

## Responses to supplementation by dairy cows given low pasture allowances in different seasons 2. Milk production

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### Abstract

Two factorial experiments were designed to determine the effects of stage of lactation, and season of the year, on cow responses to supplementary feeding. These experiments were conducted over consecutive years with 128 high genetic merit multiparous Holstein-Friesian cows in early, mid and late lactation in spring, summer, autumn and winter. At each stage of lactation, and in each season of the year, cows were offered a restricted pasture allowance (25 to 35 kg dry matter (DM) per cow per day), either unsupplemented (control) or with supplement at 50 MJ metabolizable energy (ME) per cow per day in experiment 1 and 80 MJ ME per cow per day in experiment 2. The two supplements given in both years were rolled maize grain (MG) and a mixture of foods formulated to nutritionally balance the diet (BR). In experiment 2, another treatment, of a generous pasture allowance (60 to 75 kg DM per cow per day) (AP), was imposed on an additional group of early lactation cows during each season. Direct milk solids (MS) (milk fat plus milk protein) responses in experiment 1 to MG were 169, 279, 195 and 251 g MS per cow per day in spring, summer, autumn and winter, respectively, while those to BR were 107, 250, 192, 289 g MS per cow per day. In experiment 2, however, milk solids responses to both supplements during spring were slightly below the control treatment, with values similar to those in experiment 1 in summer and autumn for cows on the BR but not the MG supplement. Milk solids responses to supplementary foods were largest during seasons of the year when the quantity and quality of pasture on offer resulted in the lowest milk solids yield from unsupplemented cows. When carry-over effects of feeding MG and BR on milk solids production were detected, they were only about half the magnitude of the direct effects. Serum urea concentrations were higher in control cows than those offered MG with a similar effect for BR in all but summer in experiment 1, while serum glucose concentrations were highest in winter and lowest in summer. The most important factor influencing milk solids responses was the relative food deficit (RFD) represented by the decline in milk solids yield of the respective control groups after changing from a generous pasture allowance to restricted allowance when the feeding treatments were imposed. Total milk solids responses (direct and carry-over) to supplements were greatest when severe food restrictions, relative to the cows' current food demand, resulted in large reductions in milk solids yield of the control groups. The RFD was the best predictor of milk solids response to supplementary foods. Therefore, it is likely that cows are most responsive to supplementary foods during or immediately after the imposition of a severe food restriction.

**Keywords:** dairy cows, grazing, milk production, supplementary feeding, total solids.

### Introduction

Many research studies have measured the milk yield response of grazing dairy cows to various forms of supplementary food. Leaver *et al.* (1968) reviewed several supplementary feeding experiments and concluded that the increase in milk yield was likely to be small and uneconomic when cows were grazing generous amounts of pasture. Penno (2002) reviewed supplementary feeding experiments published since 1979 and concluded responses to

supplements were highly variable and ranged from zero to 2 kg milk per kg dry matter (DM) of supplementary food. Most of the published experiments have attempted to define only direct responses to supplementary foods rather than the more complex total response that would be most useful for farmers making supplementary feeding decisions. Despite these results, over the past 30 years the use of supplementary feeding has become an important component of pasture-based dairy farming.

When provided with additional energy and nutrients the cow uses varying proportions for increased milk yield and increased reserves of body fat and protein, with the milk solids yield response often continuing for a period after the increased feeding had ceased (Broster and Broster, 1984). Therefore, the direct milk yield response reported from the majority of experiments generally underestimates the total response to supplementary feeding.

It has often been assumed that cows in early lactation partition a higher proportion of extra energy and nutrients toward milk production and less toward live-weight gain than cows in late lactation (Broster and Thomas, 1981; Stockdale *et al.*, 1987; Stockdale and Trigg, 1989). Even if this is true, in the long-term, energy stored as body reserves as a result of supplementary feeding will probably lead to increased milk yield some time later. While these potential carry-over effects have often been discussed, they have seldom been measured in experiments (Kellaway and Porta, 1993). Partitioning of energy between milk yield and body reserves, and subsequent carry-over effects, may explain the results of recent farmlet experiments suggesting small milk yield responses to supplementary feeding in spring (early lactation), improving as the season progressed (Penno *et al.*, 1995a). Larger responses to supplements from mid and late lactation cows in summer and autumn than from early lactation cows in spring have also been reported from short-term grazing experiments (Stockdale, 1999).

Penno *et al.* (2006) demonstrated that the specific mixture of nutrients provided by supplements had little effect on the DM intake (DMI) response to supplementary feeding. However, both the food deficit imposed on the cow, and the specific nutrients provided by the supplement, may have different effects on the animals short- and long-term milk yield responses to supplementary foods from those on DMI responses. This paper reports on the effects of stage of lactation and season of the year on the direct and carry-over milk yield and live-weight responses of dairy cows, grazing restricted amounts of pasture, to rolled maize grain (MG) or a nutritionally balanced supplementary food (BR), for the experiments described in the first paper of this series (Penno *et al.*, 2006). The BR supplement catered for the nutritional requirements of the cow, while taking into account the pasture nutrient supply, and alleviated the potential to underestimate supplementary feeding responses (Edwards and Parker, 1994; Lean *et al.*, 1996). The data generated from the experiments described by Penno *et al.* (2006) are used to calculate direct milk production and live-weight changes to supplementary foods in terms of relative food deficit, a predictor of milk solids responses to supplementary foods.

## Material and methods

### *Experimental design*

Penno *et al.* (2006) gives full details of the site, cows, experimental design, and feeding treatments. In summary, two supplementary feeding experiments were conducted with cows in early, mid and late lactation in spring, summer,

winter and autumn. In experiment 1, cows at each stage of lactation were grazed on a restricted allowance of pasture (25 to 35 kg DM per cow per day) and offered pasture only or supplementary feeding treatments of 50 MJ metabolizable energy (ME) per cow per day as either rolled maize grain, or as a nutrient balancing ration. In experiment 2 the same supplementary feeding treatments were offered at 80 MJ ME per cow per day, and a fourth treatment group of early lactation cows were offered a generous pasture allowance (60 to 75 kg DM per cow per day) during each of the four test periods. Each test period was preceded by a 7-day uniformity period when all cows were grazed together and offered a generous pasture allowance (60 to 75 kg DM per cow per day) to equalize each cow's nutritional opportunity before treatments were imposed; supplements were fed according to treatments over the next 5 weeks, the first two of which provided an adjustment period for the cows before treatment milk yield and composition responses were assessed. After each supplementary feeding period, cows were grazed together in their stage of lactation group and offered a generous pasture allowance of about 60 kg DM per cow per day for a further 28 days to allow any carry-over effects to be measured. Averaged over the four seasons of the year early, mid and late lactation cows at the start of experiment 1 were 66, 157, and 251 days in milk, respectively, with comparable milk productions of 18.8, 15.2 and 12.9 kg per cow per day. Similarly, for experiment 2, there were 52, 126, 215 days in milk, with milk productions of 18.1, 14.3 and 12.0 kg per cow per day.

