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## Efficacy of pre-mating supplementation with mono propylene glycol on reproductive performance of cows in commercial dairy herds

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### ABSTRACT

Prolonged postpartum anoestrous intervals (PPAI) are the major source of infertility in New Zealand dairy cows. Postpartum supplementation with mono propylene glycol (MPG) was previously shown to reduce PPAI in heifers. The present experiment tested this treatment in four large commercial dairy herds. During the six-week period preceding the planned start of mating (PSM; week 0), cows were drenched either once (1xMPG) or twice (2xMPG) daily with 200 ml MPG, or served as untreated controls (CON). Analyses were confined to the group of cows that were anoestrous at Week - 6 or calved between Week - 6 and Week -4 relative to PSM (n = 662 to 669 per treatment). Change in body condition score between Week - 6 and Week - 1 ( $\Delta$ BCS), and proportion of cows that were anoestrous at Week -1 were influenced by herd, age and time of calving ( $P < 0.05$ ) but not by treatment ( $P > 0.1$ ). Treatment did not influence the proportion of anoestrous-treated cows inseminated after oestrus detection (n = 263), or the pregnancy rate among all cows. Pre-mating supplementation with MPG did not influence  $\Delta$ BCS, the percentage of cows that were anoestrous at Week -1, or rates of submission and conception. Change in BCS affected both milk yield and pregnancy rates, where cows that gained BCS produced less milk but showed higher 6-week pregnancy rates. This study highlighted that key factors influencing the percentage of anoestrus at PSM are cow age, time of calving and management of the herd.

**Keywords:** mono propylene glycol; anoestrus; dairy cow.

### INTRODUCTION

In the seasonal pasture-based dairy system of New Zealand, cows should have resumed oestrous activity before the planned start of mating (PSM) to maximise the chance of maintaining a 365-day calving interval. Extended postpartum anoestrous intervals (PPAI) reduce the proportion of the herd submitted and conceiving to artificial insemination (AI). Thus, extended PPAIs lead to a spread calving pattern, resulting in reduced days in milk and milk production, fewer high-genetic replacements and reduced pasture utilisation in early lactation. Pre- and postpartum levels of nutrition affect the duration of the PPAI (Chagas *et al.*, 2001). Dietary energy intake is generally insufficient to meet the high-energy demands of modern cows during the early lactation period, resulting in negative energy balance (NEB). Body condition declines as body reserves are mobilised to meet the energy deficit. Large postpartum NEB delays the interval to first ovulation (Butler & Smith, 1989). Prolonged PPAIs also arise when the NEB is extended in cows calving in poor body condition (Dunn & Kaltenbach, 1980; Wright *et al.*, 1992; Rhodes *et al.*, 2003).

Mono propylene glycol (MPG) is a propionate precursor (Sauer *et al.*, 1973). When administered orally, MPG bypasses the rumen, is absorbed in the duodenum and is metabolised to propionate in the liver (Emery *et al.*, 1964; Studer *et al.*, 1993). Propionate is in turn converted to glucose, which induces a spike in insulin release (Grummer *et al.*, 1994). Insulin has multiple effector sites (reviewed by Lucy, 2003), including

upregulation of luteinizing hormone (LH) receptors within ovarian follicles and stimulation of LH synthesis in the pituitary. Increased LH and follicle sensitivity to LH enhance establishment of the positive feed back loop between oestradiol and gonadotrophic hormones required for first ovulation postpartum (Lamming *et al.*, 1981).

Several studies have demonstrated a beneficial effect of MPG on reproductive function in cattle (Formigoni *et al.*, 1996; Miyoshi *et al.*, 2001; Chagas, 2003). In pasture-fed heifers which calved at low body condition, MPG supplementation significantly reduced the interval from calving to ovulation (Chagas, 2003). The specific objectives of the present study were to test the efficacy of pre-mating MPG supplementation on body condition and rates of anoestrus, submission and conception in commercially managed herds with a predominance of cows in low body condition.

### MATERIALS AND METHODS

The trial enrolled 2629 cows from four commercial herds within the Waikato region (Table 1). Consistent with the selection criteria, the cows were in low prepartum body condition score (40 % of the herd with  $BCS \leq 4$  at the planned start of calving; PSC). All cows were randomly allocated within each herd to one of three treatments balanced for age, breed, pre-calving BCS and expected calving date. During the six-week period preceding PSM, cows were drenched with 200 ml MPG once daily (1xMPG; n = 724), 200 ml twice daily (2xMPG; n = 714), or served as untreated controls

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(CON; n = 684). Cows were identified to respective treatments with coloured collars. Treatments were initiated immediately for those cows calving between Weeks -6 and -4 according to pre-assigned allocation as described above.

