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Effect of increasing digestible undegraded protein supply to dairy cows in late gestation on the yield and composition of milk during the subsequent lactation

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Abstract

Effects of feeding a protein supplement to dairy cows during the dry period on performance during the following lactation were investigated in two experiments. Holstein-Friesian cows were paired towards the end of lactation, and, after drying off, one of each pair received a typical dry cow management regime of ad libitum *grass silage (experiment 1), or a mix of grass silage and distillers' grains or pressed beet pulp (experiment 2). The other cows were offered restricted access to the same basal diet, together with* ad libitum *access to barley straw and 0·5 kg/day high protein maize gluten meal. During the following lactation, animals from both groups were treated without reference to dry period treatment, and were offered equal access to the same lactation diet. Data were analysed by analysis of variance of experiment means and by parallel curve analysis using sample means. In experiment 1, milk yields were similar (27·2 v. 27·9 (s.e.d. 2·12) kg/day for control and supplemented animals respectively) but milk protein yields, and hence concentrations, were significantly higher (P < 0·001) from supplemented animals (28·9 v. 31·8 (s.e.d. 0·58) g/kg). In experiment 2, milk yields were significantly higher (P < 0·001) from supplemented animals (mean 33·3 v. 35·4 (s.e.d. 1·66) kg/day; however, milk protein yields were also significantly increased (P < 0·001) and the change in milk protein concentration was small. No difference in dry-matter intake was recorded in a subset of animals during early lactation in experiment 2. It is hypothesized that the maternal labile body protein pool was maintained or replenished during the dry period by the provision of the protein supplement, and that this had a significant effect on subsequent lactation performance.*

Keywords: *dairy cows, dry period, milk production, milk protein.*

Introduction

The nutrition of the dairy cow has a large influence on the yield and composition of milk she produces. Earlier work was very successful at manipulating milk fat concentration (Sutton, 1984) but current producer and consumer requirements demand increasing milk protein concentrations. Short-term manipulation of milk yield and composition is possible by altering the animal's diet (DePeters and Cant, 1992), although a longer-term approach to the manipulation of milk composition may be more appropriate, with management of the entire lactation cycle taken into consideration.

During early lactation the high yielding dairy cow uses body fat reserves to support milk production (Garnsworthy, 1988). Body fat content is perceived to be relatively easy to measure in the dairy cow by the use of condition scoring (e.g. Lowman *et al.*, 1973). Perhaps for this reason, much work has concentrated on the residual effects of body fat condition, as affected by dry period feeding (for reviews see Broster, 1971; Broster and Broster, 1984). Early work such as that reviewed by Broster (1971) and as conducted by Frood and Croxton (1978), supported the practice of 'steaming up' — feeding concentrates during late pregnancy to increase milk yields in the forthcoming lactation. However, in a review of the more recent literature Garnsworthy (1988) found variable effects of condition score (CS) at calving (in the range 2 to 4+) on subsequent milk yields and

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concluded that factors other than CS alone must be involved.

The effects of pre-partum energy intake and CS at calving on milk protein concentration are somewhat unclear. This is partly because some workers have deliberately manipulated feeding to alter CS, whilst others have classified cows at calving. Some authors have reported changes in protein concentration during early lactation after feeding cows a higher plane of nutrition during the dry period, with both increases (Fronk *et al.*, 1980; Cowan *et al.*, 1981) and decreases (Lodge *et al.*, 1975) being found. Several others found no change in protein concentration (Davenport and Rakes, 1969; Garnsworthy and Jones, 1987; Jaquette *et al.*, 1988; Jones and Garnsworthy, 1988; Holter *et al.*, 1990). None of the above authors apparently took into consideration the effects that the dry period treatments may have had on the labile body protein content of the experimental animals.

During early lactation, when body fat is lost in the high yielding dairy cow, the body protein content of the animal may also decrease (Belyea *et al.*, 1978; Gibb *et al.*, 1992) and proportionately up to 0·27 of body protein mass may be lost under conditions of protein deficiency (Botts *et al.*, 1979). It is unclear, however, what may occur during lactation under normal circumstances, although the results of Chilliard and Robelin (1983) suggest that body protein may be mobilized if dairy cows are underfed during early lactation. Dairy cows given proportionately 0·8 of recommended crude protein requirements during the last stages of gestation yielded less milk and lower milk solids than animals given recommended quantities (Chew *et al.*, 1984b). Similarly, rats were able to utilize labile body protein (accumulated on a protein-rich pre-partum diet) to later support lactation on a protein-free diet (Pine *et al.*, 1994). Moreover, Barnes and Brown (1990) showed that dairy goats with a greater proportion of labile body protein yielded more milk and more milk protein than goats with a relatively small proportion of labile body protein. If a similar rôle for labile body protein occurs in dairy cows, the protein status of the animal in early lactation may be more important than it is presently considered to be.

This study was initiated to compare the effect of offering *ad libitum* silage with that of restricted silage, *ad libitum* straw, and a protein supplement to dairy cows during the dry period on milk production during the subsequent lactation, with particular reference to the yield and concentration of milk protein. Two similar experiments were carried out, the first to investigate the effect of protein supplementation on milk production only, and the second with more emphasis on the study of food intakes and the protein fractions of milk.

Material and methods

Animals and management

In experiments 1 and 2 respectively, 22 and 36 multiparous Holstein-Friesian cows were drawn from the Scottish Agricultural College (SAC) Auchincruive herd and dried off about 56 days prior to predicted calving date. Animals were paired up shortly before being dried off according to condition score and predicted calving date, and for experiment 2, where available, using their cow genetic index scores for milk protein concentration. In both experiments, each group of animals was housed separately in cubicles and had constant access to fresh water. Calvings were managed without reference to dry period treatment; animals were removed to a straw pen just prior to parturition. They were milked using a 20/20 herringbone milking parlour twice daily, at approximately 05.00 to 08.00 h and 14.30 to 17.00 h.