### *Measurements*

*Milk yield, milk composition, and live-weight.* At two consecutive milkings each week, milk volumes for each cow were recorded from the meter flask post-milking. At the same time, Tru-Test™ in-line milk meters were used to take a representative sub-sample of 2.5% of the total milk yield of each cow. Following stirring by bubbling air through the flask, a 30 ml aliquot was taken and analysed for fat and protein concentrations by calibrated Fossomatic milk-o-scan (Foss Electric, Hillerod, Denmark).

Calibrated Tru-Test™ electronic scales were used to measure the live weight of each cow, immediately after the morning milking on day seven of the uniformity week, on day 35 of the test period, and on day 28 of the post-experimental carry-over period (experiment 1 only).

*Blood metabolites.* Blood samples were collected by coccygeal venipuncture from each cow, into 10 ml evacuated plain glass tubes at between 14:00 to 15:00 h on day 33 of each test period, and allowed to clot at room temperature for 60 to 90 min. Samples were centrifuged at 2800 r.p.m. for 15 min. Aspirated serum was immediately assayed for concentrations of albumin, beta hydroxybutyrate (BOH), non-esterified fatty acids (NEFA), glucose and urea using a Hitachi 717 auto-analyser.

### *Calculations*

Milk solids (MS) responses during each test period (direct responses) were calculated as the difference in mean daily

## Supplementation and pasture intake by dairy cows – 2

milk solids yield between treatment groups and their respective control groups, and were expressed as g MS per MJ ME by dividing by the daily ME intake from supplementary food. In the same way, live weight (LW) gain treatment responses were calculated and expressed as g LW per MJ ME. Similarly, carry-over milk solids responses were calculated for each early and mid lactation treatment group during the 4 weeks after the cessation of supplementary feeding, and using the daily ME intake from supplementary food during the preceding test period as the divisor. Total MS responses (direct plus carry-over) were calculated for cows in early and mid lactation in the same way. Carry-over effects for late lactation cows could not be obtained because they were approaching the end of their lactation.

Relative food deficit (RFD) was calculated as the average MS yield of each control group at the end of the uniformity week (immediately before the start of the test period) minus the average milk solids yield measured during the final 3 weeks of each 5-week test period, the latter being referred to as the 'unsupplemented MS yield'. This provides an indirect estimate of RFD which in energy terms was defined as the daily ME intake that the cow required to meet the energy costs of maintenance and pregnancy, milk yield, and the rate of change in body reserves.

### Statistical analysis

Experiments 1 and 2 were analysed separately. Residual maximum likelihood (REML) procedures of Genstat (Genstat Committee, 1997) were used for analysis of production and live-weight variables, using stage of lactation, season, food and their interactions as fixed effects. In addition, appropriate covariates from the uniformity week and random effects were specified for each particular variable. For production and milk components over the various periods, stage of lactation, season/week, food and their interactions were specified as fixed effects; milk production for the uniformity week as a deviation from the stage by season mean was used as a covariate and cow/season/week were specified as random effects.

Milk yield data presented for the test periods are the predicted means, adjusted for imbalance in covariates and the number of observations, of three herd tests over the last three weeks of each test period in experiment 1, and from two herd tests over the last two weeks of each test period in experiment 2. Milk yield data presented from the carry-over responses are the predicted means of weekly herd tests during the 28 days after each test period in both experiments.

The prediction of mean MS and live-weight gain responses were calculated as above for each experiment, were analysed as a  $3 \times 2 \times 4$  factorial design, with three feeding treatments imposed on two groups of cows (no. = 8) (early and mid lactation) at four times of the year, using the linear model of Data Desk 6.0 (Velleman, 1997). Non-significant interactions ( $P > 0.05$ ) were removed from the model. Data are presented as the predicted means with standard errors of the difference using the interaction between stage of lactation, season and food as the error term.

The combined data from experiments 1 and 2 were subject to multiple regression analysis using Data Desk 6.0 (Velleman, 1997). Combinations of factors were alternatively analysed to establish models of best fit to the calculated MS responses, as indicated by adjusted  $R^2$ . Multiple regression equations are presented with standard errors and significance levels for each coefficient, adjusted  $R^2$  and a residual standard deviation (r.s.d.).

## Results

### Experiment 1

Offering MG supplements reduced milk fat concentration relative to the control treatment in autumn and this was the case for both MG and BR supplements in winter (Table 1).

There was an interaction between the effects of stage of lactation and food type for milk protein concentration. However, the effect of food on milk protein concentration was not different between seasons. In early lactation, MG and BR supplements increased protein concentration, whereas in mid lactation only MG had this effect and in late lactation treatment differences disappeared (Table 1).

Offering MG and BR supplements increased MS (milk fat plus milk protein) yield in all seasons, with no difference between MG and BR treatments. Offering MG in spring resulted in higher MS yields than in summer, autumn and winter, whereas the milk solids yield of cows offered BR did not vary with season (Table 1).

Comparing MS yields of cows offered the control treatment with those offered the MG supplement over the different stages of lactation, showed direct responses of 169, 279, 195 and 251 g MS per cow per day in spring, summer, autumn and winter, respectively. Comparable data for BR were 107, 250, 192 and 289 g MS per cow per day. This shows these responses were not consistent across seasons, but were greatest in summer and winter and smallest in spring.

The main effects of stage of lactation, season of year and feeding treatments on the concentration of blood metabolites are presented in Table 2. Although there were some significant ( $P < 0.05$ ) interactions, especially between the effects of season and food, these are not reported because of inconsistent trends between experiments. Serum glucose concentrations were higher during winter than during spring, summer and autumn. During winter, cows offered the BR supplement had lower serum glucose than cows offered the control treatment.

There were inconsistent effects of supplementary foods and season on average NEFA and BOH concentrations in serum (Table 2).

Serum urea concentrations of cows on the control treatment were higher than in cows offered the MG and BR supplements in most seasons. Serum urea concentrations of cows on the control treatment and those offered MG supplements were highest in autumn and lowest in spring.

