio to intake of fermentable metabolizable energy (FMEI) on barley- and sugar-beet pulp-based diets.

increased intake of eRDP but not of FME; in other words, the limiting factor on microbial protein synthesis was the availability of nitrogen rather than of an energy substrate. Moreover, no treatment effects on milk urea concentrations were found, indicating that differences in ammonia absorption from the rumen were small (Oltner and Wiktorsson, 1983; Reynolds, 1992; Gustafsson and Palmquist, 1993; Roseler *et al.*, 1993). This lends further support to the suggestion that the important difference between diets in terms of the eRDP/FME ratios was that of supply of RDP rather than FME.

Treatment effects apparently attributable to dietary energy source had a much clearer effect on milk CP concentrations than dietary protein, although the effects were rather small. Slightly higher protein concentrations were achieved on the B diets than on the S diets. However, intake of CP was slightly higher on the B diets, and eRDP intake was substantially higher, as were the A/C ratios. Again, this indicates an overall greater supply of protein to the animal from the rumen which may have resulted in the increased milk protein production; however, due to the confounding of energy source and protein intake it is difficult to attribute the increased milk protein production to either a protein or an energy effect.

In terms of gross relative efficiency of dietary protein utilization for milk protein production (CPI *v.* milk protein production), there were no differences between dietary treatments. Since the major effects attributable to both diet energy source and protein level may have been a result of differences in eRDP supply, the effect of microbial protein synthesis on milk protein production may be examined in relation to rates of PD excretion (Table 11). When this was done, significant differences between dietary treatments were lost. If the original objectives of supplying equal quantities of eRDP with differing levels of DUP had been met, this would not be expected since A/C is a reflexion of microbial utilization of nitrogen and not of rumen undegradable nitrogen. This implies that dietary effects on milk protein production were brought about to a large extent by changes in microbial protein production.

Conclusion

Milk yield and milk protein concentration were significantly increased by an increase in protein supply to the animal. It is suggested that this was achieved largely through an increase in the production of microbial protein, since the rate of urinary allantoin excretion was seen to increase, and was greatest on the barley-based diets and the high protein diets. It is concluded that all diets were deficient in eRDP in relation to FME, with the sugar-beet pulp diets being more deficient than the barley diets, and that these differences in the supply of eRDP were the cause of the effects seen on milk protein.

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