During the second experiment, a small subset of animals (no. = 12) was used for intensive food intake and production studies at two points during the lactation/reproduction cycle: for 2 weeks approximately halfway through the dry period (weeks –5 to –3 of lactation) and for 2 weeks during early lactation (animals within weeks 5 to 12 of lactation). One week of each of these 2-week periods was used for adjustment to the buildings; the 2nd week was used for the collection of data. Animals were housed in individual stalls fitted with de Boer yokes. They were offered the same diets and milked *in situ* at times as close as possible to those of the main group of animals. Due to an incorrect pregnancy diagnosis and illness, only nine animals were used during the dry period. Recovery from ill health and the addition of an extra pair of animals meant that 12 animals were used during lactation.

Diets

Experimental dry period diets were allocated to one animal of each pair at random. In experiment 1, the control diet was grass silage offered *ad libitum* at the clamp face (Table 1); this meant that silage voluntary intakes could not be measured during the dry period. In experiment 2, the control diet was a mix of first-cut grass silage and distillers' grains or pressed beet pulp in the ratio of 3 : 1 (Table 1) offered *ad libitum*. The experimental (supplemented) diets were formulated to meet metabolizable energy (ME) requirements (Agricultural and Food Research Council (AFRC) 1992) using SAC advisory software (N.W. Offer, personal communication). They consisted of a restricted quantity of the same grass

Table 1 *Composition of the silages/silage mixes offered to cows during experiments 1 and 2 (a.m. mix only offered to dry cows during experiment 2)*

	Experiment 1 grass silage		Experiment 2 grass silage mix	
	Dry period	Lactation	a.m.	p.m.
Dry matter (DM) (g/kg)	149	254	312	252
Organic matter (OM) (g/kg DM)	903	932	911	928
Crude protein (g/kg DM)	202	182	161	178
Metabolizable energy (MJ/kg DM)	10·5	11·5	11·4	11·3
Neutral-detergent fibre (g/kg DM)		435	369	489
Acid detergent fibre (g/kg DM)		261	196	289
Water-soluble carbohydrates (g/kg DM)		40	21	19
Acid hydrolysis ether extract (g/kg DM)		56·3	46·8	63·4
In vitro organic matter digestibility (g/kg OM)	725	784	810	761
D-value (g/kg DM)	655	718	690†	690†
NH_4–N (g/kg total N)	224	89	116†	116†
pH	4·5	3·7	4·2†	4·2†
Calcium (g/kg DM)	6·7	6·2	8·9	4·8
Phosphorus (g/kg DM)	3·4	3·4	4·7	3·1
Magnesium (g/kg DM)	2·5	2·5	2·4	2·1
Sodium (g/kg DM)		4·0	4·0	3·5

† Silage alone.

silage or silage mix, with a daily ration of 0·5 kg high protein maize gluten meal (prairie meal, international feed number 5-09-318) (Table 2), and *ad libitum* access to barley straw. Straw was offered *ad libitum* to the protein supplemented animals during the dry period of both experiments as a filler, since access to silage was available for only part of each day. Based on ME requirements, silage intakes over the dry period were predicted by the software to be 4·0 kg silage dry matter (DM) per day and 4·1 kg silage mix DM per day for experiments 1 and 2 respectively. Animals were fed on a group basis, with the exception of the maize gluten meal protein supplement, which was offered to animals individually by hand.

During lactation, both groups of experimental animals were given the same diet. In experiment 1, this consisted of *ad libitum* access to a mix of first-cut grass silage and distillers' grains (Supergrains; Borthwick, Glasgow) in the ratio of 3 : 1 on a fresh-matter basis (Table 1) offered at 08.00 h and 16.00 h,

Table 2 *Composition of the concentrates used in the experiments*

	High protein maize gluten meal		Parlour concentrate	Beet blend
Experiment:	1	2	1 and 2	2
Dry matter (DM) (g/kg)	913	880	882	874
Organic matter (OM) (g/kg DM)	983	988	894	864
Crude protein (g/kg DM)	577	705	216	207
Metabolizable energy (MJ/kg DM)	13·4	14·2	13·4	9·1
Neutral-detergent fibre (g/kg DM)		41·0	275	312
Acid-detergent fibre (g/kg DM)	28·0	32·0	158	223
Starch (g/kg DM)	212	118	151	28
Water-soluble carbohydrates (g/kg DM)	1·4	1·4	68	79
Acid hydrolysis ether extract (g/kg DM)	85	88	61	74
In vitro organic matter digestibility (g/kg OM)	850	853	751	657
Potassium (g/kg DM)	2·6	1·0	13·4	15·8
Calcium (g/kg DM)	0·2	0·4	14·3	19·3
Phosphorus (g/kg DM)	5·3	2·5	7·1	6·0
Magnesium (g/kg DM)	0·6	0·6	8·8	4·2
Sodium (g/kg DM)	0·1	2·2	4·9	8·5

supplemented with 3 kg/day mix of ground barley/soya-bean meal (2·7 : 0·3 fresh matter) at 12.00 h, and in-parlour concentrates (Table 2) offered automatically in equal portions at each milking at the stepped-flat rate of 5·6 kg/day until day 100 of lactation, 3·2 kg/day from days 101 to 200 and 0·8 kg/day thereafter. For experiment 2, the winter diet consisted of *ad libitum* access to the same silage/distillers' grains mix as offered to the dry animals, supplemented with 3 kg/day mix of ground barley/white-fish meal (2·7 : 0·3) in the afternoon silage mix, in-parlour concentrates offered at the rate of 3·2 kg/day and an additional 3 kg/day concentrate based on sugar-beet pulp ('beet blend') (Table 2) fed at 09.00 h from days 0 to 100 of lactation. After turn-out to pasture during both experiments, concentrates were given in-parlour according to yield, with animals yielding over 30 kg/day receiving 3·2 kg/day and those below 30 kg/day receiving 0·8 kg/day. Since the calving dates of animals on both experiments were spread over a number of weeks, the stage of lactation at which the animals were turned out to pasture differed from animal to animal; the effect of this was taken into account by the pre-experimental pairing of animals.