Penno, Macdonald, Holmes, Davis, Wilson, Brookes and Thom

**Table 1** Mean milk production and composition, and live-weight changes measured during each test period of experiment 1 and average milk solids yield during each carry-over period, when all cows were offered a generous pasture allowance and no supplement

Food (F)	Stage (St)									s.e.d <sup>‡</sup>	Significance <sup>†</sup>				
	Early			Mid			Late				Season (S)	St	F	S x F	St x F
	Control	MG	BR	Control	MG	BR	Control	MG	BR						
<b>Spring</b>															
Milk yield (kg per cow per day)	17.4	19.9	18.3	13.2	15.7	15.2	11.5	12.8	11.9	0.80	**	**			
Milk solids yield (g per cow per day)	1258	1471	1363	1001	1206	1194	985	1078	1008	66.3	**	*			
Milk fat concentration (g/kg)	41.6	42.0	41.1	43.9	42.0	44.2	50.0	47.0	49.0	2.07	**	*			
Milk protein concentration (g/kg)	31.4	32.9	33.6	33.7	35.2	34.8	36.8	37.7	37.4	0.83				**	
Live-weight change (g per cow per day)	83	164	495	1221	1533	1195	237	1436	1347	294	**	**			
<b>Summer</b>															
Milk yield (kg per cow per day)	12.3	16.3	15.7	12.4	15.0	15.7	7.2	11.0	10.2						
Milk solids yield (g per cow per day)	896	1234	1151	988	1205	1256	673	950	897						
Milk fat concentration (g/kg)	44.2	43.8	42.5	46.7	46.1	46.4	55.9	50.9	51.9						
Milk protein concentration (g/kg)	31.1	31.8	31.6	33.9	35.3	34.4	39.1	38.5	38.5						
Live-weight change (g per cow per day)	-929	-710	-1014	-205	46	-336	-163	126	33						
<b>Autumn</b>															
Milk yield (kg per cow per day)	12.4	14.1	13.7	11.7	13.2	13.9	10.0	14.1	13.2						
Milk solids yield (g per cow per day)	994	1184	1147	995	1116	1170	859	1135	1111						
Milk fat concentration (g/kg)	48.9	48.2	47.8	49.5	48.2	48.6	51.4	44.3	48.8						
Milk protein concentration (g/kg)	32.5	36.8	35.3	35.7	37.2	36.6	37.7	37.5	37.8						
Live-weight change (g per cow per day)	74	223	311	676	407	319	821	1197	736						
<b>Winter</b>															
Milk yield (kg per cow per day)	10.9	14.3	15.5	9.4	11.9	13.5	8.1	11.9	11.6						
Milk solids yield (g per cow per day)	890	1131	1203	810	1005	1101	741	1053	993						
Milk fat concentration (g/kg)	49.7	44.4	44.4	51.6	47.0	46.9	55.4	51.5	47.5						
Milk protein concentration (g/kg)	32.4	34.6	33.9	35.9	38.0	35.9	39.1	39.8	39.0						
Live-weight change (g per cow per day)	-890	-612	-350	-266	50	548	-498	-350	86						
<b>Carry-over period</b>															
Milk solids yield (g per cow per day)															
Spring	1360	1494	1486	1174	1238	1348				68.2	**	**	**		
Summer	959	1094	1031	856	914	903									
Autumn	927	1011	976	850	761	882									
Winter	1002	1112	1181	1033	1016	1132									

<sup>†</sup> There were no season x stage x food interactions ( $P > 0.05$ ).

<sup>‡</sup> Maximum standard error of the difference for all paired comparison of interaction means.

**Experiment 2**

There were feeding treatment effects on the milk fat and milk protein concentrations but no interaction. Average milk fat concentrations were 44.3, 46.3 and 50.1 g/kg and milk protein concentrations were 32.3, 35.7 and 38.6 g/kg, in milk produced by early, mid and late lactation cows, respectively (Table 3).

The milk produced by cows offered the AP treatment had a similar milk fat concentration and a higher milk protein concentration when compared with the milk produced by early lactation cows offered the control treatment.

There were significant interactions between the effects of stage of lactation and those of food, and between the effects of season and those of food for milk, milk fat, milk protein and MS yield (Table 3). Therefore, data from each test period are presented separately.

**Spring.** Offering MG and BR supplements or the AP treatment had no effect on MS yield at any stage of lactation in spring. Cows at all stages of lactation and on all feeding treatments had higher MS yields during spring than during autumn and winter. Early and late lactation cows offered the control, AP and MG feeding treatment had higher MS yield in spring than summer, whereas mid-lactation cows showed no response to any feeding treatment.

**Summer.** The BR treatment increased MS yields of early and mid lactation cows in summer, with AP having a similar effect on early lactation cows; MG only affected cows in mid lactation.

Early and mid lactation cows had higher MS yields during summer than cows at the same stage of lactation and feeding treatment during autumn and winter. Late lactation cows

## Supplementation and pasture intake by dairy cows – 2

**Table 2** Concentrations of blood serum metabolites in cows at different stages of lactation, when offered a restricted pasture allowance, with or without supplemental foods of rolled maize grain (MG) or a nutritional balancing ration (BR) in spring, summer, autumn and winter in experiments 1 and 2

	Glucose (mmol/l)	NEFA <sup>†</sup> (mmol/l)	BOH <sup>†</sup> (mmol/l)	Albumin (g/l)	Urea (mmol/l)
<b>Experiment 1</b>					
Early	2.89	0.052	0.95	30.7	7.26
Mid	2.82	0.043	0.92	30.2	7.20
Late	2.84	0.037	0.94	30.5	6.64
s.e.d.	0.042	1.09 <sup>‡</sup>	1.05 <sup>‡</sup>	0.204	0.101
Significance		***		*	***
Spring	2.56	0.036	0.88	30.5	5.24
Summer	2.81	0.056	0.92	30.5	5.74
Autumn	2.74	0.051	0.98	30.7	8.99
Winter	3.29	0.049	0.96	30.2	8.16
s.e.d.	0.060	1.12 <sup>‡</sup>	1.07	0.300	0.141
Significance	**	***	**		***
Control	2.89	0.060	0.97	30.6	7.78
MG	2.84	0.042	0.92	30.2	6.10
BR	2.83	0.043	0.92	30.6	7.23
s.e.d.	0.043	1.09 <sup>‡</sup>	1.05 <sup>‡</sup>	0.211	0.103
Significance		***			***
<b>Experiment 2</b>					
Early	2.82	0.074	0.81	29.9	6.56
Mid	3.03	0.042	0.81	29.5	6.68
Late	2.76	0.038	0.80	29.5	6.29
s.e.d.	0.047	1.13 <sup>‡</sup>	1.07 <sup>‡</sup>	0.280	0.136
Significance	***	***	***		**
Spring	3.47	0.048	0.78	29.7	7.27
Summer	2.04	0.059	0.94	29.4	5.45
Autumn	2.54	0.042	0.76	28.7	5.95
Winter	3.45	0.049	0.75	30.5	7.37
s.e.d.	0.061	1.17 <sup>‡</sup>	1.10 <sup>‡</sup>	0.368	0.167
Significance	***	***	***	***	***
Control	2.74	0.058	0.86	30.0	8.08
MG	3.00	0.044	0.72	29.1	4.65
BR	2.87	0.038	0.84	29.7	6.80
s.e.d.	0.048	1.13 <sup>‡</sup>	1.08	0.286	0.138
Significance	***	***	***	**	***

