

Commercial-Scale Trials of Lactomin™ Products

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A report prepared for
Commercialisation Australia



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Effect of a crude magnesium lactate preparation on milk yield and composition: summary of findings.

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Introduction

Lactomin-Mg and Lactomin-DDG are derivatives of fuel ethanol by-products, produced by a patented procedure, that were originally developed to enhance the shelf-stability and convenience of handling of those by-products. It was immediately evident that it would be important to confirm that the process did not affect the nutritional value of the raw materials, so a feeding trial in a commercial dairy herd was initiated.

Trial one

The likely volume of product was such that it was prudent to conduct investigations in New Zealand, where the number of milking cows is large enough to provide a worthwhile market, and the importance of good magnesium nutrition is well understood by farmers. The trial was therefore designed to show that the use of these products could provide sufficient Mg at a limited dose rate, while overcoming issues of palatability and convenience of dosing found with conventional approaches. The trial achieved the hoped-for result, and independent review of the findings confirms that the product can properly be recommended for the purpose of ensuring efficient magnesium supplementation. The report about this facet of the trial outcome is attached as appendix 1.

The conduct of the trial included monitoring of individual-cow milk production, and surprisingly, it was found that consumption of the experimental diet led to a small, but significant and potentially-valuable increase in daily milk yield (Appendix 2). Therefore, it was concluded that further trial work should be conducted to see if this increase in productivity could be repeated.

Trial two

This trial was conducted in the same broad environment as trial one, except that it was carried out in the spring instead of the autumn, and the cows were fed pasture instead of conserved forage. Nearly three times as many cows were used in this trial as in trial one, and once again, a small, but significant, increase in milk yield was obtained. In contrast to the first trial, this trial also showed a substantial increase in milk solid content, giving two sources of increased return to the farmer (Appendix 3). This result was quite unexpected, and it would be desirable to confirm it, but further work in this area would be better conducted in more closely controlled circumstances, such as a dairy metabolic unit.

Appendix 1:

Lactomin-Mg is a safe, effective alternative to magnesium oxide in perinatal dairy nutrition

Introduction

According to DairyNZ, it is essential to supplement dietary Mg in dairy cows for at least the last three months of gestation and the first two months of lactation. This is due to the high demand for magnesium to support calcium metabolism during this part of the cow's life cycle, and the relatively low level of magnesium available from forage grown under temperate and cool-temperate conditions. Therefore, it is routine practice to supply extra magnesium by one or more of a number of means, including incorporation of magnesium oxide into the diet.

It is commonplace to attempt to improve the palatability of MgO by mixing it with enhancers, including molasses and Distiller's Condensed Solubles, and it is known to use chelates of magnesium with organic acids in monogastric nutrition to improve bioavailability of the mineral. MgO is known to be poorly bioavailable, in addition to its poor palatability, so the development of a crude magnesium lactate preparation appeared to offer a useful alternative to conventional means of magnesium supplementation. This crude preparation was previously shown to be palatable to a wide range of production and companion animals, and its composition includes no obvious component likely to interfere with magnesium bioavailability, since it is produced solely from a reaction between DCS and MgO. Therefore, it was decided to conduct an investigation of its efficacy and safety as a replacement for MgO in commercial dairy cow mineral supplementation, analogous to a phase two human clinical nutrition trial.

Experimental design

General

The trial was conducted in a winter milking herd in Central Southland, New Zealand. This region is in the cool-temperate climate zone, and pastures in the locality are known to be Mg-deficient for ruminants for most of the year. The herd owner routinely supplements his herd with MgO to provide 22g of supplementary magnesium daily, as recommended by DairyNZ and his nutrition consultant. The product used is of low bioavailability, however, so we proposed a Lactomin-Mg supplementation regime delivering 10g of elemental Mg daily as our experimental contrast.

Supplements were formulated based on feed wheat. For the experimental diet, 20% of Lactomin-Mg was mixed with 80% of ground wheat, which was then pelletised. The control diet consisted of 4% of MgO, mixed with 93% wheat and 3% soybean meal (used to ensure supplements were isonitrogenous) which was also pelletised.

Animals

A mating block of 180 multiparous cows was divided in two. Animals were allocated at random to the experimental or control diet. Feeding began one week before the first animals were due to calve, and continued for three months. For various reasons, late calving animals

were removed from the herd, leaving a total of 65 animals consuming the experimental diet, and 66 consuming the control diet.

Endpoints

Endpoints in the study were adverse events (whether related to the trial product or not), and body condition score and daily milk yield, used as indications of subclinical effects.

Animals were evaluated at the start of the trial, and fortnightly thereafter. All assessments were conducted under veterinary supervision, and the supervising veterinarian had absolute authority to halt the trial at any time.

Results

Adverse events

The frequency of occurrence of adverse events was similar in animals fed the two diets. One animal was lost from the control group due to pneumonia. Anecdotally, the occurrence of adverse events was relatively low in this herd compared to the few others locally being managed for winter milk.

Body condition score

Animals on both treatment arms recovered body condition after calving at the same rate, so that by the time of first oestrus, mean condition score was between 4.5 and 5 for both groups

Daily milk production

Inspection of daily milk records gave no indication of subclinical effects in individual cows that were not evident from other observations, such as body condition score, or incidence of mastitis or lameness.

Discussion and conclusions

It is the experience of dairy farmers in NZ that failure to provide adequate magnesium to dairy cows in late gestation and early lactation gives rise to significant problems of animal health and welfare. Therefore, it is ubiquitous practice to provide a daily magnesium supplement, since New Zealand pastures do not provide sufficient bioavailable magnesium to meet the needs of the high-performance dairy cow.

Recommendations for Mg supplementation using MgO (used for the sake of convenience) may lead to the provision of more than 35g Mg daily, since low bioavailability and administration losses may lead to as little as 6 g/day reaching the cow's circulation. Clearly, this is wasteful and costly, so more effective alternatives are continually sought.

In this study, conducted in a practical, commercial setting, the provision of 10g of supplemental Mg from a crude Mg-Lactate proved as effective and safe as providing 22g of elemental Mg from MgO in preventing adverse events and maintaining animal condition. Given that the trial was designed to avoid the need for ethical approval, and that it was managed to mimic standard commercial practice, it is not possible to say from the data obtained that this outcome would be achieved under different circumstances, such as in much warmer environments, where forages are derived primarily from C₄ plants. However, wherever magnesium supplementation is indicated based on research or empirical experience, Lactomin-Mg can be expected to provide a practical, effective alternative to other methods.

Appendix 2



Rich Technology Solutions Confidential Report No. 1

Effects of a crude preparation of magnesium lactate on dairy cow performance and welfare

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A report prepared for Lactomin Australia Pty Ltd

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Abstract

A more convenient, reliable, low-cost means of magnesium supplementation for dairy cows was sought. Crude magnesium lactate (10g Mg/day) derived from distillation coproducts was fed as part of a supplement to 65 multiparous cows in a large dairy herd, with a control group fed a supplement including magnesium oxide (22g Mg/day). Subgroups ($n_{\max} = 10$) within these larger groups were sampled fortnightly for milk, faeces and urine, approximately 2 hours after morning milking.

Control animal urine contained significantly higher Mg and Ca concentrations than that of experimental animals, suggesting that animals consuming the experimental diet were retaining absorbed cations better. No significant differences were observed in faecal cation concentrations. Given the reduced intake of Mg allowed by the experimental diet, this implies reduced faecal output on this diet, and increased efficiency of dietary Ca utilisation.

Animals consuming the experimental diet yielded significantly more milk (26.95L/day vs 24.89L/day, or 8%) than those consuming the control diet, and there were no significant differences in milk composition between diets with respect to fat, protein or lactose content. Milk urea content (a marker of efficiency of dietary protein use) was significantly lower in milk from cows consuming the experimental diet. Together, these observations strongly suggest a role for the crude magnesium lactate in improving efficiency of utilisation of the entire diet.