Data collection and analysis

Food intakes. During experiment 1, intakes were measured only for the supplemented animals during the dry period on a group basis by recording the quantities of food offered and refused. Food intakes were measured during experiment 2 on a group basis during the dry period, and individually over a period of 7 days on a sample of animals during the dry period and during early lactation.

Food DM content was determined by oven drying at 100°C, organic matter (OM) by difference after ashing at 500°C. Silage digestibility was determined by a modified version of the Tilley and Terry (1963) *in vitro* method (Alexander, 1969); ME was estimated using the digestible organic matter in the dry matter multiplied by a factor of 0·16 (Thomas and Chamberlain, 1982). Concentrate ME was determined using the E3 equation of Thomas *et al.* (1988) using the neutral cellulase gamanase digestibility (Ministry of Agriculture, Fisheries and Food (MAFF), 1992) and acid hydrolysis ether extract content (MAFF, 1992). Food starch content was determined by the method of Wainman *et al.* (1981) and water-soluble carbohydrate content 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). The composition of the silage mixes was estimated from analyses of the individual constituents.

Metabolic profiles. Blood samples were taken from the coccygeal vessels by venipuncture into two Vacutainer tubes (Becton, Dickinson and Co., Rutherford, New Jersey), each one containing lithium heparin and potassium oxalate/sodium fluoride, at four times during each experiment for metabolic profile analysis (Payne *et al.*, 1970). Blood was analysed for protein, albumin, urea, glucose, β-hydroxybutyrate (BOHB), non-esterified fatty acids (NEFA), magnesium and phosphorous by the Dairy Herd Health and Productivity Service (The Royal (Dick) School of Veterinary Studies, Veterinary Field Station, Easter Bush, Roslin). Target sampling dates for experiment 1 were 14 days after drying off, 7 days before predicted calving date, and 10 and 42 days after actual calving date. For experiment 2, these were 7 days after drying off, 10 days before predicted calving date, 42 and 60 days after actual calving. In practice, samples were collected on a week day nearest the target date to allow immediate (i.e. next day) analysis where possible. Samples were taken between 09.00 h and 10.00 h. If next day analysis was not possible, the blood was immediately centrifuged at 4°C for 20 to 30 min at about 1700 x**g**. Plasma was decanted into fresh tubes, frozen, and stored at –20°C until it was sent for analysis.

Lactation data. Milk samples were collected from lactating animals at two consecutive milkings, p.m. and a.m., and were preserved with a Lactab milk preservative tablet (Thompson and Capper Ltd, Runcorn, Cheshire) and stored at 4°C until analysed. Samples were collected fortnightly and weekly for experiments 1 and 2 respectively. Lactating animals housed in the Metabolism Unit were sampled over four consecutive milkings. Milk was analysed for protein, fat and lactose concentration using a Milko-Scan 203 analyser (Foss Electric, Denmark). Refrigerated unpreserved milk was collected and bulked according to yield at the time of collection, then frozen and stored at –20°C. This was analysed for crude protein (N × 6·38; BS 1741 section 5 : 2 1990 modified), casein (FIL-IDF 29: 1964), urea by Sigma test kit no. 640 (Sigma Chemical Company Ltd, Poole) and total non-protein nitrogen (N) by a Kjeldahl digestion after precipitation of protein-N by trichloroacetic acid.

Live weights and condition scores. Animals were scored for condition on a scale of 0 to 5 to the nearest half point (Lowman *et al.*, 1973) and weighed after the

afternoon milking approximately every 4 weeks during experiment 1. During experiment 2, animals were scored for condition as they were dried off, and as they calved.

Purine derivatives. Spot urine samples of approximately 100 ml were collected by vulval stimulation from animals housed individually at about 10.30 h and 15.30 h for 2 days. These were frozen and stored at –20°C until analysed by the high-performance liquid chromatography method of Balcells *et al.* (1992) for creatinine (C) and the purine derivatives (PD) allantoin (A) and uric acid (U).

Statistical analysis

Statistical analysis was carried out using GENSTAT 5 (Lawes Agricultural Trust, 1990), Maximum Likelihood Program (MLP; Rothamsted Experimental Station, 1991) and Minitab (Minitab, Inc., State College, Pennsylvania, USA). The data obtained from blood samples were analysed using analysis of variance with a blocking structure of pair/cow and treatment structure of treatment × (state/sample), where state was either dry or lactating, and sample was one of two samples taken in each state. Differences in group condition score were analysed using the Mann-Whitney signed rank test, and differences between group live weights were analysed using analysis of variance using a blocking structure of pair/cow and a treatment structure of dry period treatment.