<sup>†</sup> Calculated from natural log transformed data. NEFA = non-esterified fatty acid; BOH = beta-hydroxy-butyrate.

<sup>‡</sup> Minimum significant ratio calculated from natural log transformed s.e.d.

offered the control treatment had higher MS yield in summer than in autumn and winter but the MS yields of late lactation cows offered the MG and BR supplements were similar in summer, autumn and winter.

**Autumn.** Offering AP, MG and BR feeding treatments to early lactation cows increased MS yield, especially those offered the BR treatment. Both the MG and BR supplements increased the MS yield of mid lactation cows, while BR had a similar effect on late lactation cows.

Early lactation cows in autumn had higher MS yields than similar cows in winter. However, mid lactation cows offered the MG and BR feeding treatments had lower MS yields than mid lactation cows in winter. There was no difference between the MS yields of late lactation cows in autumn and winter.

**Winter.** In winter, only the BR treatment increased the MS yield of the early lactation cows, while both the MG and BR treatments increased the MS yields of mid and late lactation cows.

As reported for experiment 1, there was a tendency for serum glucose concentrations to be highest in winter and lowest in summer (Table 2). Cows offered the MG and BR treatments had higher glucose concentrations than cows offered the control treatment during spring and autumn. During summer, cows offered the BR treatment had lower serum glucose than cows offered the control and MG treatments.

There were few effects of season and type of food on NEFA and BOH concentrations (Table 2).

The MG treatment resulted in lower serum urea concentrations than the control treatment in all seasons, while this was true for cows offered the BR treatment in spring the opposite was true in summer. Cows offered the control and MG treatments had higher serum urea concentrations in spring and winter than in summer and autumn (Table 2).

In experiment 1 (averaged over the 4-week carry-over period) the cows offered the MG and BR supplements produced another 73 and 100 g MS per cow per day, respectively, compared with those on the control treatment (Table 1). In experiment 2 there were significant season × food interactions for all MS variables, but not for stage of lactation by food type (Table 3).

### Predicting milk solids responses to supplementary feeding

Using the results of both experiments, the relationship between the RFD and the direct and total MS responses of the appropriate supplemented herds are shown in Figures 1 and 2, respectively. The association between the RFD, the total yield of the respective control group (unsupplemented MS yield), supplement intake and stage of lactation, and the direct MS response to supplementary feeding are described by the multiple regression equation presented in Table 4. The association between the RFD, pasture ME allowance at the time of supplementary feeding, supplement intake and the stage of lactation, and the total MS response to supplementary feeding are described by the multiple regression equation presented in Table 5.

The magnitude of the direct and total MS responses to supplementary foods increased as the magnitude of the decline in MS yield in the unsupplemented cows following food restriction (RFD) increased. The direct and total MS response increased as the level of feeding of the unsupplemented cows decreased, as measured by unsupplemented MS yield or pasture allowance. As the amount of supplement eaten increased the direct MS response decreased, however, this relationship was not significant for total MS response. Direct MS response was not affected by stage of lactation.

## Discussion

When the effects of stage of lactation were separated from those of season of the year in the current experiments, stage of lactation had no effect on the direct responses in MS yield or in live-weight gain. These results support some

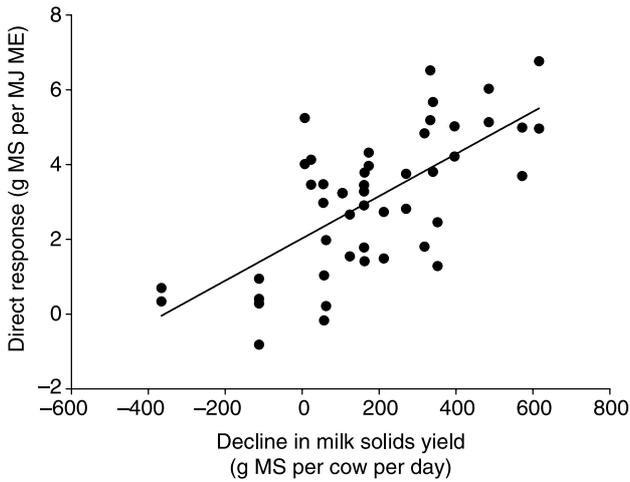
**Table 3** Mean milk production and composition, and live-weight changes measured during each test period and milk solids changes during each carry-over period, when all cows were offered a generous pasture allowance and no supplements in experiment 2