Animals consuming the experimental diet suffered fewer incidences of parturient paresis, relative to those consuming the control diet, and those that occurred were mild, not requiring intervention. The incidence of mastitis was also lower among the animals on the experimental diet, although a subset of these animals did have higher somatic cell counts, normally indicative of increased likelihood of udder infection.

This trial demonstrates that it is possible to provide essential supplementary Mg to dairy cows in the period just before to just after calving in a manner that is convenient, cost effective and reliable. The vehicle for this also delivers significant extra advantage through additional yield of milk and milk solids, enhanced efficiency of Ca utilisation and of protein utilisation.

Introduction

It is estimated that, worldwide, 75% of intensively-managed dairy cattle require magnesium supplementation for at least three months prior to calving and two months thereafter (Roche, J, *pers. comm.*). If not appropriately managed, magnesium deficiency can have a wide range of effects, from minor impacts on production, through "milk fever", to paralysis, collapse and death (Sansom, Manston, & Vagg, 1983). These impacts arise primarily as a result of the negative effect of magnesium deficiency on calcium metabolism. This syndrome thus causes major economic and animal welfare concerns if not appropriately managed. It is usual, therefore, to supplement animal diets with various forms of magnesium in an effort to mitigate these consequences.

The least reliable, but least costly, and therefore most widely-used magnesium supplement is magnesium oxide provided as a dry powder spread on forage or on pasture. The effectiveness

of this approach is limited by the palatability of magnesium oxide, the ability of the animal to recover the desired supplement of product due to the feeding mechanism, and the relatively low bioavailability of magnesium from magnesium oxide in the rumen, hind stomachs and small intestine (Davenport, Boling, & Gay, 1990).

Of similar cost is the provision of magnesium salts such as magnesium chloride and magnesium sulphate in drinking water. However, these salts are distinctly unpalatable, causing water uptake, and consequently milk production, to be limited. Furthermore, at the time when magnesium is most needed, over winter, cows are normally able to obtain the majority of their water requirement from their forage.

Alternative, more reliable interventions may cost at least 3 to 4 times as much as supplementation of forage with magnesium oxide or drinking water with magnesium salts. Even so, the cost of lost production, and the impact on animal health and welfare are such that farmers often choose to meet those costs. There is, naturally, considerable economic incentive to find a means of magnesium supplementation that is both reliable and low cost.

Amongst the more reliable interventions, provision of magnesium in the form of chelates with organic acids has been found to be workable, but sufficiently expensive that this option is not widely used. It has been discovered (Coles & Pearce, 2011) that it is possible to prepare crude salts of lactic acid with dibasic cations by reacting inorganic compounds of these cations with the organic acids found in distiller's byproducts. By manipulating the level of lactic (and other) acids in these byproducts, materials with useful levels of mineral chelates can be manufactured. It has been discovered that these materials can be converted to convenient dry powders, and, because those powders contain worthwhile levels of fermentation byproducts such as glycerol, they are very palatable to all production animals. However, it is necessary to determine whether these materials would prove convenient in commercial practice, and provide, in particular, adequate protection against magnesium deficiency at a commercially-realistic cost.

A commercial-scale feeding trial, conducted under continuous veterinary supervision, was therefore carried out in a large commercial dairy herd, which was being managed for winter milking, and in which a high proportion of the forage was being provided in an untethered-barn situation. The majority of the animals were supplied with a conventional magnesium supplement, while a selected proportion was provided with magnesium by supplementation with the crude magnesium lactate preparation. The goal of the supplementation was to elicit similar performance from the control and experimental animals, with close attention paid to any adverse events, whether related to magnesium nutrition or not.

Materials and methods

Trial herd

The trial herd was a winter milking herd of New Zealand Friesians owned by Southern Centre Dairies Ltd. The dairy platform is located at East Limehills, just north of Winton in Southland, New Zealand. Approximately 800 cows, calving from February to April, were milked twice daily. Prior to the commencement of the trial, dry cows were conventionally grazed on pasture with supplementation when necessary, and magnesium supplementation was supplied to these dry cows in the form of broadcast magnesium oxide.

Within this herd, a mating block of 180 third and fourth parity cows, due to start calving on March 18th 2012, was selected to provide animals for detailed investigation. On March 14th, these cows were separated into two groups as follows. All the cows were brought into the yard of the milking platform, and allowed to make their normal social arrangements. Cows were then run on to the rotary platform in the order that suited them. This order was recorded, and cows allocated to either the control group or experimental group alternately (the "condition" groups). After the 30th cow had come off the platform each fifth cow was alternately allocated to further smaller experimental groups (sampler cows). In this way, two groups of 80 condition cows and two groups of 10 sampler cows were created. The process was intended to provide a random allocation of cows to groups by splitting an entire population in half, and abstracting a further subpopulation at random.

As the animals came off the rotary platform, their ear tag numbers were recorded in order. Sampler cows were provided with coloured ear tags (blue for experimental diet, red for control diet), and drafted out of the main block. The condition cows were then individually judged for body condition by a veterinarian, and returned to grazing. The sampler cows were allowed a respite on pasture, and then returned to the platform for sample collection prior to going back to the main mob.

Allocation of animals to groups was recorded in the herd management database, and these allocations were used thereafter to determine which of the two dietary supplements each animal was fed, and to aid drafting at each sampling visit.

For practical reasons, not all of the animals in the mating block assigned to treatments entered the milking herd. The final numbers in each treatment block were: 10 experimental diet sampler cows; 10 control diet sampler cows; 55 experimental diet condition cows and 55 control diet condition cows.

Diets

Lactomin-Mg

Unless otherwise stated, all materials included in animal diets were of feed grade.

LactominTM-Mg was prepared according to Coles and Pearce (op. cit.), modified as follows.

Distillers condensed solubles (DCS) syrup was collected from normal production from the facilities of Shoalhaven Starches Pty Ltd at Nowra, New South Wales, Australia, and transferred to the premises of Halcyon Products Pty Ltd in Melbourne, Victoria. Under normal conditions, DCS from this source contains approximately 25% lactic acid (dmb), but this shipment was somewhat depleted in this material, so was supplemented with technical grade lactic acid (All Raw Materials Pty Ltd, Young, NSW).

DCS was heated to 55°C, and reacted with commercial feed grade magnesium oxide (Causmag International Pty Ltd, Melbourne, Victoria) with continuous stirring and monitoring of pH. When pH reached 7, addition of magnesium oxide ceased, and the product was prepared for drying. Small-scale spray drying studies had revealed the need to incorporate a quantity of maltodextrin to improve flow properties, so 10% additional maltodextrin was mixed with the slaked DCS.

The resulting mixture was spraydried using conventional techniques, and packaged for storage and transport in 20 kg quantities in moisture-proof plastic bags in cartons. Its composition is given in table 1. (Dairy One, Inc, Ithaca, New York, USA)

Table 1: Proximate composition of Lactomin™-Mg

Component	Content as fed
Moisture (%)	10.5
Drymatter (%)	89.5
Crude protein (%)	16.7
Available protein (%)	13.6
ADICP (%)	3.0
Adjusted crude protein (%)	14.5
Acid detergent fibre (%)	1.8
Neutral detergent fibre (%)	4.2
Nonfibre carbohydrate (%)	58.2
Starch (%)	0.5
Water-soluble carbohydrates (%)	20.1
Crude fat (%)	1.9
Ash (%)	9.44
Total digestible nutrients (%)	68.0
NE _L (Mcal/kg)	1.57
NE _M (Mcal/kg)	1.62
NE _G (Mcal/kg)	1.06
Calcium (%)	0.11
Phosphorus (%)	0.55
Magnesium (%)	2.82
Potassium (%)	0.93
Sodium (%)	0.782
Iron (ppm)	115.0
Zinc (ppm)	26.0
Copper (ppm)	6.0
Manganese (ppm)	57.0
Molybdenum (ppm)	1.3
Sulphur (%)	0.21
Chloride ion (%)	0.59
pH	8.6
Dietary cation-anion difference (mEq/100g)	31.0

Digestible energy was estimated by NIR to be 14.4 MJ/kg.

