EFFECT OF ENERGY AND PROTEIN NUTRITION IN LATE GESTATION ON IMMUNOGLOBULIN G IN THE COLOSTRUM OF DAIRY COWS WITH VARYING BODY CONDITION SCORES

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SUMMARY
An experiment considered whether nutrition of dry cows or their body condition (thin or fat) affected the concentration of immunoglobulin (Ig) G in the colostrum produced by freshly calved dairy cows. Three diets were offered during the last 3-4 weeks before expected calving date: 1) a total mixed ration (TMR) containing maize silage, barley grain, canola meal and hay, fed at 1.5% of body weight (control diet), 2) the TMR plus 4 kg of pellets (energy diet), and 3) the TMR plus 3.5 kg of soyabean meal (protein diet). On average, the thin cows were 111 kg lighter (561 v. 672 kg; $P<0.05$), and were 1.8 units of body condition lower (3.9 v. 5.7 units; $P<0.05$), than the fat cows. Colostrum yields from cows with low body condition, averaged across the first 6 milkings after calving, were 10.4, 10.0 and 11.2 kg/cow.day for the control, energy and protein diets, respectively, while equivalent yields for the cows with high body condition were 10.5, 10.6 and 11.8 kg/cow.day ($P>0.05$). In all treatments, IgG concentrations were highest at the first milking and diminished exponentially. There was little effect of any treatment on colostral IgG concentration or yield. The main exception was that IgG at the first milking was significantly decreased by feeding the control diet to cows with low body condition. When regression analysis was undertaken, this effect of body condition on the IgG concentration in colostrum from the first milking ([IgG]) was not significant, but those of energy (total MEI; MJ/cow.day) and protein (total CPI; kg/cow.day) intake during the last month of gestation were, as described in the following weak significant regression equation:

$$[IgG] = 50.4 - 0.36\ (\pm 0.127)\ \text{total MEI} + 10.0\ (\pm 3.87)\ \text{total CPI}$$
$$100\ r^2 = 11.1\ (P = 0.022);\ \text{r.s.d.} = 11.1;\ \text{c.v.} = 37.4\%;\ n = 68$$

This regression indicates that each additional MJ of metabolisable energy consumed by cows was associated with a reduction of 0.4 g of IgG per kg of colostrum and that each additional kg of crude protein in the diet increased IgG concentrations by 10.0 g/kg.

Keywords: colostrum, immunoglobulin G, IgG, dairy cows, body condition, late gestation diets

INTRODUCTION
Bioactive substances, such as immunoglobulins, lactoferrin, growth factors and hormones, in the colostrum of dairy cows have attracted interest in commercial exploitation of colostrum for pharmaceutical and dietary uses in the last decade. Several milk factories have been collecting colostrum from farmers to harvest immunoglobulin (Ig) G that is marketed as nutrition and health products for elite athletes (Buckley and Scammell 2000). There have been reports of variation in colostral Ig, of which IgG constitutes the predominant proportion, in dairy cattle due to the effects of breed, age and season (e.g. Muller and Ellinger 1981; Shearer *et al*. 1992; Morin *et al*. 2001). However, there are few data on the effects of either nutrition or body condition score of dairy cows on Ig concentrations. The exception was a report by Shearer *et al*. (1992) suggesting that nutrition may influence Ig concentration because they found a significant increase of Ig in the colostrum from cows where body condition score increased from drying off to calving in contrast to where it remained stable or decreased. An experiment was conducted to test the hypotheses that cows in better body condition would produce colostrum with higher concentrations of IgG, and that IgG concentration would also be positively related to energy intake before calving.

MATERIALS AND METHODS
Seventy two multiparous cows were fed differentially from April 2002 to achieve body condition scores at calving of about 4 (3.5 to 4.5) or 6 (5.5 to 6.5) on an 8-point scale (Earle 1976). The cows, which had a Holstein-Friesian background, were selected on the basis of body weight, body condition, expected calving date (all in August) and milk production and composition in the first 3 months of the previous lactation. All cows had achieved their target condition scores by 1 month before their expected calving date. They were individually fed 1 of 3 diets during the last 3-4 weeks before

expected calving date. The 3 diets were 1) a total mixed ration (TMR) containing maize silage, barley grain, canola meal and hay, fed at 1.5% of body weight (control diet), 2) the TMR plus 4 kg (as fed) of pellets (energy diet), and 3) the TMR plus 3.5 kg (as fed) of soyabean meal (protein diet). Cows being fed the control diet were offered a diet with 10.6 MJ/kg DM of metabolisable energy, 11.8% crude protein and 43.6% neutral detergent fibre. Equivalent information for the cows supplemented with pellets was 10.9 MJ/kg DM metabolisable energy, 12.3% crude protein and 37.0% neutral detergent fibre, while those supplemented with soyabean meal consumed a diet with 11.4 MJ, 23.5% and 33.6%, respectively. Cows were fed their total diet as 1 meal, during the morning each day. The cows in each condition score group were allocated to treatments by stratified randomisation based on milk yield in the previous lactation, liveweight and age using Genstat V. The daily DM intake of each cow was measured through to calving.