Food (F)	Stage (St)												Significance <sup>†</sup>							
	Early						Late													
	Control	AP	MG	BR	Control	MG	BR	Control	MG	BR	Control	MG		BR	s.e.d. <sup>‡</sup>	Season (S)	St	F	S × F	St × F
Spring																				
Milk yield (kg per cow per day)	24.0	23.0	23.4	22.9	15.8	16.5	17.0	15.0	15.9	14.5	1.12								*	
Milk solids yield (g per cow per day)	1889	1852	1758	1798	1285	1342	1350	1319	1371	1282	84.3								**	
Milk fat concentration (g/kg)	48.7	46.8	44.7	43.0	44.2	44.0	42.2	50.7	46.9	49.6	2.29			**	**	**			**	
Milk protein concentration (g/kg)	32.1	33.9	32.4	34.4	36.2	38.1	38.0	37.5	39.2	39.7	1.20			**	**	**			**	
Live-weight change (g per cow per day)	250	717	305	597	854	1038	415	381	834	1123	312			**	**	**			**	
Summer																				
Milk yield (kg per cow per day)	19.1	22.1	19.7	23.1	15.6	17.1	18.3	12.0	12.9	13.1										
Milk solids yield (g per cow per day)	1463	1635	1487	1723	1229	1414	1495	1066	1058	1141										
Milk at concentration (g/kg)	47.5	43.9	43.1	45.0	46.0	48.7	47.5	52.0	47.1	48.7										
Milk protein concentration (g/kg)	30.6	31.3	32.4	31.9	33.4	34.3	35.2	37.5	37.0	38.4										
Live-weight change (g per cow per day)	-665	-100	-228	-215	337	281	267	-363	-134	43										
Autumn																				
Milk yield (kg per cow per day)	14.6	16.1	16.8	19.5	8.7	10.8	13.5	8.1	10.1	11.1										
Milk solids yield (g per cow per day)	1075	1235	1302	1441	713	910	1098	760	912	965										
Milk fat concentration (g/kg)	43.4	44.4	44.6	43.4	50.5	46.7	47.6	57.8	51.3	49.4										
Milk protein concentration (g/kg)	30.1	30.9	32.4	32.1	35.4	38.0	35.1	39.7	39.9	39.8										
Live-weight change (g per cow per day)	-368	-14	351	428	28	520	442	693	594	1059										
Winter																				
Milk yield (kg per cow per day)	11.6	13.6	13.7	15.2	10.3	15.3	15.3	9.1	12.7	12.3										
Milk solids yield (g per cow per day)	865	1038	1022	1172	864	1189	1186	782	1114	1037										
Milk fat concentration (g/kg)	44.1	42.0	40.5	44.1	50.8	42.3	44.6	50.3	49.7	47.3										
Milk protein concentration (g/kg)	31.5	33.4	34.3	33.3	34.4	36.4	34.2	36.6	39.4	38.6										
Live-weight change (g per cow per day)	502	1690	976	1120	146	382	681	1217	1569	1526										
Carry-over period																				
Milk solids yield (g per cow per day)											85.3							*		
Spring	1658	1384	1446	1595	1264	1309	1336													
Summer	1361	1481	1457	1481	1272	1339	1413													
Autumn	807	874	868	895	712	743	890													
Winter	807	874	868	895	712	743	890													

<sup>†</sup> There were no season × stage × food interactions ( $P > 0.05$ ).

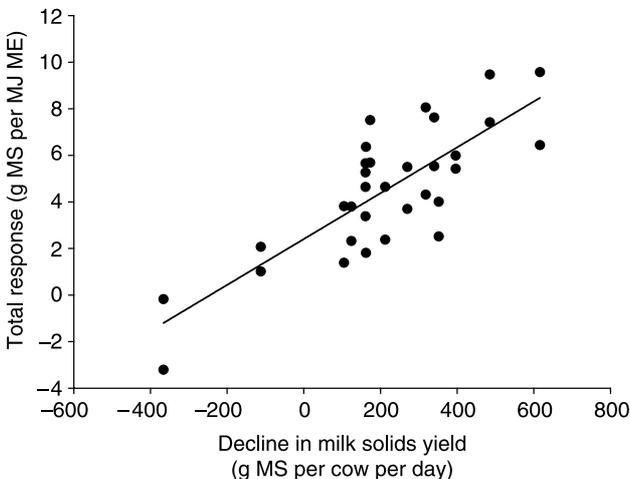
<sup>‡</sup> Maximum standard error of the difference for all paired comparison of interaction means.

## Supplementation and pasture intake by dairy cows – 2



**Figure 1** The relationship between the decline in milk solids (MS) yield of the control herd as treatments were imposed and the direct milk solids response.

recent studies that have demonstrated large increases in milk yield when supplements have been offered to late lactation cows (Stockdale and Dellow, 1995; Robaina *et al.*, 1998). However, they contrast with earlier indoor studies which were designed to make direct comparisons between the supplementary feeding responses of cows in early or mid lactation (Stockdale *et al.*, 1987; Stockdale and Trigg, 1989). However, these indoor supplementary feeding studies imposed common feeding treatments on cows at different stages of lactation, despite large differences in actual and potential milk yield. For example, Stockdale *et al.* (1987) compared early and late lactation cows consuming a severely restricted allowance of pasture (about 7 kg DM per cow per day) plus different amounts of concentrates, with control groups consuming only the restricted allowance of pasture. This common restricted food allowance imposed a more severe food restriction on the higher yielding cows in early lactation than those in late lactation, resulting in a much larger reduction in MS yield. Thus, as feeding levels



**Figure 2** The relationship between the decline in milk solids (MS) yield of the control herd as treatments were imposed and the total milk solids response.

were increased with supplementary foods, the early lactation cows partitioned a greater proportion of the additional energy toward milk yield and a lesser proportion to live-weight gain. Further, when the amount of pasture offered to the control cows was more generous, and the decrease in milk yield of the early and late lactation cows became less severe, the responses attributed to supplementary feeding also declined (Stockdale and Trigg, 1989).

The changes in MS yield that occurred as the control treatments were imposed during the present study were generally much smaller than those of Stockdale *et al.* (1987) and Stockdale and Trigg (1989), as was also reported by Grainger (1990). Further, the differences between groups at different stages of lactation, within each test period were also small. Thus, the potential of early and late lactation cows to increase milk yield, back to their pre-treatment yield, was similar. Grazing also creates higher energetic requirements and provides the cow with an opportunity to respond to the imposition of food restrictions by grazing more intensely, to maintain pasture intake. This may buffer the different relative food restrictions that were imposed on early and mid-lactation cows during the present experiments.

The MS increases from cows offered MG and BR supplements were largest in summer, autumn and winter, rather than spring, in agreement with results of farm systems experiments conducted by Penno *et al.* (1995b). These experiments suggest that large increases in MS production are closely associated with periods of low milk yields (relative to their potential) from the control cows. Again, when the control cows are placed under more severe nutritional restrictions, the potential to increase milk yield is larger, and these cows are likely to partition a higher proportion of additional food energy to milk yield, rather than live-weight gain.

In both experiments, cows in late and mid lactation showed larger changes in live weight than those in early lactation. Season and supplementary food effects were inconsistent between years (Tables 1 and 3).