Due to the repeated measures nature of the lactation data, antedependence testing indicated that analysis by split-plot analysis of variance was not a suitable test of the significance of treatment effects. Therefore, the results of milk sampling were analysed using analysis of variance of mean data (i.e. mean of the whole experiment) for each animal to give estimates of treatment mean effects. To obtain more information from the data, parallel curve analysis using Dhanoa's modification of Wood's curve ($y = an^{mc}e^{-cn}$; Dhanoa, 1981) was carried out on the same data set using treatment means for each sampling week; mean data were used since preliminary analyses indicated that the lack of fit error about fitted values was not significantly different from the pure error around each mean and was therefore a valid estimate of the error for subsequent analyses (Ross, 1990). Wood (1976) showed that his curve was a suitable model for describing milk constituents as well as milk yields. However, for the variables of fat and lactose concentrations and protein and fat yields of experiment 2, where the Wood's model was not suitable (i.e. the model did not fit the data well), a straight line ($y = a + bn$) was fitted. Parallel curve analysis gave estimates for treatment effects on changes in lactation performance over the whole of the experimental sampling periods, indicative of the effects of treatment on shift displacement (Wood's scaling factor a or the linear regression y-intercept) and curve shape (Wood's parameter c or the linear regression slope).

Table 3 *Experiment 1: mean condition scores shortly before drying off, shortly after calving and at peak lactation*

	Condition score†	
	Control	Supplemented
Before drying off	2·8	3·0
Post calving	1·8	2·1
Peak lactation	1·7	1·9

† There was no significance of differences between condition scores of groups as tested by Mann-Whitney signed rank test.

Results

Experiment 1

The mean silage intake of supplemented dry cows was 2·68 kg DM per day. The straw intake of these animals was 1·96 kg DM per day. Silage intake of the control dry animals could not be measured due to the manner in which they were housed with other, non-experimental animals. Condition scores of animals shortly before being dried off and shortly after calving are presented in Table 3. There was no significant difference between the group mean CS at either time, although both groups lost 1 CS point during the dry period and into early lactation. Mean live weights were not significantly different between the two groups of animals over the first 6 months of lactation (Figure 1).

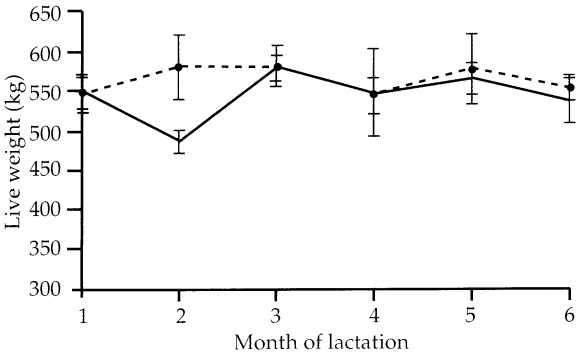

Figure 1 Treatment mean live weights (with standard error bars) of dairy cows during the first 6 months of lactation in experiment 1 (+—+, control animals; •– –•, supplemented with additional protein during the dry period).

Table 4 *Experiment 1: summary of results of lactation (mean of weeks 3 to 31) following different treatments during the dry period*

	Dry period treatment			
	Control	Supplemented	s.e.d.	Significance
Milk yield (kg/day)	27·2	27·9	2·12	
Protein concentration (g/kg)	28·9	31·8	0·58	***
Fat concentration (g/kg)	40·2	42·7	2·40	
Lactose concentration (g/kg)	45·5	46·5	0·60	
Protein yield (g/day)	783	883	66·2	
Fat yield (g/day)	1086	1190	121·6	
Lactose yield (g/day)	1232	1291	96·5	

Mean effects of dry period treatment on milk yield and milk protein, fat and lactose concentrations and yields are presented in Table 4 and effects on model parameters are given in Table 5. Mean milk yields were not significantly affected by dry period diet but the shape parameters of the models for both protein concentrations and yields were affected significantly by dry period treatment ($P < 0{\cdot}01$ and $P < 0{\cdot}05$ respectively). Lactose concentrations and hence yields also tended to be higher ($P = 0{\cdot}051$ and $P = 0{\cdot}051$ respectively for shift displacement). Mean milk yields, and milk protein concentrations and yields over the course of the first 31 weeks of lactation are presented graphically in Figures 2 to 4. The effect of dry period treatment on milk protein concentrations apparently lasted until approximately the end of the sampling period.

No significant treatment differences were observed in the blood metabolic profile data except for albumin concentration which was significantly higher in supplemented animals (37·5 *v.* 39·2 g/l for control and supplemented animals respectively; s.e.d. 0·65; $P < 0{\cdot}05$) and phosphorus which was significantly lower (1·9 *v.* 2·3 (s.e.d. 0·10) mmol/l, $P < 0{\cdot}05$).

Table 5 *Experiment 1: summary of significance levels of shape and shift displacement effects of parallel curve analysis of lactation data following dry period treatments. A significant effect indicates a significant difference in the shape or scaling parameters of the curve used in the analysis*

	Shape†	Shift displacement
Milk yield (kg/day)		
Protein concentration (g/kg)	**	***
Fat concentration (g/kg)		
Lactose concentration (g/kg)		
Protein yield (g/day)	*	***
Fat yield (g/day)	*	**
Lactose yield (g/day)		

† $y = an^{mc}e^{-cn}$

Experiment 2

Mean group intake and PD excretion data for dry and lactating animals are presented in Table 6. Although the dry period diet CP concentration was slightly higher for control dry animals it is estimated

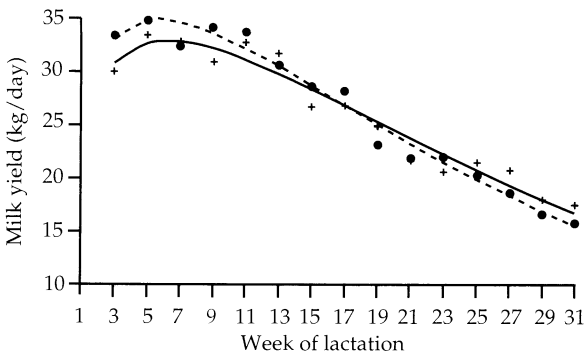

Figure 2 Model mean daily milk yields for each dry period treatment, experiment 1 (+—+, control animals; •– –•, supplemented with additional protein during the dry period).