Feed supplement manufacture

Experimental diet

For the purposes of this experiment, it was estimated that magnesium availability from Lactomin™-Mg would be similar to availability of magnesium from magnesium chloride or sulphate, i.e. approximately 65%. Provision of 6.5 g of magnesium absorbed therefore required daily intake of sufficient Lactomin™-Mg to provide a total of 10 g of magnesium, and this quantity was available from 400 g of the spray dried product.

For convenience, the 400 g of Lactomin™-Mg was incorporated in 2 kg of diet, the balance consisting of 1600 g of ground locally-grown new season's winter wheat (12.2% protein dmb). This mixture was supplemented by the manufacturer (Seales Winslow Ltd, Tinwald, New Zealand) with 0.1% of the manufacturer's proprietary palatability enhancer. 19.9 t of this diet was manufactured and fed.

Control diet

The control diet consisted of 40 g of dairy nutritional grade magnesium oxide (Causmag International Pty Ltd, Melbourne, Victoria) in 2 kg of supplement. The supplement consisted of the same wheat as used in the experimental diet, augmented by sufficient soy bean meal (46% protein dmb) (Viterra Ltd, Auckland, New Zealand) to compensate for the difference in the protein content between the wheat and the Lactomin-Mg displaced from the experimental diet formulation. This diet was also supplemented with the same proprietary palatability enhancer. 119.0 t of this diet was manufactured and fed.

Composition

Composition of the two supplements was determined by an independent laboratory (Nutrition Laboratory, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand). Results are given in table 2.

Table 2: Supplement composition

Analyte	Experimental	Control
Drymatter content (%)	89.4	89.8
Ash (%)	3.1	3.9
Protein (%)	10.9	11.9
Fat (%)	1.8	1.6
Crude fibre (%)	2.2	2.4
NDF (%)	6.5	8.5
ADF (%)	1.2	1.6
Calcium (g/100g)	0.070	0.100
Magnesium (g/100g)	0.77	1.35
ME (MJ/kg)	>13	>13

Feeding

For practical reasons, it proved necessary to provide the required magnesium to the entire herd through the feeding facilities on the rotary platform. It was for this reason that considerably more of the control diet was fed, as it was supplied to all members of the milking herd not being fed the experimental diet. Feeding of supplements commenced five days after animals were allocated to experimental groups.

Pre-parturition.

Animals yet to calve were maintained in a herd separate from other animals on the farm. Each day this mob of cows was brought into the milking shed, and fed their daily ration of the diets to which they were allocated.

As each cow calved, she was admitted to a post-calving group as part of the main herd, and was supplemented according to the regimen described below.

Cows in milk.

From calving, cows were included in the main herd. This herd was milked twice a day, and each animal received supplementation according to the group in which it was included. This took the form of 1 kg of the appropriate diet at each milking, with the addition of 100 mL of molasses to further enhance acceptability. Animals suffering adverse events (e.g. mastitis or lameness) were separated from the main herd, coming into the parlour for milking after the main herd was finished. From the end of calving, the herd began to be fed undercover untethered, so these animals suffering adverse effects were fed the same basal diet in a different pen within the main barn.

In the last three weeks of the trial, all animals in the herd yielding above-average amounts of milk were supplemented with additional soy bean meal, initially at the rate of 500 g per day, and where this induced a yield response, with a further 500 g. Animals receiving this supplement were identified each day.

Data collection

Daily milk yield records were collected for all condition and sampler cows. At fortnightly intervals, condition cows were individually assessed for body condition score on a scale of 1 – 10 by an experienced veterinarian (Anon., 2012).

Sampler cows were drafted from the main herd after morning milking, and placed on pasture with free access to water for approximately 90 min. These animals were then brought back to the milking parlour, placed on the platform, and voluntary urine samples collected. Animals not immediately providing a urine sample were induced to do so where possible by moderately vigorous subvulval massage, under the supervision of a veterinarian. Milk samples (~100 mL) were obtained manually from the two rear quarters of the udder. Faeces samples were then collected, where necessary intra-rectally, by a veterinary technician.

Collected samples were divided into two subsamples, one for immediate analysis, and one for archiving. Urine and faeces samples were analysed for calcium and magnesium content by an external laboratory, and urine pH was determined. Milk samples were analysed for total solids, fat, protein lactose and urea, and somatic cell count by an external laboratory. Data were obtained by use of a Foss CombiFoss+ (Foss, Hillerød, Denmark). Note that since milk samples were collected approximately 120 minutes after complete udder stripping, it was expected that milk would be somewhat concentrated, relative a full 24hr collection, but that relative values would still be informative.

Adverse events

All adverse events occurring during the period of the trial were recorded. Adverse events considered to be related to the objects of the trial include incidence of milk fever, "Downer" cows, incidence of mastitis, lameness, loss of condition and delayed conception. Other adverse events were noted.

Data analysis

Data were organised for statistical analysis using Microsoft Excel 2007. Organised data were transferred to Minitab 16 (Minitab Inc www.minitab.com) worksheets for statistical investigation. Due to the investigation environment, the experimental design was necessarily unbalanced, so analysis of variance was conducted using the GLM (general linear model) procedures in Minitab.

Results and discussion.

The target of the experiment was to provide control and experimental diets which would lead to similar nutritional and performance outcomes.

Supplement composition

Apart from magnesium content, the differences in composition between the experimental and control supplements were not significant, given that in both cases, the supplement provided less than 10% of total dry matter intake daily.

Body condition

The original group of cows prior to allocation to experimental subgroups had a range of condition scores, from several at 3.5 (significantly underweight: all cows at this score had been recently purchased) to 5 (ideal weight). Animals with an initial condition score below 4.0 were not included in the trial, and the high condition animals were observed prior to calving. As expected, calving induced a loss of condition of around about 0.5 condition score units (Sheppard, *pers. comm.*).

Subsequent estimations of mean body condition score showed no significant differences between groups. As expected, all animals rapidly recovered condition after calving, so that after one month of lactation, almost all animals comfortably fell into a condition score range of 4.5 to 5.0. Thus, it appears that the experimental diet caused no differences in body condition score, or the rate of recovery after calving.

Urine pH

In this experiment, urine pH was used as a surrogate estimate of blood pH (Vagnoni & Oetzel, 1998). Reduced blood pH is associated with reduced risk of milk fever, and management techniques have been developed to shift the balance of strong cations and anions in the diet towards the latter, as a means of achieving a desirable reduction in blood pH.

The presence of a worthwhile quantity of lactate ions in the experimental supplement was predicted to have an effect on blood pH. However, there was only a very minor, statistically insignificant shift overall between treatments. There was, however, an indication that there was a beneficial shift in urine pH prior to calving, which is thought to prime calcium remobilisation, reducing the risk of postparturition hypocalcaemia. (Vagnoni & Oetzel, 1998) Further investigation, particularly including higher levels of lactate supplementation, is required.

Mineral excretion

Urinary magnesium

Contrary to expectations, urinary magnesium content was higher in cows fed the control diet (8.50 mmol/L vs 7.13 mmol/L; $p=0.003$). Magnesium excretion was a minimum at day 14 of the trial (2.705 mmol/L) and reached a peak of 20.10 mmol/L at day 70 (Figure 1).