Within the experimental design, comparisons between seasons present the most difficulty. In addition to changing pasture allowances, necessitated by changing pasture structure, pasture quality also varied between seasons (Penno *et al.*, 2006). Both these factors are known to have a large affect on pasture DMI and subsequent MS yield (Holmes, 1987). However, environmental factors associated with season of the year, other than nutrition, may also have affected milk yield (Garcia and Holmes, 1999). In particular, seasonal changes in photoperiod are known to affect food intake and milk yield of cows at all stages of lactation (Peters *et al.*, 1981). Typically, cows under winter photoperiods produce 7 to 10% less milk than cows under summer photoperiods at the same level of nutrition (Peters *et al.*, 1981; Bilodeau *et al.*, 1989). However, these results are not consistent with those of Auld *et al.* (1998) who demonstrated higher MS yield from early, mid and late lactation cows in summer and autumn than in winter and spring.

**Table 4** The effect of the reduction in milk solids (MS) yield of the unsupplemented cows immediately preceding supplementary feeding, pasture allowance and supplement intake on the direct milk solids response to supplementary foods (g MS per MJ metabolizable energy (ME))<sup>†</sup>

Variable	Coefficient	s.e. of coefficient	Significance
Constant	11.1	1.87	***
Reduction in MS yield (kg per cow per day)	2.1	1.00	*
Unsupplemented MS yield (kg per cow per day)	- 4.1	0.99	***
Supplement intake (MJME per cow per day)	- 0.06	0.014	*
Stage of lactation (DIM)	- 0.005	0.0029	

<sup>†</sup> Adjusted  $R^2 = 0.688$ ; residual s.d. = 1.09.

The present experiments suggest that the different levels of performance observed between seasons and in particular the low milk production levels measured in autumn and winter are largely a reflection of the relative levels of nutrition immediately before and during the test periods.

Cows become increasingly responsive to cereal grain supplements as the ME concentration of the pasture on offer declines (Stockdale *et al.*, 1997). In the present experiments, ME concentration in pasture was consistently lowest in summer (Penno *et al.*, 2006) but these differences were not well correlated with the magnitude of supplementary feeding responses. For example, the ME concentration of spring and winter pastures were similar, yet supplementary feeding resulted in small responses in spring and large responses in winter (although pasture intake was also lower during winter). The differences in pasture quality observed by Stockdale *et al.* (1997) were much larger (8 to 12 MJ ME per kg DM) than the between season variation that occurred in the present studies. Nevertheless, the small changes in pasture quality that were observed may have contributed to the differences in response between seasons by increasing the magnitude of underfeeding during the summer. Offering BR supplements to early lactation cows in summer, autumn and winter of experiment 2 resulted in larger increases in MS yield than offering the same amount of ME in the form of MG supplement. The main difference between the two supplements was that BR contained extra protein as both rumen degradable (soybean meal) and undegradable protein (fish meal) in summer, and undegradable protein and effective fibre (chopped hay) in autumn and winter (Penno *et al.*, 2006).

Nutritional treatments and time of the year had little effect on the longer-term protein status of the cows as indicated by serum albumin concentration. However, serum urea concentrations varied with different crude protein (CP) concentrations in the pasture (Penno *et al.*, 2006). High CP concentrations in autumn and winter pasture

(experiment 1) resulted in elevated rumen ammonia N and blood urea concentrations and similarly, in spring, autumn and winter of experiment 2 (Penno, 2002). Offering MG and BR supplements decreased serum urea concentration, probably by reducing CP intake and increasing the supply of readily fermentable carbohydrate (Kolver *et al.*, 1998). Based on these metabolic indices, it is likely that the treatment groups offered the MG supplement in the summer and autumn (experiment 2) did not receive adequate protein nutrition. The low serum urea and albumin concentrations in the summer of experiment 2 corresponded with the lowest pasture CP concentration (17.1 g per 100 g DM).

Offering undegradable protein supplements to cows grazing generous amounts of pasture have usually not increased milk yield (Brookes, 1984; Penno *et al.*, 1995b; Rusdi and Van Houtert, 1997; Stockdale *et al.*, 1997), the opposite being the case when pasture availability was restricted (Rogers *et al.*, 1980; Minson, 1981), as in the current experiments. The total diets of cows offered the MG supplements in the autumn and winter of experiment 2 were likely to have exceeded a CP concentration of 17 g per 100 g DM, which should have been adequate for milk production (National Research Council, 1989). Undegradable protein (fish meal) was more effective than degradable protein (soya-bean meal) when supplementing grazing cows consuming large amounts of maize silage (Macdonald *et al.*, 1998). Ørskov *et al.* (1981) suggested that when cows were in energy deficit, undegradable protein supplements may provide amino acids that were limiting milk yield, thereby stimulating the mobilization of body condition to provide glucose for increased milk yield, although there was no difference between the live-weight changes of cows offered the MG and BR supplements in the current experiments. Nevertheless, the possibility exists that the dietary levels of essential amino acids limiting the milk yield of the control cows could have been countered by the addition of fishmeal.

**Table 5** The effect of the reduction in milk solids (MS) yield of the unsupplemented cows immediately preceding supplementary feeding, pasture allowance, supplement intake and stage of lactation during the period of supplementary feeding, on the total milk solids response to the supplementary feeds (g MS per MJ metabolizable energy (ME))<sup>†</sup>

Variable	Coefficient	s.e. of coefficient	Significance
Constant	10.2	2.30	***
Reduction in MS yield (kg per cow per day)	9.1	1.18	***
Pasture allowance (MJME per cow per day)	- 0.02	0.006	**
Supplement intake (MJME per cow per day)	- 0.02	0.022	
Stage of lactation (DIM)	0.007	0.005	

<sup>†</sup> Adjusted  $R^2 = 0.794$ ; r.s.d. = 1.35.

## Supplementation and pasture intake by dairy cows – 2

Overall, the incremental benefits from providing supplements that were formulated to balance the diet of grazing cows were small when compared with maize grain supplements. These findings contradict recent suggestions that many published supplementary feeding studies have grossly underestimated potential supplementary feeding responses by ignoring the detailed nutritional requirements of the cows and the nutrient composition of the pasture (Edwards and Parker, 1994; Lean *et al.*, 1996).

One of the limitations of the present experiments is the difficulty in accurately measuring carry-over effects so most researchers have just reported the direct response. In these experiments MS yield remained elevated during the 4 weeks following supplementary feeding of MG in the spring, summer and winter of experiment 1, and in winter of experiment 2. MS yield also remained elevated following the supplementary feeding of BR in the spring and summer of experiment 1, and after the summer, autumn and winter of experiment 2. The magnitude of carry-over effects when present was on average about half the direct effect, and diminished over time. MS yield increases after stopping supplementary feeding usually follows a period in which supplementary feeding had increased body weight. The extra MS produced after supplementary feeding has often been attributed to the mobilization of body reserves (Kellaway and Porta, 1993). However, several authors have also suggested that nutritional history can affect future MS yield (Broster and Broster, 1984; Oldham and Emmans, 1989). In addition to the benefits of additional body reserves that usually result from a higher level of feeding, a higher absolute milk yield may predispose the cow to higher future MS yield, should level of nutrition allow.