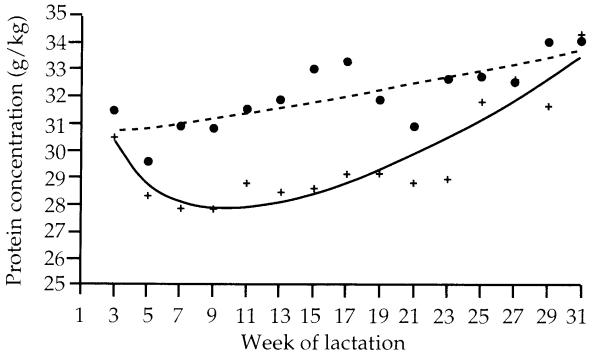

Figure 3 Model mean milk protein concentrations for each dry period treatment, experiment 1 (+—+, control animals; •– –•, supplemented with additional protein during the dry period).

Table 6 *Experiment 2: mean daily intakes of silage and total dry matter, crude protein, and metabolizable energy of group-fed animals during the dry period (all animals) and during early lactation (12 animals), and urinary excretion of purine derivatives (allantoin + uric acid) expressed in relation to creatinine (AU : C; 12 animals)*

	Dry period			Early lactation		
Dry period treatment:	Control	Supplemented	s.e.d.	Control	Supplemented	s.e.d.
Silage dry matter intake (kg/day)	10·0	5·0		13·8	14·8	0·66
Total dry matter intake (kg/day)	10·0	7·6		19·2	20·1	0·66
Crude protein intake (kg/day)	1·8	1·3		3·4	3·6	
Metabolizable energy intake (MJ/day)	113	76		200	209	
Urinary AU : C excretion (mmol : mmol)	1·16	1·09	0·078	3·04	3·16	0·167

that the supplemented animals consumed approximately 100 g digestible undegraded protein (DUP) per day more than the control animals. This is based on assumed CP degradabilities of 0·85, 0·40 and 0·60 (AFRC, 1992), and digestibilities of 0·75, 0·90 and 0·60 of undegraded protein for the silage, prairie meal and straw respectively. Urinary PD excretion (AU/C) was not significantly different between the two groups of dry animals when measured in the smaller subset of animals. During early lactation, no differences were seen in dietary intakes or PD excretion from the 12 animals studied. A summary of mean CS of animals at the start and end of the dry period is given in Table 7; the supplemented group lost a small amount of condition over the dry period. The mean CS of the 12 animals observed individually in early lactation was 2·4 for control and 2·1 for supplemented animals.

Mean effects of dry period treatment on mean milk yields and mean milk protein, fat and lactose concentrations and yields are presented in Table 8 and effects on model parameters are given in Table 9. Model milk yields were significantly higher from the cows that were offered the dry period treatment ($P < 0·001$ for model shift displacement), with a mean of over 2 kg/day more milk during the whole of the sampling period. Milk protein concentrations were slightly higher for supplemented animals ($P < 0·01$ for model shift displacement), so that milk protein yields were highly significantly higher ($P < 0·001$ for model shift displacement). Milk yields and the protein concentrations and yields for weeks 1 to 18 of lactation are presented in Figures 5 to 7. No significant treatment differences were observed in milk production data, including N fractions, in the subset of 12 animals studied more closely during

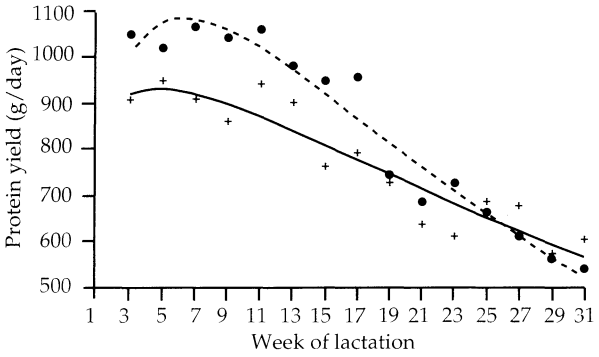

Figure 4 Model mean daily milk protein yields for each dry period treatment, experiment 1 (+—+, control animals; •– –•, supplemented with additional protein during the dry period).

Table 7 *Experiment 2: mean condition scores of animals at the start and end of the dry period*

	Condition score†		
	Control	Supplemented	Significance†
At drying off	2·9	2·6	
At calving	2·9	2·5	*

† Significance of differences between condition scores of groups as tested by Mann-Whitney signed rank test.

Table 8 *Experiment 2: summary of results of lactation (mean of weeks 1 to 18) of all animals following different treatments during the dry period*

	Dry period treatment†		
	Control	Supplemented	s.e.d.
Milk yield (kg/day)	33·3	35·4	1·66
Protein concentration (g/kg)	31·0	31·2	0·62
Fat concentration (g/kg)	45·1	44·6	1·92
Lactose concentration (g/kg)	45·8	46·0	0·43
Protein yield (g/day)	1031	1104	53·7
Fat yield (g/day)	1505	1581	113·1
Lactose yield (g/day)	1528	1629	79·2

† No significant treatment differences were observed.