The BCS of each cow was visually assessed prepartum and at weeks -9, -6 and -1 relative to PSM. The same person performed these assessments using a scale of 1 to 10 (1 = emaciated and 10 = obese; Dexcel Farm Facts, 2001). Two fore milk samples were collected from a single quarter of each cow, 7 to 10 days apart, from Week -7 to Week -6. Progesterone (P4) concentrations were determined using an enzyme-linked immunosorbant assay kit (Ridgeway Sciences, Gloucestershire, UK) validated for use in cattle (Sauer *et al.*, 1986). Anoestrous cows were identified before treatment initiation as having P4 < 3.5 ng/ml in either milk sample. From Week -6 relative to PSM, farm staff observed the cows for signs of behavioural oestrus in the milking shed and in the paddocks twice a day, based on tail paint removal and overt oestrous activity. Research staff collected oestrous data weekly.

Veterinarians diagnosed anoestrous cows after rectal palpation 7-10 days before PSM. Anoestrous cows (excluding those cows that veterinarians identified as not suitable for treatment; n = 17) received an intravaginal device containing 1.38 g of P4 (CIDR; Pfizer Animal Health, Auckland, New Zealand; n = 687) for eight days, with an intramuscular injection of 2 mg oestradiol benzoate (ODB; Intervet Ltd, Auckland, New Zealand) at device insertion and 1 mg ODB 24 hours after device removal. Two of the farms inseminated cows at a set-time, 48 hours after CIDR removal, while the other two inseminated on oestrous detection. A single herd test was performed in each of the four farms in early October (Week -2 relative to PSM). Veterinarians diagnosed pregnancies in January (Week 10 after PSM) by ultrasonography.

Final analyses were confined to the group of cows which were anoestrus at Week -6 or calved between Week -6 and Week -4 relative to the PSM (Week 0; n = 2122). Data were analysed to test the fixed effect of

treatment on BCS, change in BCS between Week -6 to Week -1 ( $\Delta$ BCS), proportion of cows remaining anoestrus before PSM, submission and pregnancy rates by Week 3, pregnancy rate by Week 6, milk production and AI submission rate of CIDR-treated cows. Herd test data, BCS data and the interval from PSM to conception were analysed for linear effect of treatment. Proportional data (anoestrous cows at vet check, submission rate to CIDR treatment, submission and pregnancy rates by Week 3, and pregnancy rate by Week 6) were analysed using generalised linear models with a binomial error distribution. All data were analysed using GenStat, Release 6.1 (Lawes Educational Trust, Oxford, UK). Calving group (n = 5) was defined by time of calving prior to PSM and categorised as: i) > 12 weeks, ii) 10-12 weeks, iii) 8-10 weeks, iv) 6-8 weeks, and v) 4-6 weeks. Age groups were defined as 2, 3 and  $\geq 4$  years old. Interactions of treatment with age group and calving group were included in the statistical model. Data are presented as the main effect means from the fitted model.

## RESULTS

Treatment did not influence  $\Delta$ BCS, BCS at Week -1, proportion of cows which were anoestrus at Week -1, three-week submission rate or the three- and six-week pregnancy rates ( $P > 0.1$ ; Table 2), although three-week pregnancy rate was increased slightly by MPG treatment. Milk protein yield at Week -2 increased ( $P < 0.02$ ) with MPG supplementation in a linear fashion, whereas milk fat yield tended ( $P = 0.06$ ) to decline such that there was no effect of treatment on milksolids yield as measured at this single herd test (Table 2). More than 90 % of the anoestrous-treated cows (n = 263) were submitted to AI in the six days after device removal, with 34 % of these conceiving. Neither response was influenced by treatment.

**TABLE 1:** Effect of Herd on body condition score (BCS) at Week -1 relative to the planned start of mating (PSM), BCS change from Week -6 to Week -1, percentage of cows in anoestrus at Week -1, 3-week submission rate, 3-week and 6-week pregnancy rates, and milksolids yield per cow in herd test by Week -2, in the four farms enrolled in the trial. Standard errors of the mean (SEM) are added into brackets.