The carry-over effects of supplementary feeding appear to differ both within and between seasons. Bryant and Trigg (1982) suggested that the use of supplements during spring food deficits would not result in any significant carry-over effects. However, Clark (1993) found that 0.68 of the additional milk production that resulted from feeding silage in spring occurred after the feeding period. It would appear that carry-over effects could be both animal and pasture related. Within farm systems, substitution of pasture by supplements may increase the pasture allowance and DMI for some time after supplementary feeding ceases, increasing MS yield and contributing to carry-over effects (Kellaway and Porta, 1993). Although derived from short-term experiments the present data suggests that the contribution of the cow to carry-over effects is only about half the 7 kg MS per cow reported by Clark (1993) after feeding silage in spring and summer and measuring the total carry-over effects for the remainder of the milking season.

### *Prediction of milk solids responses to supplementary feeding*

While there were often differences between the MS and live-weight responses that resulted from offering supplements to cows at different stages of lactation, no consistent patterns emerged.

The effects of stage of lactation and of season have usually been confounded in previous experiments, because of the seasonal calving pattern of most grazing dairy herds.

Nevertheless, experiments have usually not demonstrated large seasonal effects. In the present study, there was no association between pasture quality parameters and the magnitude of the measured responses. The lowest ME concentration of pasture offered was 10.9 MJ/kg DM, and concentrations were often greater than 12.0 MJ ME per kg DM (Penno *et al.*, 2006).

Stage of lactation and season of the year were shown to have only small, inconsistent effects on the response of dairy cows to supplementary food. It has been demonstrated that the level of pasture feeding, as measured by pasture allowance, has a large influence over the extent to which cows substitute pasture for supplement. In the current study, this effect is shown as a reduction in the total and direct MS response with increasing pasture allowance or as higher daily MS yield of the control cows reflecting a higher pasture allowance. Higher pasture allowances and greater supplementary food intake in the second than in the first experiment, provides an explanation for the lower MS responses measured in experiment 2.

During the experimental periods, the pasture allowance offered, represented a restriction relative to that offered up to and during the uniformity week. The severity of this food restriction varied between test periods and, to a lesser degree, between different stages of lactation. It has been assumed that the magnitude of the loss of MS yield that occurred as the feeding treatments were imposed provides a measure of the severity of the food restriction relative to current food demand and, therefore, is a measure of the potential energy deficit for that particular treatment group. Thus Figures 1 and 2 demonstrate the association between potential energy deficit and the direct and total MS responses. The lowering of MS yield (as a measure of potential energy deficit) increased total MS responses by 9 g MS per MJ ME per 1.0 kg decline in MS yield. Nevertheless, in developing the relationships illustrated in Figures 1 and 2, we recognize that using the control group's milk yield in both the calculation of the cow response and the relative food deficit (represented by the loss in MS yield by the control group) may cause spurious correlations because of variation in the data.

In the literature a wide range of experimental techniques have been used and conditions under which these experiments have been undertaken, the model of direct MS yield responses closely predicted the results of the published work. This suggests that the factors considered by the model, particularly the decline in MS yield, the unsupplemented MS yield, and the amount of supplement offered may account for a high degree of the variation between published results. Therefore, the level of feeding of experimental treatments, relative to the level of feeding immediately before treatments have been imposed, is an important factor in determining the MS response to supplementary foods.

### *Carry-over responses*

The magnitude of the carry-over responses we measured was generally about 50% of the direct responses. While the data of Clark (1993) support these estimates, the current data suggests that the contribution the cow makes directly

to the carry-over response is likely to provide only half of the response measured within systems experiments, with the remainder being provided by the increased pasture intake at a later date, resulting from pasture substitution.

### Conclusions

The present studies showed that stage of lactation has little effect on the milk yield responses to supplementary foods of high genetic merit cows grazing restricted amounts of pasture. Likewise, formulating supplementary foods to complement the pasture on offer is of little benefit when those supplements are primarily used to overcome a total food deficit. In contrast, season of the year can affect the responses to supplementary feeding. Differences between seasons were closely associated with the level of production achieved by cows receiving the pasture only control, with responses being larger at times when MS yield was reduced by pasture characteristics such as quantity and quality. Perhaps these low absolute MS yields are the best measure of the difference between the energy required for the cows to attain their potential MS yield, and the actual energy intake allowed by the amount and quality of pasture on offer. Therefore, although it is difficult to define quantitatively, the concept of a potential energy deficit should be developed as a predictor of the likely response of the grazing dairy cows to supplementary foods.

This work suggests that the magnitude of the total MS response to supplementation at pasture can largely be predicted by the magnitude of the potential energy deficit as indicated by recent change in daily MS yield and the ME allowance from pasture and supplement. These are all factors that can be estimated by farmers in advance of supplementary feeding decisions and they may provide the basis for a tool to allow the MS responses of grazing dairy cows to supplements to be predicted more accurately. Irrespective of season of the year and stage of lactation, the largest total MS responses are likely to occur when cows have suffered a sudden decline in pasture allowance and when small quantities of supplementary food are offered. On well-managed farms, the decline in pasture allowance and the introduction of the supplements will occur simultaneously, so that the decline in milk yield is prevented and high responses to the extra food are obtained.