Urinary mineral concentration with time

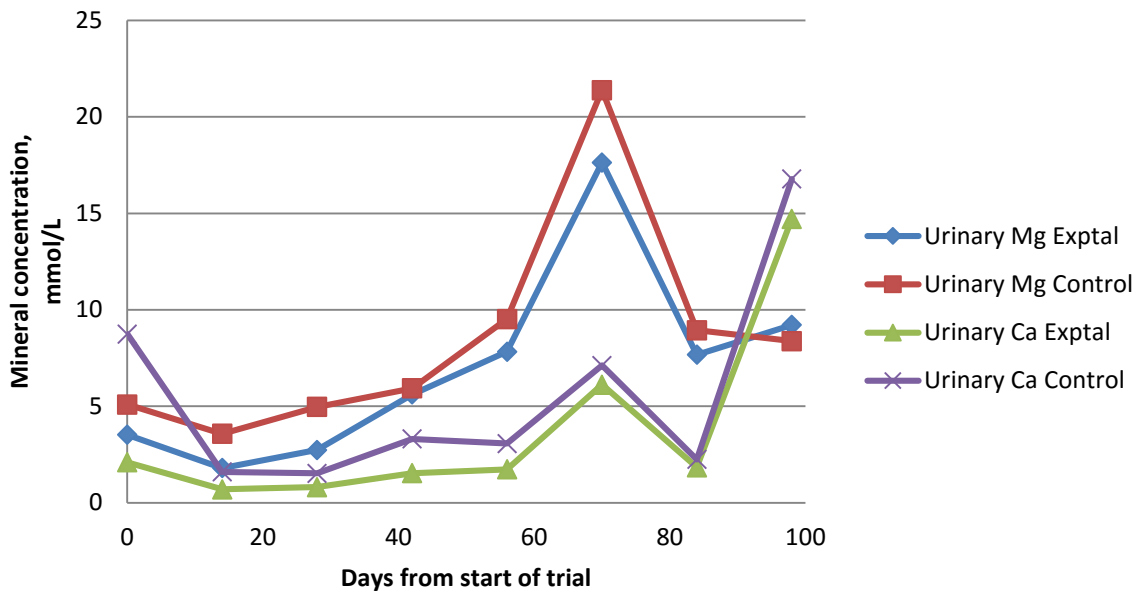


Figure 1: Changes in urinary magnesium and calcium concentration with time from trial commencement

It is not clear whether this result is an artifact, as it was at this sampling date that most sampler cows were at their first oestrus after calving. As a result, it may be that the urine samples were significantly contaminated with vaginal secretions at this sampling date. However, even if the data for day 70 are excluded from analysis, the differences in urinary magnesium content between sampling dates are highly significant. There were no interactions observed between diet and sampling time.

Urinary Calcium

As with urinary magnesium, significantly more calcium was excreted by the animals consuming the control diet relative to the experimental diet (5.60 mmol/L vs 3.57 mmol/L; $p=0.005$), and once again, there were significant changes in the amount of calcium excreted with time (figure 1). Differences between treatments were greatest at the beginning of the trial: once milk production was established, the differences between treatments progressively diminished.

Faecal Magnesium

There was no statistically significant difference in the faecal concentrations of magnesium between the two groups, although the animals consuming the control diet had substantially higher faecal magnesium content (7.18, vs 6.84 g/kg drymatter; $p=0.16$). There was a significant spike in concentration at day 98 (figure 2), but when data from this sampling time were excluded from analysis, there were no other significant differences with time. Nevertheless, figure 2 does indicate that animals on the control diet were excreting considerably more magnesium prior to calving and during establishment of milk flow.

Faecal mineral concentration with time

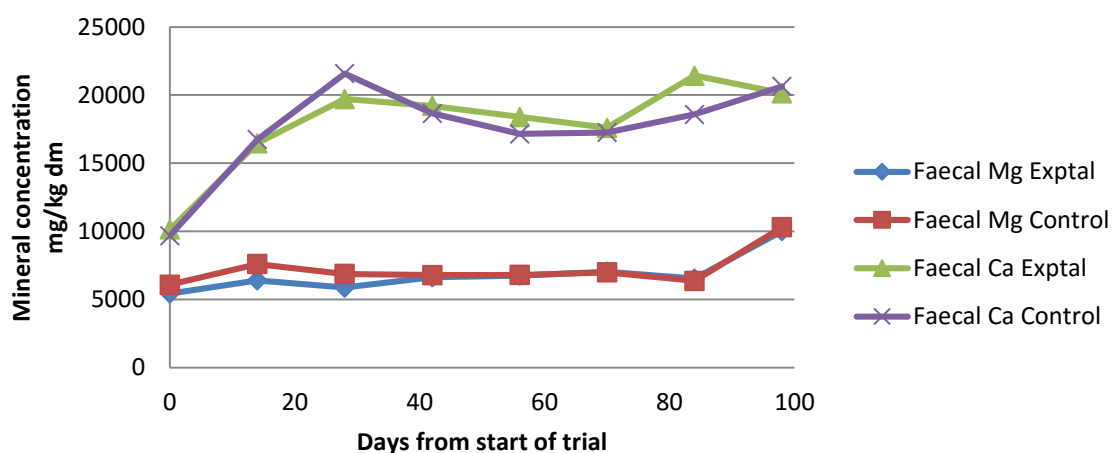


Figure 2: Faecal excretion of magnesium and calcium with time

Faecal Calcium

As with faecal magnesium, there was no difference between treatments in terms of overall faecal calcium loss. There was a rapid increase in calcium excretion once cows calved (figure 2), consistent with the increase in calcium supplementation that is conventional practice in dairy nutrition.

Once gross supplement magnesium content is taken into account, the significant but limited difference between control and experimental diet urinary magnesium is seen in a different light. Given that the control supplement was nearly twice as concentrated in magnesium (13.5g/kg vs 7.7g/kg (table 2), or 1.8 fold) as the experimental supplement, it is significant that the ratio of urinary magnesium concentrations was considerably lower, at 1.2 fold. This is suggestive, but not conclusive of enhanced magnesium bioavailability in the experimental diet.

Interestingly, given that both treatments received the same form and amount of calcium supplementation, the enhanced calcium excretion on the control diet compared to the experimental diet is strongly suggestive of a role for magnesium lactate, either in aiding calcium retention or stimulating alternative demand for calcium.

In light of the relationship between treatments with respect to urinary magnesium, which suggests that there should still be a surplus of magnesium in the control treatment for faecal excretion, one would expect a significantly higher faecal magnesium concentration in that treatment. That this is not the case is suggestive of mineral dilution due to significantly higher faecal output from cows on the control treatment. Regrettably, it was not possible to include a marker in diets fed, but other evidence (discussed later) points to a role for magnesium lactate in enhancing fermentative efficiency, with consequent improved diet utilisation.

Given the indicated disproportionation of magnesium excretion between treatments, the observed relationship between treatment faecal calcium concentrations suggests enhanced

recovery from the diet, and either retention in body depots or secretion in milk. Other data from this trial suggest the latter.

Production data

Milk production data in the form of 24 hour milk volume for each cow were collected daily. Conducting ANOVA using a General Linear Model to permit analysis of unbalanced data indicated a small but significant overall advantage in milk yield from cows on the experimental diet (25.01L/day vs 24.21L/day; $p=0.001$). However, there was a very significant interaction between diet and time (Figure 3).

