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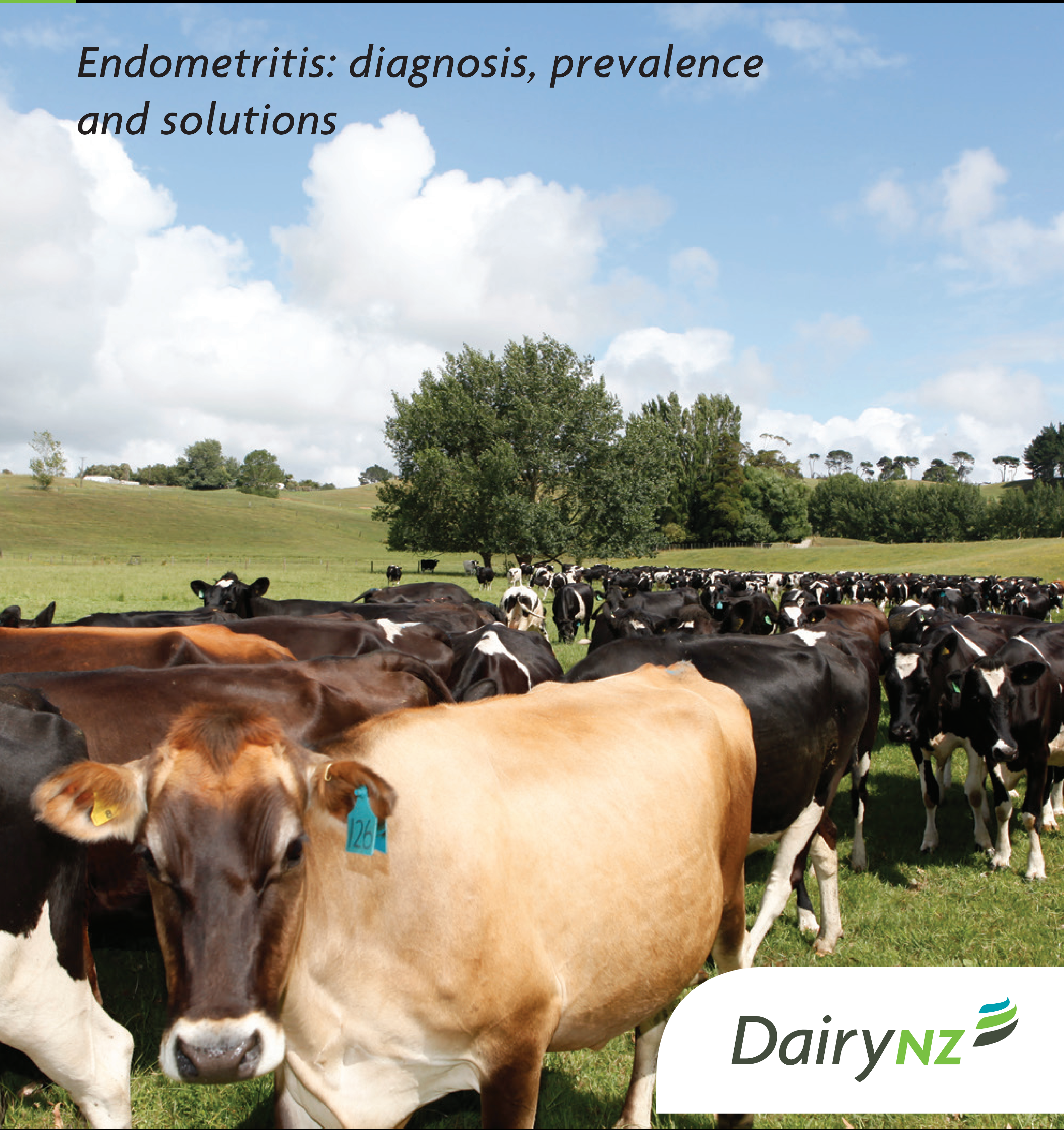
IN BRIEF

Endometritis: diagnosis, prevalence and solutions



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Endometritis: diagnosis, prevalence and solutions

Endometritis is an infection or inflammation of the uterus that persists beyond the third week after calving[1]; it will reduce reproductive performance[2,3,4]. Here we describe how endometritis is detected, its likely prevalence in the national herd, and its effect on reproductive performance.