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### References

- Auldish, M. J., Walsh, B. J. and Thomson, N. A.** 1998. Seasonal and lactational influences on bovine milk composition in New Zealand. *Journal of Dairy Research* **65**: 401-411.
- Bilodeau, P. P., Petitclerc, D., Pierre, N. St., Pelletier, G. and Laurent, G. J. St.** 1989. Effects of photoperiod and pair-feeding on lactation of cows fed corn or barley grain in total mixed rations. *Journal of Dairy Science* **72**: 2999-3005.
- Brookes, I. M.** 1984. Effects of formaldehyde-treated and untreated casein supplements on performance of dairy cows offered ryegrass-clover pastures. *New Zealand Journal of Agricultural Research* **27**: 491-493.
- Broster, W. H. and Broster, V. J.** 1984. Long term effects of plane of nutrition on the performance of the dairy cow. *Journal of Dairy Science* **51**: 149-196.
- Broster, W. H. and Thomas, C.** 1981. The influence of level and pattern of concentrate input on milk output. In *Recent advances in animal nutrition* (ed. W. Haresign), pp. 49-69. Butterworths, London.
- Bryant, A. M. and Trigg, T. E.** 1982. The nutrition of the grazing dairy cow in early lactation. In *Dairy production from pasture* (ed. K. L. Macmillan and V. K. Taufa), pp. 185-207. Clark and Matheson Ltd, Hamilton.
- Clark, D. A.** 1993. Silage for milk production. *Proceedings of the Ruakura farmers' conference, vol. 45*, pp. 41-46
- Edwards, N. J. and Parker, W. J.** 1994. Increasing per cow milk solids production in a pasture-based dairy system by manipulating the diet: a review. *Proceedings of the New Zealand Society of Animal Production* **54**: 267-273.
- Garcia, S. C. and Holmes, C. W.** 1999. Effects of time of calving on the productivity of pasture-based dairying systems: A review. *New Zealand Journal of Agricultural Research* **42**: 347-362.
- Genstat Committee.** 1997. *Genstat for Windows command language manual, third edition, release 4.2b*. Rothamstead Experimental Station, Harpenden.
- Grainger, C.** 1990. Effect of stage of lactation and feeding level on milk yield response by stall-fed dairy cows to change in pasture intake. *Australian Journal of Experimental Agriculture* **30**: 495-501.
- Holmes, C. W.** 1987. Pastures for dairy cows. In *Livestock feeding on pasture* (ed. A. M. Nicol), *New Zealand Society of Animal Production occasional publication no. 10*, pp. 133-143.
- Kellaway, R. C. and Porta, S.** 1993. Factors affecting the response to supplementation. In *Feeding concentrates: supplements for dairy cows* (ed. R. Hopkins), pp. 117-147. Agmedia, Melbourne.
- Kolver, E. S., Muller, L. D., Varga, G. A. and Cassidy, T. J.** 1998. Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. *Journal of Dairy Science* **81**: 2017-2028.
- Lean, I. J., Parker, W. J. and Kellaway, R. C.** 1996. Improving the efficiency of pasture-based dairying. *Proceedings of the New Zealand Society of Animal Production* **56**: 270-275.
- Leaver, J. D., Campling, R. C. and Holmes, W.** 1968. The use of supplementary feeds for grazing dairy cows. *Dairy Science Abstracts* **30**: 355-361.
- Macdonald, K. A., Penno, J. W., Kolver, E. S., Carter, W. A. and Lancaster, J. A.** 1998. Balancing pasture and maize silage diets for dairy cows using urea, soybean meal, or fishmeal. *Proceedings of the New Zealand Society of Animal Production* **58**: 102-105.
- Minson, D. J.** 1981. The effect of feeding protected and unprotected casein on the milk production of cows grazing ryegrass. *Journal of Agricultural Science, Cambridge* **96**: 239-241.
- National Research Council.** 1989. *Nutrient requirements of dairy cattle, sixth revised edition*. National Academy of Science, Washington, DC.
- Oldham, J. D. and Emmans, G. C.** 1989. Prediction of responses to required nutrients in dairy cows. *Journal of Dairy Science* **72**: 3212-3229.
- Ørskov, E. R., Reid, G. W. and McDonald, I.** 1981. The effects of protein degradability and food intake on milk yield and composition in cows in early lactation. *British Journal of Nutrition* **45**: 547-555.
- Penno, J. W.** 2002. The response by grazing dairy cows to supplementary feeds. Ph.D. thesis, Massey University, New Zealand.

## Supplementation and pasture intake by dairy cows – 2

- Penno, J. W., Thomson, N. A. and Bryant, A. M.** 1995a. Summer milk – supplementary feeding. *Proceedings of the Ruakura farmers' conference, vol 47*, pp. 17–24
- Penno, J. W., Bryant, A. M., Carter, W. A. and Macdonald, K. A.** 1995b. Effects of fishmeal supplementation to high genetic merit cows grazing temperate spring pastures in early lactation. *Journal of Dairy Science* **78**: (suppl. 1) 295.
- Penno, J. W., Macdonald, K. A., Holmes, C. W., Davis, S. R., Wilson, G. F., Brookes, I. M. and Thom, E. R.** 2006. Responses to supplementation by dairy cows given low pasture allowances in different seasons. 1. Pasture intake and substitution. *Animal Science* **82**: 661-670.
- Peters, P. R., Chapin, L. T., Emery, R. S. and Tucker, H. A.** 1981. Milk yield, feed intake, prolactin, growth hormone, and glucocorticoid response of cows to supplemented light. *Journal of Dairy Science* **64**: 1671-1678.
- Robaina, A. C., Grainger, C., Moate, P., Taylor, J. and Stewart, J.** 1998. Responses to grain feeding by grazing dairy cows. *Australian Journal of Experimental Agriculture* **38**: 541-549.
- Rogers, G. L., Porter, R. H. D., Clarke, T. and Stewart, J. A.** 1980. Effect of protected casein supplements on pasture intake, milk yield and composition of cows in early lactation. *Australian Journal of Agricultural Research* **31**: 1147-1152.
- Rusdi, and Van Houtert, M. F. J.** 1997. Responses to protected amino acids or protected protein in dairy cows grazing ryegrass pastures in early or late lactation. *Proceedings of the New Zealand Society of Animal Production* **57**: 120-125.
- Stockdale, C. R.** 1999. The nutritive characteristics of herbage consumed by grazing dairy cows affect milk yield responses obtained from concentrate supplementation. *Australian Journal of Experimental Agriculture* **39**: 379-387.
- Stockdale, C. R., Callaghan, A. and Trigg, T. E.** 1987. Feeding high energy supplements to pasture-fed dairy cows. Effects of stage of lactation and level of supplement. *Australian Journal of Agricultural Research* **38**: 927-940.
- Stockdale, C. R. and Dellow, D. W.** 1995. The productivity of lactating cows grazing white clover and supplemented with maize silage. *Australian Journal of Agricultural Research* **46**: 1205-1217.
- Stockdale, C. R., Dellow, D. W., Grainger, C., Dalley, D. and Moate, P. J.** 1997. *Supplements for dairy production in Victoria*. Dairy Research and Development Corporation, Glen Iris, Melbourne.
- Stockdale, C. R. and Trigg, T. E.** 1989. Effect of feeding levels on the responses of lactating dairy cattle to high energy supplements. *Australian Journal of Experimental Agriculture* **29**: 605-611.
- Velleman, P. F.** 1997. *Data Desk, statistics guide, version 6*. Data Description, Ithaca, New York.

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