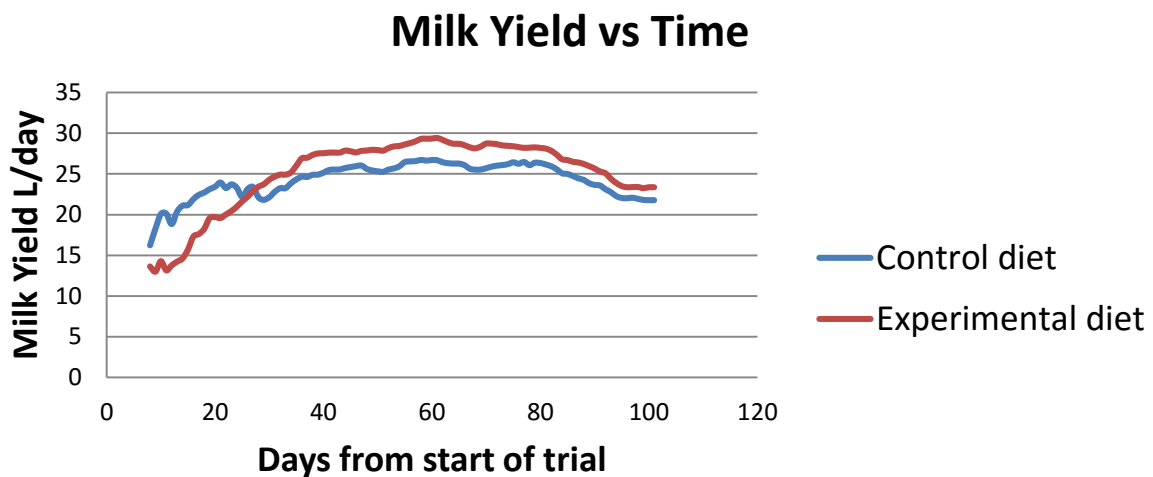


Figure 3: Relationship between milk yield and time

It seems possible that this interaction is due to a requirement for adaptation to one or more components of the experimental diet, but that once this adaptation is achieved, there is a sustained, significant advantage in milk yield. If the first four weeks of the trial (during which cows calved) are ignored, the interaction between diet and time disappears completely, as expected, and the advantage conveyed by the experimental diets increases (26.95L/day vs 24.89L/day, or 8%).

In this trial, the sampler cows were managed as a separate group. Despite the small number of animals included in this group, a similar difference between treatments remained highly significant (23.6L/day vs 21.6 L/day ($p=0.000$), or 9%)

Milk composition.

Because it was necessary to restrict milk sample collection to a time shortly after a complete milking, composition data were generally substantially elevated compared to those normally found from bulk milk collections. Importantly, however, no differences were found between treatments for a given day for milk fat content, protein content or total solids, indicating that differences in milk yield are likely to be accompanied by difference in milk solids production. As expected, the concentration of each of the milk components discussed declined with time. The experimental diet led to a significantly lower milk urea content (35.99 mg/dL vs 40.84 mg/dL; $p=0.029$) than was observed for the control diet, and there was

a statistically significant ($p=0.000$) decline with time, from 40.94 mg/dL at day 28 to 25.66 mg/dL at day 98.

Milk urea content is strongly correlated with plasma urea content, and elevated plasma urea content is associated with degradation of protein for energy, thus wasting this relatively costly diet component (Baker, Ferguson, & Chalupa, 1995; Frank & Swensson, 2002). Baker *et. al (op.cit)* suggest that the most useful metric is the ratio of milk urea to milk protein, with higher values indicating reduced efficiency of dietary protein utilisation. If ratios of milk urea to milk protein are compared according to diet, the experimental feed provides a significant advantage in this regard (10.77 vs 12.32; $p=0.027$). The mechanism by which this is achieved is unclear.

The somatic cell counts were significantly higher for the experimental diet-fed cows than for the control diet-fed animals. Examination of the data indicated strong heterogeneity of variance, so data were log-transformed. Upon analysis, transformed data still showed a significant advantage to the control treatment (5.239 vs 5.669; $p=0.000$). When back-transformed, the trial grand mean for the control diet was 173,380 cells/ml, whereas the figure for the experimental treatment was 466,659 cells/ml – above the limit of 400,000 cells/ml set for bulk milk samples in New Zealand. Further inspection of the data indicated that three outlier observations strongly influenced the analysis, and when those values were excluded, the experimental treatment mean cell count (after back-transformation) fell to 377,572 cell/ml.

Milk composition data suggest that the milk collected for analysis was at least twice as concentrated as would be the case from a 24 hour collection, indicating that neither treatment would cause concern with respect to somatic cell count from a production perspective. This was confirmed by independent monitoring results received for the herd from the dairy company.

Cows had ad lib access to forage, in addition to the feed supplement provided in the bail, so the increase in milk production may well simply have been due to improved appetite, and thus, increased feed intake. However, the mineral concentration data discussed above strongly suggest that faecal flow is significantly reduced from animals fed the experimental diet, indicating that those cows are utilising their diets more efficiently. While there is evidence that lactate residues enhance fermentation efficiency in *in vitro* studies (Newbold *et al.*, 2005), there are a number of other possibilities, such as provision of a significant quantity of yeast non-starch polysaccharide, or of significant quantities of high quality protein. Further experimental work is required to understand these observations. The results relating to milk protein:urea ratio are suggestive of enhanced rumen efficiency, in line with the suggested improvement in efficiency of fermentation mentioned above.

Adverse events

Hypocalcaemia

Clinical symptoms of hypocalcaemia include generalised paresis and collapse, but generally respond well to intervention (Barrington, 2011). At the request of the managing veterinarian, the herd manager paid particular attention to this syndrome, noting the degree of effect, not just presence or absence. Four control-diet-fed animals displayed normal symptoms of milk fever, whereas only one animal fed the experimental diet displayed full-blown symptoms, due

to a dramatically-complicated calving. Two other experimentally-fed animals displayed mild or very mild symptoms, not requiring intervention.

Mastitis

Thirteen control-fed cows contracted mastitis, including 5 animals suffering multiple infections. Twelve animals on the experimental diet also contracted this disease, but only 2 cows had repeat infections. Four infections happened to occur among the sampler cows, providing a possible explanation for the elevated somatic cell counts observed among those animals.

Other disorders

One experimentally-fed cow died of pneumonia, an event not considered to be due to diet. Otherwise, three animals on the control diet, compared to one on the experimental diet, suffered bouts of diarrhoea.

Conclusions

Conventional low-cost approaches to dairy cow magnesium supplementation late in pregnancy and in early lactation can be replaced by inclusion of crude magnesium lactate preparations in dietary supplements. These crude preparations appear to deliver adequate magnesium uptake if fed to provide a gross daily magnesium intake of 13 g, as animals consuming the experimental diet in the trial reported appeared not to exhibit any significant symptoms of parturient paresis. Consumption of the experimental diet appeared to improve feed utilisation, leading to increased milk yield of approximately 8%, with no apparent reduction in milk quality. Consumption of the experimental diet appeared to improve calcium utilisation, reduce urine pH at a critical point around calving, and to improve protein utilisation. The increase in milk somatic cell count observed in the sampler cows appears to be an artifact of an imbalance in mastitis infections not observed in the broader experimental herd.

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Appendix 3:



Rich Technology Solutions Confidential Report No. 2

Effect of a crude magnesium lactate preparation on milk yield and composition in a New Zealand commercial dairy herd

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April 2013

A report prepared for Lactomin Australia Pty Ltd

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February 2013

Introduction

Magnesium deficiency is a significant issue for profitable dairy production in New Zealand and elsewhere where intensive practices are used (<http://www.dairynz.co.nz/file/fileid/36336>). It should be noted that the recommendations are made not because New Zealand pastures are deficient in magnesium: rather, they have high levels of nitrogen and potassium (vital for milk production) which interfere with magnesium absorption (John Roche, DairyNZ, *pers. comm*). Consequently, it is common practice to provide magnesium supplements to late-gestation and lactating dairy cows, particularly when the risks of milk fever and grass tetany are considered to be elevated. Present supplementation practices are largely based around whole-herd provision of magnesium through forage, with the majority of supplemental magnesium being supplied as magnesium oxide, either dusted on pasture or supplied with supplemental forage such as hay or silage. The provision of magnesium by this means is imprecise, leading to significant wastage, and the potential for over-supplementation in some animals in a herd. However, these deficiencies are outweighed by the consequences of under-supplementation, so are reluctantly-accepted practice. However, if a more precise means of supplementation were available at reasonable cost, it is expected that it would enjoy widespread uptake by dairy farmers.