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There are two forms of endometritis. The first, and more familiar, is when there is gross purulent material visible at the vulva (purulent vaginal discharge; PVD). Often, however, there is no externally visible discharge, and this disease is only diagnosed with additional testing. There are several ways to do this[5], but the most commonly used method is with the metricheck device (Figure 1). Metrichecking is a fast and clean way of diagnosing PVD-endometritis. The appearance of material recovered from the vagina using a metricheck device can be scored on a 1 (i.e. clear oestrus-like mucus) to 5 (greater than 50% particulate material with a foul odour) score. A score of 2 or more is used to define PVD-endometritis[2]

Key findings

- Endometritis is a uterine disease that affects a quarter of the national herd
- The 6-week in-calf rate is 8% lower in cows with endometritis
- There are two forms of endometritis: one is detected by metri-check but the other cannot be readily detected on farm
- Prevention is best approached through effective management of the transition period, by optimising BCS and minimising disease around calving
- The metri-check procedure is the best way to diagnosis PVD endometritis
- Treatment is best done in conjunction with your vet
- Current research is seeking better solutions to reduce the impact of endometritis on reproductive performance.

Figure 1. Metrichecking is a fast and clean way to diagnose endometritis associated with a purulent vaginal discharge (PVD-endometritis).

In some cases, inflammation is limited to the uterus and no purulent material is present in the vagina or on the vulva. Under research conditions, we can diagnose this type of endometritis by the presence and proportion of immune defence cells (neutrophils) present in smears taken from the lining of the uterus (the endometrium). The neutrophils (polymorphonuclear cells or PMN's) have distinct dark staining multi-lobed nuclei, as indicated in Figure 2. If this test is done four weeks before mating start, a cut-point of more than 2% of the cells being PMN's indicates that the cow has cytolological (CYTO-) endometritis. Whereas PVD-endometritis is detected by metrichecking, there is no practical way to routinely check cows for CTYO-endometritis under normal field conditions.

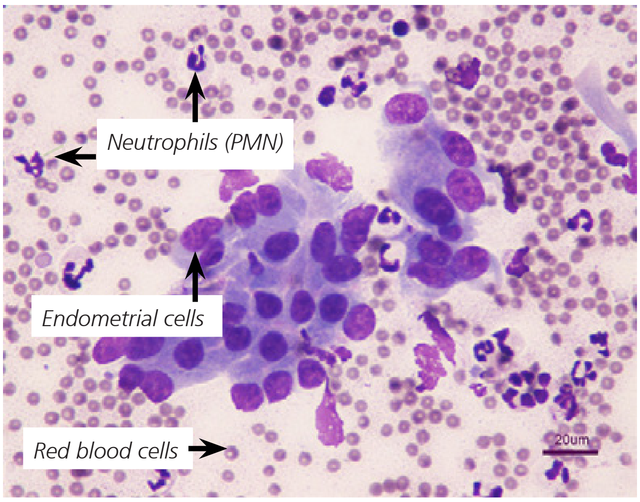


Figure 2. Photograph of a microscopic slide prepared from a smear of the endometrium (uterine lining) used to diagnose cytological (CYTO-) endometritis.

Endometritis is a widespread problem in New Zealand

Endometritis is regarded as a significant disease affecting dairy cattle in the USA (12 to 50% of cows in the herd[6]) and Canada (4 to 64% of cows in the herd[3]). Until recently, there was no such comparable information available for commercial New Zealand herds, and only limited data was available from research herds. This changed with a field study in 2015 involving 1,806 cows assessed about 30 days before the start of mating, across 100 herds in the North and South Islands. Median pre

valence rates were about 25% for PVD-endometritis, based on the routinely used metricheck score of 2 or more indicating PVD2, and 27% for CYTO-endometritis using a diagnostic cut-point of more than 2% PMN cells; 10% of cows had both PVD and CYTO-endometritis. On this basis, only 58% of the cows had neither of these conditions (Figure 3) four weeks out from the start of mating.