Farm	1	2	3	4
BCS at Week -1	3.97 (0.02)	3.92 (0.02)	3.80 (0.02)	3.74 (0.02)
$\Delta$ BCS from Week -6 to Week -1	0.26 (0.02)	0.33 (0.02)	0.27 (0.02)	0.38 (0.02)
Anoestrus at Week -1 (%)	14.5 (1.6)	43.3 (2.2)	33.6 (1.9)	36.1 (1.8)
3-week submission rate (%)	75.6 (1.9)	95.5 (1.0)	87.9 (1.3)	88.8 (1.2)
3-week pregnancy rate (%)	51.8 (2.2)	56.7 (2.3)	50.6 (2.2)	46.6 (2.0)
6-week pregnancy rate (%)	68.5 (2.0)	73.4 (2.0)	68.5 (2.0)	68.8 (1.9)
Milksolids yield (Kg/cow/day)	1.65 (0.01)	1.98 (0.01)	1.40 (0.02)	1.50 (0.01)

**TABLE 2:** Effect of treatment with mono propylene glycol (MPG) on body condition score (BCS) at Week -1 relative to the planned start of mating (PSM), BCS change from Week -6 to Week -1, percentage of cows in anoestrus at Week -1, 3-week submission rate, 3-week and 6-week pregnancy rates, and milk protein, milk fat and milksolids yield per cow in herd test by Week -2. Standard errors of the mean (SEM) are added into brackets.

Treatment group	MPG2	(SEM)	MPG1	(SEM)	Control	(SEM)
BCS at Week - 1	3.80	0.02	3.80	0.02	3.80	0.02
$\Delta$ BCS from Week-6 to Week -1	0.32	0.02	0.30	0.02	0.32	0.02
Anoestrus at Week -1 (%)	31.9	1.60	31.4	1.60	32.0	1.60
3-week submission rate (%)	85.6	1.25	86.6	1.23	87.6	1.22
3-week pregnancy rate (%)	52.5	1.90	51.5	1.90	49.1	1.90
6-week pregnancy rate (%)	68.9	1.60	74.6	1.50	67.7	1.70
Milk protein yield (Kg/cow/day)	0.74	0.01	0.73	0.01	0.71	0.01
Milk fat yield (Kg/cow/day)	0.90	0.01	0.91	0.01	0.92	0.01
Milksolids yield (Kg/cow/day)	1.63	0.01	1.63	0.01	1.63	0.01

**TABLE 3:** Effect of age and time of calving relative to the planned start of mating (PSM) on the proportion of cows in anoestrus one week before PSM, 3-week submission rate, and 3-week and 6-week submission rates. Standard errors of the mean (SEM) are included into brackets.

	Age			Time of calving (weeks before PSM)				
	2	3	4 +	12 +	12 - 10	10 - 8	8 - 6	6 - 4
Anoestrus at Week -1 (%)	53 (2)	37 (3)	25 (1)	25 (2)	24 (2)	30 (2)	41 (2)	61 (3)
3-week submission rate (%)	80 (2)	91 (2)	88 (1)	90 (2)	89 (1)	86 (1)	85 (2)	79 (2)
3-week pregnancy rate (%)	39 (2)	56 (3)	53 (1)	58 (2)	58 (2)	49 (2)	42 (2)	40 (3)
6-week pregnancy rate (%)	54 (2)	75 (2)	73 (1)	79 (2)	76 (2)	70 (2)	63 (2)	57 (3)

Cow age and time of calving were significant determinants of several reproductive endpoints (Table 3). Younger and later calving cows had the greatest ( $P<0.01$ ) anoestrous rates at Week - 1. Fewer two-year old cows (81%) than mature cows (94%) were inseminated in the six days after CIDR removal in the two herds that inseminated on detected oestrus ( $P<0.05$ ). The percentage of cows pregnant by Week 3 and Week 6 was also greater in older cows and in earlier calvers (Table 3; treatment, age group, calving group and herd are included in the statistical model).

Mean BCS at Week - 1 and  $\Delta$ BCS were affected by herd ( $P<0.01$ ). Percentage of cows that were anoestrus at Week -1 was different between herds ( $P<0.01$ ). Herd did not influence submission rate to CIDR (in the farms that inseminated on oestrous detection after device removal), 3-week or 6-week pregnancy rates. However, herd did influence ( $P=0.06$ ) milksolids production per cow in Week -2 (Table 1). Interactions between herd and treatment were not detected.