It is evident that a substantial increase in precision could be obtained by providing a supplement that could be directed to individual animals, and a further increase obtained if the bioavailability of the chosen supplement were higher than is normally expected from magnesium oxide. Development of a crude magnesium lactate preparation (LactominTM-Mg) offers the possibility of achieving this desired increase in precision at reasonable cost. In a prior study, (Coles, Sheppard, & Pearce, 2012) the ability to provide adequate supplementation in this way was tested, by developing two diet supplements for comparison. The control supplement was developed to be able to supply a fixed amount of elemental magnesium from magnesium oxide to each subject animal each day, and the experimental supplement was formulated to provide an amount of elemental magnesium from crude magnesium lactate expected to achieve the same magnesium uptake, and a similar incidence of magnesium deficiency symptoms and other adverse events.

The outcome of the trial conducted was to achieve the expected result, but there were a considerable range of additional observations made, to do with changes in milk production, milk composition and protein utilisation, which demanded confirmation in a further trial

designed for the purpose. Therefore, a second trial was designed and implemented, using the same dairy business, albeit with a different herd, and during a spring lactation rather than an autumn lactation.

In this study, a crude magnesium lactate preparation (Lactomin™-DDGS) was compared with magnesium oxide, provided at the standard daily dose rate for the farm in question in a wheat-based pellet. Three experimental treatments were contrasted: a control supplement, an experimental supplement and a 1:1 blend of the two. **The expected outcome of the trial was that the experimental supplement would produce a significant increase in daily milk yield, with no change in milk composition, relative to the control supplement.** It was expected that the intermediate (1:1 mixture) supplementation regimen would produce results intermediate between the two other treatments, allowing determination of a dose: response function.

Materials and methods

Experimental animals and facilities

As with the previous trial, the investigation was conducted in a dairy herd owned by Southern Centre Dairies Ltd, East Limehills, Southland, New Zealand. The animals in the herd were New Zealand dairy Friesians, and only animals bearing their second or later calves were incorporated in the trial. The entire herd of approximately 800 animals was grazed throughout the trial period in three main mobs, with small mobs maintained separately for animal health and welfare reasons: these small mobs included animals still producing colostrum, being treated for mastitis, or for lameness.

Based on the results of the previous trial (in which groups of approximately 55 animals gave a statistically-significant difference in daily milk yield over approximately 2 months) groups of 150 cows were considered ample to detect the same significant difference, if it exists. Because of the unbalanced nature of the design required for the previous trial, it proved impractical to carry out a formal power analysis. A total of 450 members of the herd complying with the above requirement were selected for randomisation to 3 trial groups. At the commencement of feeding, these animals had calved between seven and 35 days previously.

Subgroups of seven animals were randomly selected from each main experimental group, and separately identified. These animals were used throughout the trial to provide samples of milk, urine and faeces according to the trial protocol. Their milk yield data were analysed separately.

Diets

Basal diet

The main diet of the herd consisted of grazed pasture, with some PKE supplementation, and a further supplement of rolled wheat in the bail during milking. 1 kg of the rolled wheat was replaced by the experimental diets, which were fed at the rate of 500 g per milking.

Trial diets

The trial diets consisted of a control feed and an experimental feed. The control feed consisted of rolled wheat, supplemented with magnesium oxide and other components as in

table 1, and the experimental feed was based on LactominTM-DDGS, similarly supplemented with other components.

Component	Control Diet (g/g)	Experimental Diet (g/g)
Lactomin TM –DDGS		0.823
Wheat	0.783	
Rumifat*	0.025	0.025
MagOx	0.040	
Limeflour	0.080	0.080
Salt	0.005	0.005
Molasses	0.050	0.050
High 5 Premix*	0.007	0.007
Mycosorb*	0.010	0.010
Rumasweet*	0.0003	0.0003
Total	1.000	1.000

Table 1: trial diet formulations. *Proprietary products.

Trial diets were introduced to the experimental groups four days after the first milk and excreta samples were collected. A total of 500g of supplement was provided to each animal during milking, together with 100g of molasses, and rolled wheat. Initial supply of rolled wheat was 1 kg per milking, but animals whose daily production exceeded 30 L were provided with a further 250g per milking.

Sample collection

At the start of the trial, and fortnightly thereafter, the subgroups selected for sampling were drafted from their respective mobs after evening milking, and then grazed separately overnight. After morning milking was completed for the rest of the herd, these subgroups were brought on to the milking platform, and milked to completion into test buckets. Once milking was complete for a particular cow, the milk collected was thoroughly mixed, subsampled to produce two samples for analysis, and the balance retained for calf feeding. At the start of the trial, and four-weekly thereafter, urine and faeces samples were collected as for trial one. The subgroups of animals to provide the samples were drafted as above and urine samples collected as soon as they arrived on the platform, where necessary with the aid of moderately vigorous infra-vulval massage. Milking was then carried out as described above, before the animals had faecal samples collected, usually intra-rectally. Duplicate samples of both urine and faeces were collected, one for immediate analysis and one for archiving retention. Similarly, one milk sample was sent for analysis immediately, while the other was retained for archiving purposes.

Sample analysis

Milk

Milk samples were forwarded to an independent laboratory for analysis. Analytes were milk fat percentage, milk protein percentage, milk lactose percentage, total solids percentage, somatic cell count and milk urea content.

Urine

Urine samples were forwarded to an independent laboratory for measurement of elemental magnesium and calcium content.

Faeces

Faecal samples were forwarded to an independent laboratory for measurement of elemental magnesium and calcium content.

Excreta samples from the first collection were immediately analysed, and the data used to check that the three sampling subgroups were not statistically distinguishable from each other. Milk composition data were similarly used for this purpose. No significant differences were found between groups for any analyte (Table 3).

Milk yield

Daily milk yield is a standard output of the dairy shed management system used by Southern Centre Dairies Ltd. Data are summed for individual cows from 12 p.m. to 12 p.m. Milk yield analyses were conducted on data collected from the time that all animals had converted from colostrum to milk production.

Data management and analysis

Data were received from the herd management system or the independent laboratories immediately on completion of analysis. The individual managing the computer system for the dairy shed management system was blinded to the trial, as were the analysts carrying out the analysis of the urine, faeces and milk.

As data were received, they were transferred to a Minitab 16 project, with intermediate manipulation using Microsoft Excel 2010 if necessary. Data for each milk analyte, and for milk yield were analysed using the General Linear Model (GLM) procedure in Minitab, as, particularly with respect to milk yield, animals were removed from the trial herd for various reasons, leading to unbalanced data.

Results and discussion

Diet composition

Diets as formulated were sent to an independent laboratory for proximate analysis, estimation of ME, and determination of calcium and magnesium content. Results are presented in table 2. Protein was estimated by the Leco total combustion method (AOAC 968.06). Protein estimation coefficients were 6.25 for diets and LactominTM-DDGS, and 5.83 for wheat. Fat was determined by acid hydrolysis and Mojonnier extraction (AOAC 954.02) and ash by combustion at 550°C (AOAC). Mineral content was by ICP-OES, and ME was estimated using NIR.