About a quarter of affected cows were diagnosed with both conditions, representing 10% of the 1,806 cows examined. We would have expected that most cows with PVD-endometritis would also test positive with CYTO-endometritis (i.e., an infected uterus should also be inflamed with an immune response). This low level of overlap indicates that some PVD-endometritis cows may be suffering an infection of the cervix or vagina, rather than the uterus. Another possibility is that neither test is perfect; in some cows, purulent material in the vagina discharging from an infected uterus is not supported with a positive test for uterine inflammation.

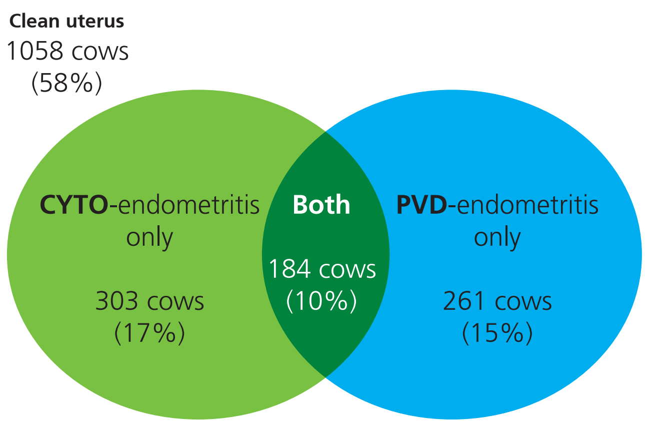


Figure 3. The number and percentage of 1,806 randomly selected cows diagnosed with cytological (CYTO-) and purulent vaginal discharge (PVD-) endometritis at approximately 30 days before the planned start of mating in 100 North and South Island herds.

Endometritis reduces reproductive performance

Table 1 and 2 describe the reproductive outcomes for the 1,806 cows involved in the 2015 study. Increasing PVD score was associated with declining conception rate to first service, and lower 3- and 6-week in-calf rates (Table 1). In this study, the fertility of cows diagnosed with PVD-endometritis[2] was substantially reduced with a metricheck score of 3 or more.

Similarly, cows with CYTO-endometritis (more than 2% PMN cells) had poorer reproductive outcomes than those without endometritis (Table 2). Cows that had both conditions had poorer reproductive performance, than cows with only one of these conditions[7].

Table 1. The relationship between metricheck score 30 days before mating start and reproductive performance. Reproductive performance declined with increasing score, most notably when the score was greater than 2.

	PVD Score				
	1	2	3	>3	P-value
No. cows	1361	317	72	56	
% of cows	75.4	17.6	4.0	3.1	
First service conception rate (%)	52.9	54.6	47.9	31.5	0.01
3-week submission rate (%)	87.8	86.9	90.5	80.0	0.30
In calf by 3 weeks (%)	53.3	53.3	46.6	35.2	0.05
In calf by 6 weeks (%)	75.6	74.2	68.5	50.0	0.00

Table 2. Reproductive performance of cows diagnosed with CYTO-endometritis (PMN% greater than 2) compared with those assessed as having a normal, uninflamed uterus (PMN% 2 or less) 30 days before the start of mating.

	PMN%		
	2 or less	More than 2	P-value
No. cows	1318	488	
% of cows	73.0	27.0	
First service conception rate (%)	54.5	45.8	0.001
3-week submission rate (%)	89.3	83.7	0.001
In calf by 3 weeks (%)	55.4	44.4	0.000
In calf by 6 weeks (%)	76.4	67.5	0.000

Risk factors for endometritis

A UK study reported that retained fetal membranes, assisted calving, stillbirth, vulva angle, first calving, and delivery of a bull calf increased the odds for cows developing endometritis, but not having had either mastitis or milk fever[8]. Ketosis and acute metritis were detected as risk factors in a USA study[6]. This same study reported that average herd milk yield was not a risk factor, but that as individual milk yields increased, heifers were more vulnerable, while mature cows were less vulnerable, to being diagnosed with endometritis. Cows with endometritis are often reported to be in deeper negative energy balance[9], and as such, will appear to lose more BCS than their herd mates.