Anoestrous and pregnancy rates, and milksolids production were affected by  $\Delta$ BCS. Cows that lost 0.5 to 1.5 points of BCS during the 6-week period of treatment were grouped as 'loss' ( $n = 147$ ), cows with no  $\Delta$ BCS as 'no change' ( $n = 624$ ), and cows that gained BCS were grouped as 'gain' ( $n = 1088$ ). Rate of anoestrus was significantly affected by  $\Delta$ BCS ( $P < 0.032$ ) in farm #2, where, of the cows that lost, maintained or gained BCS, 68%, 48%, or 30% were anoestrus correspondingly. Pregnancy rate up to Week 3 was not significantly

affected by  $\Delta$ BCS. However, there was a significant effect of BCS loss on pregnancy rate by Week 6 ( $P<0.005$ ). For cows that lost, maintained or gained BCS throughout the treatment period, 64%, 70% or 77% were pregnant by Week 6 respectively.

Cows that maintained or gained BCS tended to produce less milk compared with cows that lost BCS ( $P=0.01$ ). This difference was significant in farm #4 ( $P<0.001$ ), where cows that lost, maintained or gained BCS from Week -6 to Week -1 produced 1.52 kg, 1.50kg or 1.47kg of milk solids in that order.

## DISCUSSION

This study tested the hypothesis that supplementing dairy cows with MPG during the six-week period preceding the PSM would improve their reproductive performance. The rationale was based on the outcomes of a previous study demonstrating positive effects of MPG on restoring LH pulse frequency in heifers calving in sub-optimal body condition (Chagas, 2003). In the present study, supplementation with MPG altered milk composition but had no measurable benefit on reducing PPAAI. It remains unclear why MPG had no positive effect on reproductive performance variables.

Key differences between the previous study (Chagas, 2003) and the present one were that this time MPG supplementation was: administered to the entire herd, comprising mixed age and breed animals; initiated on a calendar date (i.e. six weeks before PSM); and

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drenched for a limited duration (i.e. six weeks), at a slightly reduced dose.

The dose-dependent increase in milk protein yield with MPG supplementation is evidence that MPG did have some metabolic effects in the cows. The conversion of MPG into propionate in the liver results in an increase in blood glucose (Studer *et al.*, 1993; Grummer *et al.*, 1994). Increasing glucose availability to the mammary gland results in increased protein synthesis and protein yield, due to a lower need to break down amino acids for gluconeogenesis (Emery, 1978). This is a potential mechanism that could explain the effect of MPG on milk composition, and perhaps argues that the extra energy provided by MPG was partitioned into milk rather than to 'activities' that might lead to improved reproductive performance.

The widely recognised risk factors associated with reduced fertility in seasonal pasture-based dairy systems (Rhodes *et al.*, 2003; McDougall *et al.*, 1995; Rhodes *et al.*, 1998) were evident in this study. A greater proportion of first-calving heifers were anoestrus at Week -1, and in agreement with McDougall (2001), submission rate to AI following CIDR treatment was reduced for the heifers. Consequently, the three-week submission rate, and the three- and six-week pregnancy rates for this age group were reduced as compared with older cows. Cows calving within six weeks before PSM were twice more likely to be anoestrus at Week -1 than those that had calved greater than eight weeks before PSM. Subsequent differences in pregnancy rates amounted to approximately 20% in favour of the earlier calving animals.

Change in BCS during the six weeks preceding PSM influenced both milk production and reproduction. Cows that lost BCS during the six weeks preceding the PSM tended to produce more milk and had reduced pregnancy rates in the present study. This is in agreement with Butler and Smith (1989), who reported that cows which lost 0.5 to 1.0 BCS units between calving and mating achieved a greater pregnancy rate than those losing > 1 BCS units. A number of studies report a negative correlation between genetic selection for milk yield and reproductive performance (Butler and Smith, 1989; Pryce, 1999; Gillund *et al.*, 2001; Gong *et al.*, 2002; Buckley *et al.*, 2003). However, many studies report either no relationship or a positive phenotypic correlation between milk production and reproductive parameters in pasture-based systems (Fulkerson, 1984; Rhodes and Morgan, 1999). The present study is one of a few involving a pasture-based system to provide some evidence that cows with greatest milk yields lose condition during the period leading up to mating, and are less fertile.

## CONCLUSION

Six weeks drenching of 200 ml MPG either once or twice daily during the six weeks preceding the planned start of mating had no measurable benefit on reproductive performance of cows in four large

commercial herds. The widely known effects of herd, cow age and time of calving on reproductive and lactation performance were reinforced.

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