Component	Lactomin™- DDGS	Wheat	Control Diet	Experimental Diet
% Ash	7.7	1.7	15.0	15.2
% Crude Protein	22.2	13.1	12.6	22.8
% Fat	7.0	3.1	5.0	8.6
Calcium (mg/kg)	1448	533	36622	35689
Magnesium (mg/kg)	20018	1519	26634	13951
ME (Mj/kg)	10.1	13	13	9.8

Table 2: Feed composition. Note that the data for the wheat and the control diet indicate that either the diet magnesium content estimate is in error by approximately 3.5g, or that the added concentrates contained significant magnesium.

Baseline analyses

To ensure that the trial groups and subgroups were indeed random, representative samples of the herd as a whole, data collected at the beginning of the trial for the parameters measured were subjected to separate statistical analysis.

Milk yield

Data for the first three days of the trial were analysed. Table 3 shows the mean daily milk yield achieved by each group in this period.

Character	Experimental diet (% Control)	1:1 diet blend (% control)	Control diet	Significance (p)
Milk yield (L/day)	29.13 (100.9)	28.27 (97.9)	28.87	0.132
<i>Milk Composition</i>				
Fat (%)	6.73 (120.8)	6.23 (111.7)	5.57	0.606
Protein (%)	3.60 (108.1)	3.40 (102.1)	3.33	0.518
Lactose (%)	4.58 (95.2)	4.67 (97.1)	4.81	0.572
NZ Solids (%)	10.76 (120.1)	9.63 (108.1)	8.91	0.301
Urea (mM/L)	17.76 (103.7)	20.79 (121.4)	17.12	0.607
Log SCC*	5.15	4.91	5.79	0.155
N ratio	5.06 (98.0)	6.26 (122.0)	5.13	0.582
Milk Energy	20.72 (119.4)	18.68 (107.6)	17.36	0.405

*Ratio to control is not appropriate when reporting transformed data

Table 3: Baseline performance measurements

Milk Composition

Data obtained from the first sample collection were analysed using one-way ANOVA. Results are given in table 3. Note that NZ solids is the sum of Fat % and Protein %. Milk energy is calculated as the sum of lactose% + 2.25 x Fat%. The dietary energy content of fats is 2.25 times greater than carbohydrate on a weight for weight basis.

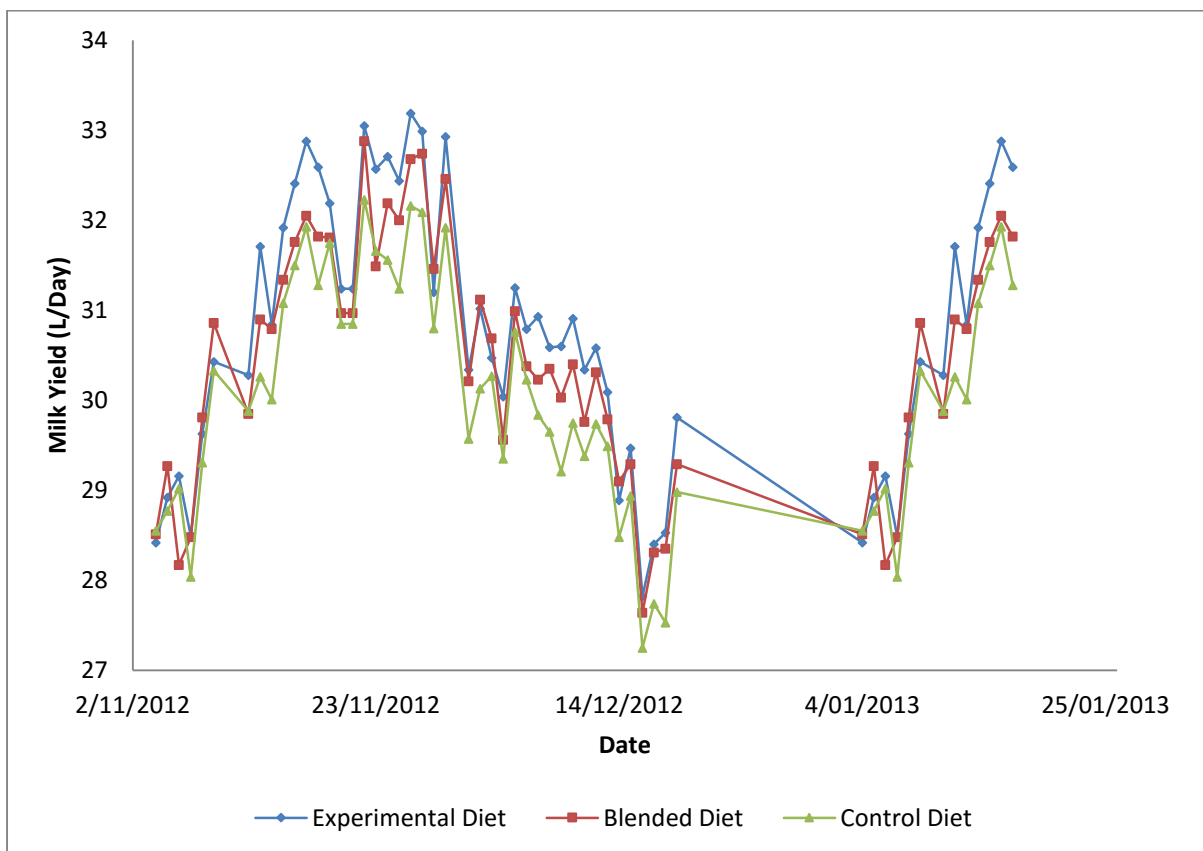
Despite substantial absolute and relative difference for most characters between groups, the within-group variability is such that the group results are not significantly different for any character at baseline. Consequently, if differences in any character achieve statistical

significance in the course of the trial, we assume adequate *prime facie* evidence for a real effect.

Trial analyses

Milk yield

For various management reasons, a small number of cows were removed from the herd during the trial period, leading to unbalanced data. Milk yield data from cows not completing the full feeding regimen were excluded from analysis. Daily yield data for individual cows were analysed using the GLM procedure in Minitab. Milk yield profiles for the three treatments are given in Figure 1. In order to exclude the possibility of a systematic bias in milk yield, the mean yield over the first three days of the trial for each cow was used as a covariate in the analysis. Consequently, the estimate of daily milk yield advantage is particularly conservative.



1622.4L (99.2%); Control diet: 1635.5L; $p=0.169$. As indicated by the previous study, the differences in the summed milk yields were slightly higher than the differences in mean daily yields, but the accumulation of data to produce the summed yield greatly reduced the number of degrees of freedom for the analysis, and concealed a small but evidently important treatment:time interaction.

Milk Yield: sampler cows

The milk yield data analysed above were derived from the main trial groups, each consisting of approximate 140 cows. Data from groups managed separately for provision of excreta and milk for composition analyses were also analysed. A larger yield advantage was achieved (Experimental diet: 29.98L/Day (104.7%); Blended diet: 26.96L/day (94.2%); Control diet: 28.63L/day ($p=0.000$)), and when mean milk production/day for the first three days was used as a covariate, the advantage to the experimental diet was increased (Experimental diet: 29.46L/day (106.2%); Blended diet: 28.31L/day (102.1%); Control diet: 27.73L/day ($p=0.000$)).

Milk Composition

Milk composition data were analysed as for milk yield, using a factorial analysis in GLM in Minitab. There was no significant interaction between treatment and sampling date for any character. Because of the likelihood of sampling error in a single collection, and because of the known fall in milk component concentration immediately after calving, no analysis of covariance using baseline data was conducted.