Table 9 *Experiment 2: summary of significance levels of shape and shift displacement effects of parallel curve analysis of lactation data following dry period treatments. A significant effect indicates a significant difference in the shape or scaling parameters of the curve used in the analysis*

	Shape†	Shift displacement
Milk yield (kg/day)		***
Protein concentration (g/kg)		**
Fat concentration (g/kg)‡		
Lactose concentration (g/kg)‡		
Protein yield (g/day)‡		***
Fat yield (g/day)‡		
Lactose yield (g/day)		***

† $y = an^{mc}e^{-cn}$.
‡ $y = a+bn$.

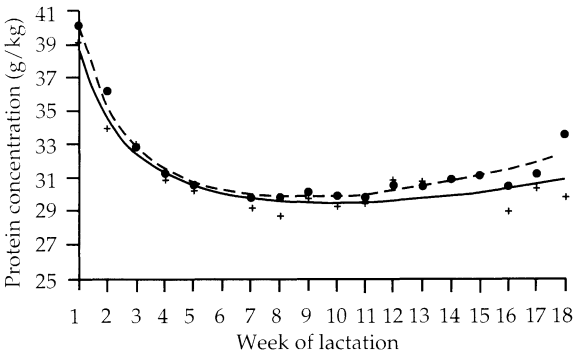

Figure 6 Mean milk protein concentrations for each dry period treatment, experiment 2 (+—+, control animals; •– –•, supplemented with additional protein during the dry period).

early lactation (37·4 *v.* 37·6 (s.e.d. 1·94) kg milk per day, 31·1 *v.* 30·4 (s.e.d. 1·43) g crude protein per kg milk, 24·2 *v.* 23·2 (s.e.d. 0·98) g casein per kg, 38·5 *v.* 40·3 (s.e.d. 2·27) g fat per kg, and 50·1 *v.* 49·5 (s.e.d. 0·67) g lactose per kg, for control and supplemented animals respectively).

The metabolic profile data for all animals during experiment 2 are summarized in Table 10. No significant effects of dry period treatment were seen for any of the variables. In contrast, the effect of state (i.e. dry or lactating) was highly significant for all variables except albumin and phosphorus. The interaction between dietary treatment and lactational status (not shown in Table 10) was significant for blood urea ($P < 0{\cdot}05$) and blood NEFA ($P < 0{\cdot}001$) concentrations. Control animals had a higher mean blood urea concentration during the dry period than supplemented animals (2·46 *v.* 2·28 mmol/l), but a lower mean blood urea concentration during lactation (2·67 *v.* 2·86 mmol/l), whereas the opposite trends were seen for NEFA concentrations (0·24 *v.* 0·30 mmol/l dry and 0·33 *v.* 0·28 mmol/l lactating).

Discussion

Significant increases in the yields of milk protein were achieved by offering a protein supplement to dairy cows during the dry period in both experiments. In experiment 1, with no differences in milk yields, this was expressed as an increase in protein concentration. In experiment 2, a concomitant increase in milk yield resulted in only a very small increase in the overall concentration of milk protein. These effects on lactation were brought about by changes in the dry period management of the animals, since all experimental animals were

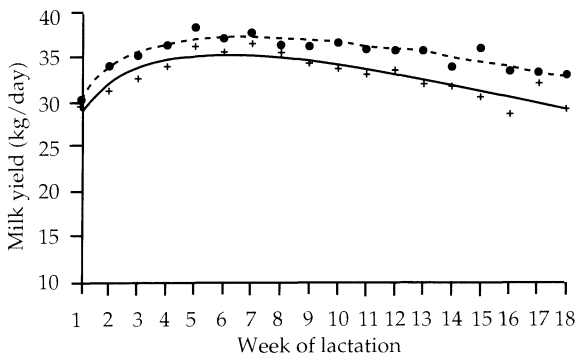

Figure 5 Model mean daily milk yields for each dry period treatment, experiment 2 (+—+, control animals; •– –•, supplemented with additional protein during the dry period).

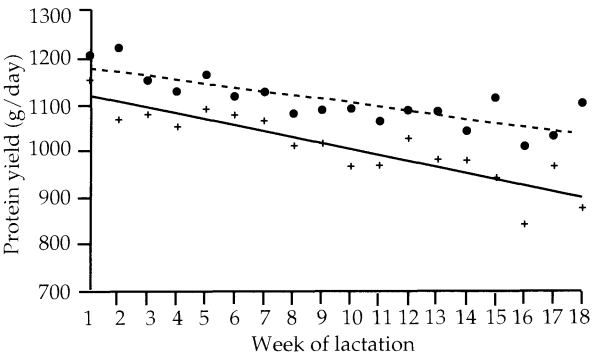

Figure 7 Mean daily protein yields for each dry period treatment, experiment 2 (+—+, control animals; •– –•, supplemented with additional protein during the dry period).

Table 10 *Experiment 2: summary of effect of experimental treatment and physiological state (dry or lactating) on blood metabolic profile analyses of all animals.*

	Dry period treatment									
	Control				Supplemented					
State:	Dry		Lactating		Dry		Lactating			Significances§ of
Sample†:	1	2	3	4	1	2	3	4	s.e.d.	state
Protein (g/l)	83·6	79·7	85·8	87·1	83·6	76·9	83·5	83·7	2·30	***
Albumin (g/l)	38·1	38·3	38·3	38·8	38·8	38·1	38·3	38·9	0·53	
Globulin‡ (g/l)	45·5	41·4	47·5	48·3	44·8	38·8	45·2	44·8	2·04	***
Urea (mmol/l)	2·61	2·31	2·80	2·53	2·13	2·42	3·05	2·67	0·180	***
Glucose (mmol/l)	4·13	4·21	3·53	3·75	4·17	3·92	3·57	3·93	0·122	***
BOHB (mmol/l)	0·48	0·49	1·02	0·78	0·34	0·42	0·89	0·81	0·119	***
NEFA (mmol/l)	0·22	0·25	0·36	0·30	0·27	0·33	0·28	0·29	0·034	**
Mg (mmol/l)	1·04	1·00	1·10	1·10	1·04	0·94	1·08	1·09	0·037	***
P (mmol/l)	2·05	1·82	1·95	1·86	1·93	1·80	1·87	1·97	0·138	