Our work in New Zealand herds has indicated that endometritis is more likely to occur in cows with a body condition score (BCS) of 4 or less at calving. A similar relationship four weeks out from the start of mating was observed in the 2015 study involving 100 North and South Island herds, where cows with BCS 4 or less had a CTYO-endometritis rate of 32% compared with 22% in those with BCS 4.5 or better. Cows with ketosis[10] and those that are older[11] are also more likely to have endometritis. Endometritis is also linked with cows having poorer liver function and lower magnesium levels around calving[12]. Although it is difficult to establish a 'cause and effect' relationship between endometritis and the numerous risk factors associated with this disease, effective management of the transition period has an important role in minimising endometritis.

Prevention and treatment of endometritis

Research into finding better solutions for farmers to minimise the impact that endometritis has on the reproductive performance of the herd is being performed within a jointly funded programme, Pillars of a Competitive and Responsible Dairy System[13], between the New Zealand Ministry of Business, Innovation and Employment and New Zealand dairy farmers through DairyNZ Inc., and includes AgResearch core funding.

The large between herd variation in the prevalence of endometritis (Figure 4 a, b) suggests some presently unknown herd management factors are influencing the prevalence of endometritis. We believe that this herd variation likely relates to how well the cows are transitioned from late pregnancy through to the first few weeks of lactation; the period of highest risk for cows succumbing to health disorders that delay the recovery of the reproductive tract to a state that can support pregnancy. Since many of these disorders around calving are preventable (e.g. under- and over-conditioned cows, difficult calvings), transition cow management offers the greatest opportunity for minimising endometritis through a managerial approach[14].

Treatment options should be discussed with your vet. Where endometritis is diagnosed either by metricheck (or cytology), on-label intrauterine antibiotics have been proven in randomised controlled studies to improve in calf rates compared with leaving them untreated[15]. Treatment of cycling cows (i.e. presence of a corpus luteum) that are metricheck-positive with a prostaglandin-F2α may be as effective as intrauterine antibiotic, and is a lower-cost option with potential advantages in synchronising oestrus. Thus, assessing metricheck-positive cows by scanning or palpating the ovaries allows cows to be allocated to an antibiotic treatment if there is no corpus luteum present, while prostaglandin may be used in those animals that do have a corpus luteum.

Figure 4 a, b. Herd level prevalence (95% confidence intervals; that is the range within which 95% of the results fall) of (a) purulent vaginal discharge (PVD)-endometritis, and (b) CYTO-endometritis (PMN% greater than 2) among 1806 randomly selected cows from 100 North and South Island herds examined 30 days before the planned start of mating.

Figure a

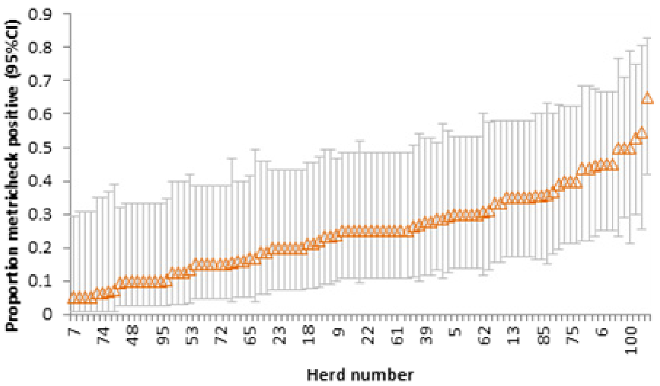
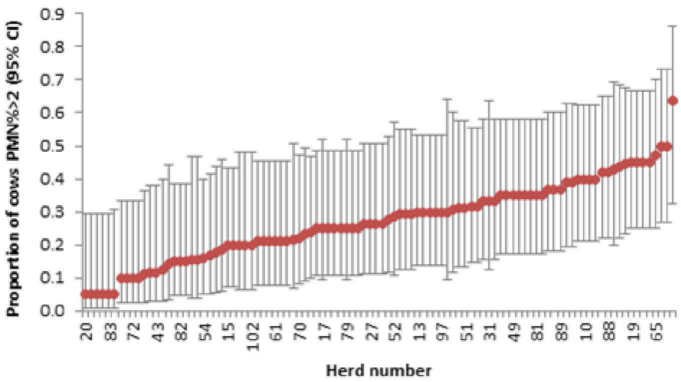


Figure b



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Protect your paddocks, and the environment

Taking cows out of the paddock can protect against treading damage and potentially increase feed grown on wet, heavy soils. Off-paddock facilities can also help farmers meet environmental regulatory requirements. Two key challenges are managing pasture surpluses in spring and the cost structures of the new system when cow grazing hours are reduced due to off-paddock facility use.