Cows were weighed in July to determine feeding levels. Body condition was scored each week, independently by 2 experienced assessors. Daily intakes of TMR and concentrates were calculated for each cow, after samples of the feeds offered and refused were dried at 100^{o}C for 48 h to determine DM content. Additional feed samples were bulked on a weekly basis and freeze-dried. After grinding through a 1 mm screen, all samples were analysed for *in vitro* digestibility (Clarke *et al*. 1982 – forages; Tilley and Terry 1963 - concentrates), total nitrogen as determined by a Leco FP-428 (Leco Australia Pty Ltd), and neutral detergent fibre using the method described by Van Soest *et al.* (1991).

Calves were removed from cows before they could suckle and immediately fed 2 litres of good quality colostrum that had been obtained from other recently-calved cows. Cows were milked twice daily, at 0600 and 1530 hours, individual colostrum yields were recorded, and samples of colostrum taken in triplicate at the first 6 milkings after calving. During this period, all cows grazed annual pasture and received high-energy concentrates after each milking.

Samples of the colostrum of the freshly calved cows were collected immediately a cow had been milked, and stored in a refrigerator at 4^{o}C until they were transferred to the laboratory for analysis. Concentrations of IgG were determined by nephelometric immunoassay. In a 96 deep-well plate, colostrum was diluted to 1/100 with buffer solution (10 µl colostrum to 990 µl buffer - phosphate buffered saline + 550 mM EDTA + 50% Triton X-100) and defatted by centrifugation at 3000 rpm for 5 minutes. A 25 µl sub-sample was taken from the defatted solution and 275 µl of an anti-IgG antibody was added, allowing antibody-antigen complexes to form. Concentrations of anti IgG-IgG complexes were then determined at 25^{o}C in a nephelometer (Nephelostar-Galaxy, BMG Lab Technologies Pty Ltd).

Concentrations and yields of IgG were analysed as a 2 * 3 factorial analysis of variance, with no blocking, using individual animals as experimental units (Genstat V). Relationships between concentrations and yields of IgG and feed intake and body condition score were investigated by multiple regression analysis (Genstat V). Only 68 of the 72 cows were included in the analyses because 4 cows suffered from metabolic disease at calving.

RESULTS

On average, the thin cows were 111 kg lighter (561 v. 672 kg; $P<0.05$), and were 1.8 units of body condition lower (3.9 v. 5.7 units; $P<0.05$), than the fat cows. Mean intakes of cows in the various treatments are given in Table 1. On a live weight basis, the fat cows had lower intakes than the thin cows. The cows supplemented with soyabean meal consistently consumed more DM, metabolisable energy and crude protein than did the cows in the control treatment. The thin cows offered pellets had similar total DM intakes to those offered soyabean meal while the intakes of the fat cows fed pellets were the same as for those on the control diet. With the cows fed pellets, the fat cows ate significantly less than did the thin cows, but the reverse tended to be true for cows fed soyabean meal. The thin cows maintained body condition during the month before parturition relative to the fat cows (+0.01 v. –0.10 units change in condition score; $P<0.05$). The cows offered the protein concentrate improved their body condition during this time, while the cows in the other 2 dietary treatments lost condition (+0.07 v. –0.10 units change in condition score; $P<0.05$).

In all treatments, IgG concentrations were highest at the first milking and diminished exponentially while colostrum yields increased after the initial milking (Figure 1). There was little effect of any treatment on colostral yield or IgG concentration, with the exception being that the concentration of IgG at the first milking was significantly ($P<0.05$) decreased by feeding the control diet to thin cows (Figure 1). There was no effect ($P>0.05$) of any treatment on IgG yield at any time.

Table 1. Effect of body condition and diet fed during late gestation on the total intake of dry dairy cows.

	Body condition	Feeding treatment in late gestation			s.e.d.
		Control	Plus pellets	Plus soyabean meal	
kg DM/100 kg LW	Thin	1.42b	1.63cd	1.68d	0.076
	Fat	1.23a	1.17a	1.52bc	
kg DM/cow.day	Thin	8.02a	9.20b	9.35b	0.541
	Fat	8.00a	7.92a	10.25b	
MJ ME/cow.day	Thin	85a	100b	111bc	5.9
	Fat	85a	86a	122c	
kg CP/cow.day	Thin	0.95a	1.14b	2.16c	0.077
	Fat	0.94a	0.97a	2.44d	

Values for a variable followed by different letters are significantly different ($P<0.05$)
ME – metabolisable energy; CP – crude protein