† Blood samples were taken from control and supplemented animals twice during the dry period and twice during lactation (BOHB=β-hydroxybutyrate; NEFA=non-esterfied fatty acids). Sample: 1 = 7 days after drying off, 2 = 10 days before calving, 3 = 42 days after calving, 4 = 60 days after calving.
‡ Globulin calculated as total protein – albumin.
§ There was no significant effect of experimental treatment.

treated without reference to the dry period treatment during lactation.

In the interpretation of the results from the present study, difficulties are encountered because of the limited amount of food intake data, particularly from experiment 1, because of the limited facilities available for the study. However, there are a number of ways in which the residual effects of dry period treatment may have been carried forward into lactation. Factors that have important effects on the dairy cow's ability to produce milk include seasonality effects, genetic background, food intake, and the nutrient supply from the animal's body nutrient depots (fat and labile protein).

In this study, animals were paired according to calving date to compensate for the effects of season: a poorer diet during the winter months, day length, and temperature all have potential effects on milk yield and composition. These factors were taken into account by the experimental design. Genetic background is also an important factor, and again this was taken into account by design in experiment 2 by pairing animals with similar genetic index scores. Preliminary statistical analysis of the data from experiment 1, using genetic indices and previous lactation records as covariates indicated these factors to have negligible effects, and they were therefore not included in the final analyses.

It is well established that the dairy cow mobilizes body fat as a source of energy during early lactation when food intake is not sufficient to supply energy requirements for lactation. However, there is a mechanism by which food intake is influenced by the animal's body condition, with thinner animals tending to eat more than fatter animals (Lodge *et al.*, 1975; Garnsworthy and Topps, 1982; Bines and Morant, 1983; Treacher *et al.*, 1986; Garnsworthy and Jones, 1987; Jones and Garnsworthy, 1988). Such an effect may be due to physical size restrictions in very fat animals, which are not applicable to the animals of the present study, and/or metabolic factors (Bines and Morant, 1983; Reid *et al.*, 1986). However, changes in food intake due to the small differences in CS in the present study are unlikely to have been large (Garnsworthy, 1988), although the animals may not have been scored for condition frequently enough for this to be a useful indicator of intake. In experiment 1, the CS values of the two dry period groups were no different shortly after calving and live-weight change during lactation was not significantly different between groups either, with only a small, non-significant drop in weight for the control group animals at about the 2nd month after calving. Even if this initial small difference in live weight was biologically significant, the differences in milk protein concentration lasted well beyond the differences in live weight. During experiment 2, the difference in group mean CS values was statistically significant although numerically small (0·4 points) and no significant differences in food intake were found in the small sample of animals observed during early lactation, the mean CS of which also differed by 0·4 points between the two groups.

However, CS is not a simple function of the fat condition of the animal, since underlying musculature will influence the apparent CS (Reid *et al.*, 1986). Loss of condition by both groups, in both experiments, indicates a deficiency of dietary energy during the dry period. This is clear since silage intake of the supplemented animals during the dry period was some 1·3 kg DM per day less than predicted; similar losses of body condition by the control animals suggest that silage intake, even with the silage offered *ad libitum*, was less than needed to meet energy requirements, and is probably a reflexion of the poor quality of the forage.

Plasma BOHB concentrations have been found to be a sensitive indication of energy intake relative to requirements in pregnant, non-lactating cattle (Russel and Wright, 1983). In experiment 2, no significant treatment differences were seen in plasma BOHB concentrations, although BOHB increased during lactation, indicating the negative energy balance of the animals at that time. During experiment 1, plasma BOHB and NEFA concentrations were relatively high during both the dry period and during early lactation, indicating negative energy balance of the animals during both periods leading to the loss of condition during the dry period/early lactation. Again, however, there was no difference due to treatment and therefore probably little difference in dietary energy supply between treatments during early lactation, although it is not certain that the analyses of the plasma metabolites were sensitive enough, or samples taken frequently enough, to be conclusive.

Since there are limited data to suggest otherwise, it could be assumed that food intake during lactation was altered in response to dry period treatment, and this was the cause of the effects seen on milk production. For this to have happened, the animals would have had to increase their consumption of silage, since concentrate rations were fixed. Changes in milk composition would then have been expected — increasing forage intake tends to increase milk fat production (Thomas and Martin, 1988; Sutton and Morant, 1989) but reduce milk protein concentration (Macleod *et al.*, 1983; Tessmann *et al.*, 1991; DePeters and Cant, 1992). Milk fat yield was indeed seen to increase by approximately 100 g/day from the supplemented animals in experiment 1, suggesting increased energy intake. Milk protein production is also sensitive to changes in energy intake and protein yields were also increased from supplemented animals in experiment 1, although this would not generally be expected as a result of increased silage intake alone, particularly since the milk yields of the two groups were unaffected by the dry period treatment. If energy intake was increased by increases in silage intake by the supplemented animals during lactation, it is considered unlikely that this factor was the main cause of the effects seen, and that some other factor was acting to compensate for the detrimental effect that increased silage intake would have on milk protein concentration.