Character	Experimental diet (% Control)	1:1 diet blend (% control)	Control diet	Significance (p)
Fat (%)	4.87 (125.2)	4.91 (126.2)	3.89	0.000
Protein (%)	3.33 (94.3)	3.60 (102.0)	3.53	0.008
Lactose (%)	4.87 (98.4)	4.87 (98.4)	4.95	0.108
NZ Solids (%)	8.26 (111.2)	8.51 (114.5)	7.43	0.000
Urea (mM/L)	28.71(110.0)	26.53 (101.6)	26.11	0.090
Log SCC*	4.804	4.719	5.186	0.001
N ratio	8.70 (118.5)	7.40 (100.8)	7.34	0.013
Milk Energy	15.95 (116.2)	15.92 (116.0)	13.73	0.000

*Ratio to control is not appropriate when reporting transformed data

Table 4: *Results of analysis of milk composition.*

The results in table 4 are quite unexpected. In the previous study (Coles et al., 2012), no evidence was obtained to suggest that the experimental diet supplement would have any impact on milk composition. As noted above, the baseline results, although variable, were not significantly different. The observed variability that developed during the trial therefore demanded closer investigation. Data obtained after feeding ceased were therefore analysed.

Washout analyses

The observed significant differences between treatments for milk composition may have been due to real diet effects, or due to non-random sampling. In human trials, it is often necessary to collect data for a period after treatment is completed, to observe washout effects. Since the first evidence of statistical significance (for milk protein) was obtained two weeks after the trial commenced, it was presumed that a washout period of four weeks would be necessary,

but sufficient, to permit milk composition to return to baseline. It is noted, however, that this period constitutes 10% of the average length of lactation in the trial herd, and that the period coincides with both physiological changes in the cow, and in this trial, a period of warm, dry weather. Results will therefore be considered in conjunction with changes in milk yield.

Milk yield

As expected, milk yield differences persisted for a period after trial feeding ceased. (Figure 2), then began to diminish. Closer inspection of the data revealed a period of considerably increased variability from approximately one week after feeding ceased. Data for a further two weeks were collected, yielding a most surprising result. Even after milk yield was corrected for baseline productivity, animals that had consumed the experimental diet maintained a continuing production advantage. (Experimental diet: 26.72L/day (105.9% of control); Diet blend: 26.07L/day (103.3% of control); Control diet: 25.24L/day (p=0.000))

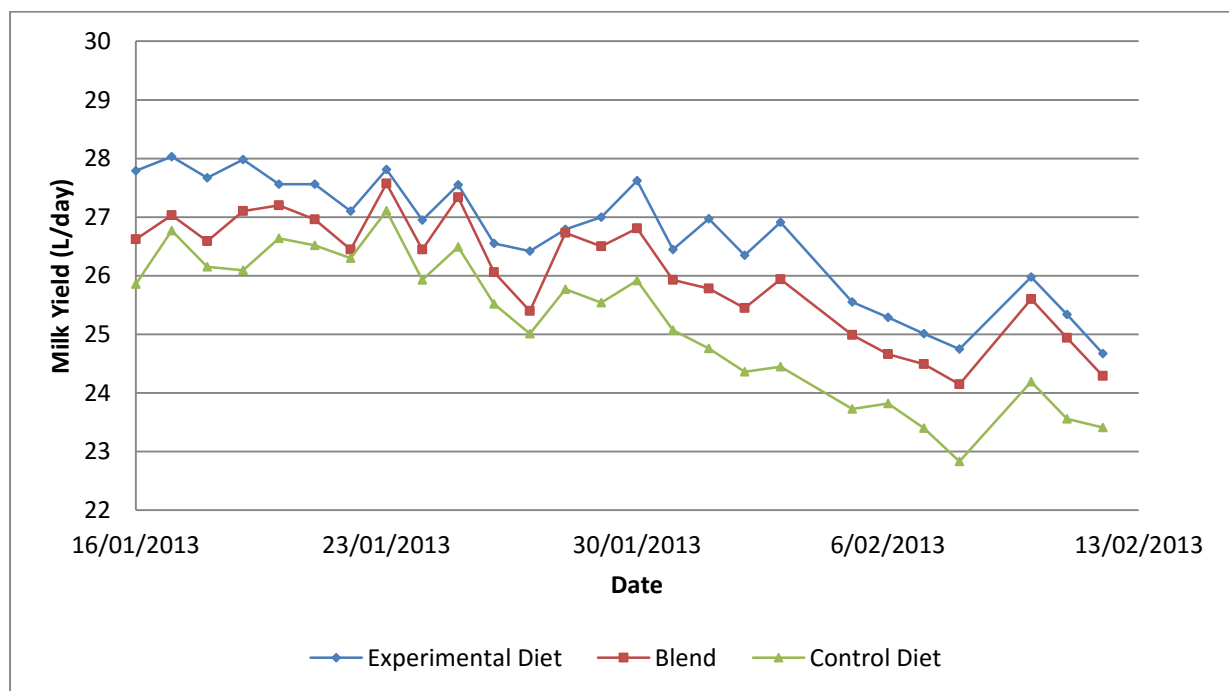


Figure 5: Daily milk production during washout. Raw data were corrected for variation in baseline milk production. Magnesium source for all animals in this phase was $MgCl_2$ in drinking water.

Data from the separate sampling cows indicated a rather quicker washout: by the end of the first week after cessation of the experimental diets, the significant differences between treatments had disappeared. However, the milk yield advantage to animals formerly fed the experimental diet had returned by one month after the end of feeding.

Milk composition

With the exception of N ratio, significant differences between treatments for all characters measured had disappeared within one month of cessation of feeding (Table 5).

Character	Experimental diet (% Control)	1:1 diet blend (% control)	Control diet	Significance (p)
Fat (%)	5.31 (128.5)	5.25 (127.1)	4.129	0.287
Protein (%)	3.50 (93.4)	3.60 (103.4)	3.75	0.094
Lactose (%)	4.78 (99.4)	4.74 (98.6)	4.82	0.711
NZ Solids (%)	8.81 (111.8)	9.13 (115.9)	7.88	0.341
Urea (mM/L)	36.17(122.3)	30.17 (102.0)	29.57	0.107
Log SCC*	4.74	4.86	5.22	0.071
N ratio	10.38 (131.2)	7.90 (99.8)	7.92	0.030
Milk Energy	16.73 (118.6)	16.56 (117.4)	14.10	0.288

*Ratio to control is not appropriate when reporting transformed data

Conclusions

As with the previous trial, there was no evidence of any ill effects on animal health and welfare when a daily supplement of 22.0g of elemental Mg from MgO was replaced with 10.0g from crude magnesium lactate. As previously observed, a supplement containing crude magnesium lactate provided for a small but significant increase in daily milk yield, even when variation in baseline milk production was accounted for through covariate analysis. There appeared to be a more or less linear dose response, as indicated by the blended diet. However, there were several important, unexpected outcomes. Firstly, the experimental diet provided a statistically-significant 15.26% increase in milk solids (when both milk yield and solids content are accounted for), and the blended diet produced a slightly higher increase in total solids. This effect was achieved **despite** an apparent **reduction** in milk protein relative to the control. Unlike the earlier trial, the experimental diet was associated with a relative increase in milk urea, which may explain the fate of protein which otherwise would have been available for secretion in milk. However, the milk urea levels observed in the study are not indicative of a major diversion of dietary N to energy production.

Note that the results above are achieved in comparison to a diet including an alternative supplement of wheat grain. When effects of that supplement are accounted for, the estimated milk solids yield increase rises to 23.0%

Excreta data from trial one suggested that a possible mechanism for increased milk yield might be increased rumen fermentative efficiency. Provided feed intakes are similar, the logical consequence of an increase in efficiency would be an increase in energy recovery from the diet. Such an outcome is supported by the relative increase in secretion of energy-containing components observed in animals consuming the experimental diet.

Reference

Coles, G. D., Sheppard, M. L., & Pearce, R. J. (2012). *Effects of a crude preparation of magnesium lactate on dairy cow performance and welfare.*