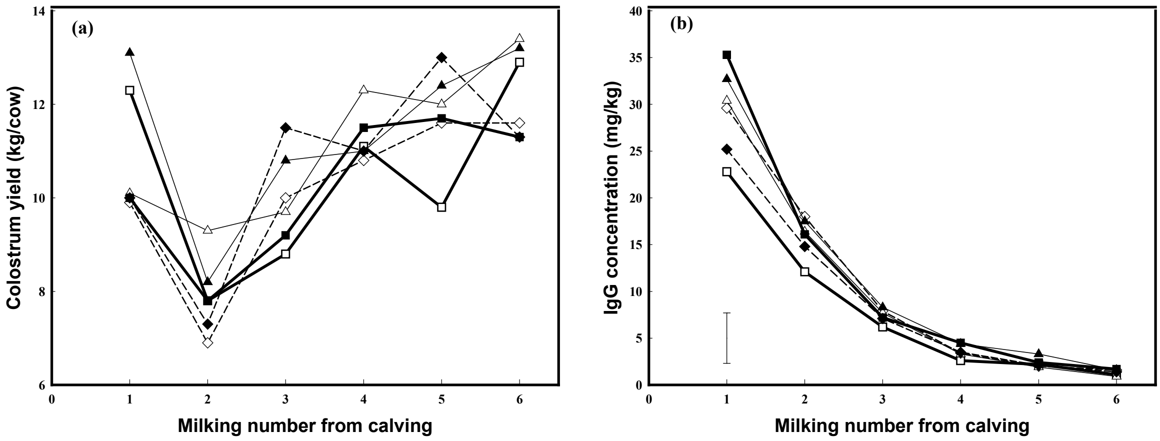

Figure 1. Yield of colostrum (a) and immunoglobulin G (IgG) concentrations in the colostrum (b) produced by cows at the first 6 milkings after calving (open symbols – cows in low body condition; closed symbols – cows in high body condition; square – control diet; diamond – the control diet with additional high-energy concentrates; triangle – the control diet with additional protein concentrates). The bar is the l.s.d. at P=0.05; otherwise there were no significant differences (P>0.05).

When regression analysis was undertaken, this effect of body condition on the IgG concentration in colostrum from the first milking ([IgG]) was not significant, but those of energy (total MEI; MJ/cow.day) and protein (total CPI; kg/cow.day) intake during the last month of gestation were, as described in the following weak regression equation:

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DISCUSSION

The results of this study support the findings of other research that IgG concentrations are highest in the first colostrum immediately after calving, and decline exponentially thereafter (Auldist *et al.* 1993; Marnila and Korhonen 2002). The concentrations of IgG in the colostrum of most cows was lower than traditionally has been considered to be acceptable for ensuring the health of newly born calves (Fleenor and Stott 1980). Good quality colostrum should have at least 50 g/kg Ig, of which the most significant proportion will be IgG, with previous research indicating that IgG will constitute more than 70% of the total Ig concentration in all dairy breeds (Muller and Ellinger 1981).

The research reported here has indicated that nutrition in the dry period can have a small influence on IgG concentrations, but only at the first milking, and then only if there are large variations in dietary energy or protein intake. Increases in energy intake reduced IgG concentration, while increases in protein intake increased concentrations. Shearer *et al*. (1992) used body condition at calving and change in body condition between drying off and calving as surrogates for nutritional status. They reported that cows that gained condition during the dry period were likely to have a significantly higher concentration of Ig in their colostrum compared with cows that lost condition. Logan (1977) reported low colostral volumes in beef cattle subjected to severe nutritional deficiencies during pregnancy, however, IgG levels were not measured in this experiment. In terms of protein nutrition, Blecha *et al*. (1981) restricted the protein fed to beef heifers during the last 100 days of gestation and found that there was no effect on Ig concentrations. However, their highest protein consumption was about equal to the lowest amount fed in the current experiment.

Analysis of variance indicated that fat cows had a higher mean IgG concentration than thin cows when they were offered the control diet. In this case, the fat cows had a lower intake than the thin ones on a live weight basis, and all the cows lost more body condition than did those in the other dietary treatments. Interestingly, in the research of Shearer *et al*. (1992), cows with a body condition score of 3.0 (4.8 on the 8-point scale of Earle (1976)) were less likely to have good colostral Ig compared with cows with a body condition score of 2.5 (4.0 on the 8-point scale). It is unclear why there should be this variation in results between experiments.

In summary, it would appear that the influence of nutrition and/or body condition on colostral IgG concentrations has not been clearly established in this or previous research, suggesting that more research could be warranted. Within the context of the diets and body conditions imposed in this experiment, nutrition did had a small effect on colostral IgG concentrations, and body condition seemed to be important, but only when the control diet was fed. However, from a practical point-of-view, it would seem that there is little scope for farmers to manipulate IgG concentrations by nutritional means.

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