Dietary protein intake can have obviously important effects on milk protein production. The major source of protein obtained by the animal from silage is through capture of rumen degradable nitrogen into microbial protein. Because dietary nucleic acids are rapidly degraded by rumen microbes (McAllan and Smith, 1973), the PD excreted in the urine of ruminants result mainly from the degradation and absorption of nucleic acids of microbial origin (McAllan, 1982). Therefore, the excretion of PD in urine can be used as an index of microbial protein yield, and relative comparisons can be made using the concentration of creatinine (de Groot and Aafjes, 1960; Albin and Clanton, 1966) in spot urine samples. In the present studies, no differences in PD excretion were seen between treatments either during the dry period or during early lactation, suggesting that the supply of microbial protein to the animals was not affected by the dry period treatments. However, there was no significant effect of dry period treatment on the food intakes of the 12 animals used to measure PD excretion and these animals did not exhibit the same milk production characteristics of the main group, i.e. there was no significant effect of dry period treatments on yield or composition of milk from those animals. Whether or not dietary protein supply differed between the two main groups of animals in experiment 2, and therefore if this could have been a factor which influenced milk compositions in the larger groups of animals, thus remains unresolved.

In the same way that body fat acts as a source of energy, body proteins may be used as a source of amino acids. The concept of body protein 'reserves' is contentious, although Swick and Benevenga (1977) point out that body protein synthesis in the dairy cow is sensitive to nutritional status; protein accretion in skeletal muscle occurs with excess protein intake, whereas repartitioning of this protein occurs with an inadequate protein intake. Work with rats (Pine *et al.*, 1994) has highlighted the potential importance of maternal body protein in the support of lactation, since rats given a high protein diet were able to lactate for about 6 days after being switched to a protein-free diet post partum, whereas those maintained on a low protein diet pre-partum were unable to do this. Van Saun *et al.* (1993) obtained a significant increase in milk protein concentration similar to the results of experiment 1 of the present study by feeding first lactation heifers increased

levels of rumen undegradable protein for 3 weeks before calving. They suggested that the provision of supplemental rumen undegradable protein during the last stages of pregnancy spared the use of maternal body protein which was otherwise being mobilized to support foetal growth. Hook *et al.* (1989) similarly gave dairy cows increased levels of rumen undegradable protein before calving, and found no difference in calf weight, although a subsequent proportional increase in milk yield of 0·084 was achieved. This was comparable to the increase in milk yields seen in experiment 2 of this study (a proportional increase of 0·11).

It is not clear what the body protein status of a typical high yielding dairy cow at the end of lactation is, and whether, therefore, the animal would respond to an increase in supply of DUP during the dry period by accreting any 'extra' dietary protein as body tissues. If the level of dietary protein supply is sufficient to support foetal growth and other peripartum protein requirements such as mammary development, it is generally considered that there is unlikely to be any net benefit in the provision of extra DUP to the dry cow. Sykes (1976) found blood albumin levels to be a good indicator of the protein 'reserves' of sheep, and Payne *et al.* (1974) found a positive correlation between blood albumin concentration and milk solids-not-fat concentration in a study of 191 dairy herds. In the present study, the plasma albumin concentration in the control group of animals during experiment 1 was lower than that of the supplemented group, particularly in late pregnancy and early lactation. This suggests a general decrease in body protein content. There was no difference in blood albumin concentrations between the two dry period groups in experiment 2, and similar concentrations of milk protein and lactose were observed, in accordance with the earlier findings of Payne *et al.* (1974).

In both this study and that of Van Saun *et al.* (1993), the protein concentration of the milk from control animals was comparatively low compared with that which may be expected during the first 6 weeks of lactation. There are a number of mechanisms by which body protein may influence milk production. The first, suggested by Van Saun *et al.* (1993), is a direct repartitioning of body proteins as amino acids from, for example, uterine tissues in early lactation or skeletal muscle, to the mammary gland for milk protein synthesis. One can speculate that the availability of certain amino acids from body tissues may compensate for limiting quantities of specific dietary amino acids. If amino acids are not being mobilized to contribute directly to milk protein production, the presence of a larger body protein mass may contribute to a greater flux of nutrients towards milk production. Wilson *et al.* (1988) demonstrated that in early lactation proportionately up to 0·34 of the carbon in casein passes through body proteins, indicating a possible rôle of body protein in support of lactation. The data of the present study do not indicate how the extra protein supplied to the animals during the dry period may have been used by them, although it is interesting that the responses lasted for several months of lactation — longer than any putative differences in body protein content between the control and supplemented animals are likely to have existed. Changes in mammary gland development during late pregnancy and early lactation have been suggested by other groups as a potential cause for differences in lactation performance in animals treated to alter endocrine profiles during late pregnancy (Chew *et al.*, 1984a; Stelwagen *et al.*, 1992). However, neither of those groups actually measured differences in mammary development; similarly, without observations to suggest otherwise, the present results may have been influenced by differences in mammary development mediated through the dry period treatments offered to the animals.

Regardless of the exact mechanisms by which the present results were mediated, it is suggested that the control animals of experiment 1 may have represented a problem area in terms of dry cow management which the protein supplemented dry period treatment rectified or prevented — a better control management system in experiment 2 meant that similar results were not obtained.

Conclusion

An increase in the milk protein yields from dairy cows was achieved by the provision of a dry period diet that consisted of restricting their energy intake and increasing their DUP intake. In experiment 1, this seems to have prevented a decrease in the protein concentration of milk, possibly rectifying a problem in the management of the animals when dry. One mechanism by which this may have been achieved is by altering the labile body protein status of the animal, with the supplemental protein minimizing the mobilization of maternal protein reserves in support of foetal growth, and therefore allowing their use in support of lactation. Further work is currently in progress to investigate this hypothesis.

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