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Dawn Dalley, DairyNZ

Introduction

Cattle grazing on wet soil can damage soil physical quality and reduce pasture production. It also increases the risk of sediment and nutrient losses by overland flow and reduces future pasture production as a result of soil compaction. Reducing the time cows spend on pastures during autumn and winter, irrespective of soil water content (SWC), will reduce urinary nitrogen (N) returns to soil thus lowering soil nitrate (NO_3–) concentration. This is particularly important during the autumn when pasture growth is limited by low temperatures and a large volume of rainfall increases drainage and leaching.

Off-paddock facilities may be used to accommodate cows periodically during spring and autumn to help protect wet soils from treading damage (spring) and reduce nitrogen leaching losses (autumn). Strategies aimed at reducing the time cows spend grazing pastures in spring and autumn are referred to as duration-controlled grazing (DCG) and have been investigated as part of the P21 programme of research conducted at Telford Dairy Farm, South Otago.

Key findings

- To reduce spring pugging damage, stand cows off pastures when soil water content is at or near field capacity.
- A short (4-5 hour) grazing after each milking per day is best.
- Using an off-paddock facility in April and May can reduce N leaching by about 10%.
- Greater complexity to the farm system results from more decisions to make and additional labour, maintenance and animal feeding requirements.

Duration-controlled grazing for soil protection

During early spring, soils usually have a high moisture content (i.e. they are at or near field capacity) and are prone to damage from animal treading, from pugging and soil compaction. Pugging takes place when soils are very wet and soil pores are likely to be full of water rather than air. Soil compaction occurs at lower soil water contents when soil pores are filled with water and air[1]. Poor soil drainage may compound these wet soil problems by not allowing water to drain.

When soils are wet, the pressure under the cow's hooves causes a breakdown of the soil structure, reducing soil drainage and aeration[2]. This increases the risk of sediment, faecal microbe and nutrient losses via overland flow, and reduces soil biological activity[3]. An immediate loss in pasture production occurs due to direct damage caused to the pasture sward and root systems (Figure 1). Long-term reductions in pasture growth rate may also occur due to the poor soil physical conditions created as a consequence of cow treading[4].

Holding cows off-paddock during 'wet' periods can help to reduce treading damage. However, a farm's capital and operating expenditure increases due to costs associated with the addition of off-paddock facilities and the cost of conserving more pasture due to inflated feed surpluses. Required upgrades to the effluent management system, potentially more labour and quality supplementary feed are also important considerations[5-8].

Figure 1. Increasing levels of treading damage to soil and pasture following grazing events that occurred at increasing (from left to right) soil water content.

When should cows be taken off pasture?

Soils on the Telford farm, in Balclutha where we have conducted experiments, are dominated by Pallic soils that are highly susceptible to structural damage due to high clay content and impaired drainage characteristics. On these, or similar clay soils, grazing time should be reduced when soil water content is at or near field capacity. In the first year of the P21 research at Telford, a soil water deficit threshold of 3 mm was used[1] i.e. soil water content was slightly below field capacity. When a lower deficit occurred, cows were stood off in a barn for 13 hours a day and offered 5 kg DM/cow of pasture silage. For soils with low clay content (i.e. < 10% clay), a higher soil water content can be tolerated (i.e. sometimes above field capacity) before the effects of treading significantly affect pasture production[7].

Treading damage that occurs in spring and early summer has significantly greater effects on annual pasture production than that in autumn, due to the greater portion of seasonal pasture production grown in spring and summer[9].

Monitoring soil water content can be useful to guide decisions about when to take cows off pasture. In most instances, good knowledge of the farm, including approximations of soil wetness on a given day, will be sufficient to guide this strategy. Alternatively, a simple calendar-based approach may be preferable in locations with predictable seasonal weather where it is logistically easier to plan and implement a DCG schedule (e.g. the cows removed for a set period every day in spring). In the second and third years of the farmlet experiment at Telford, a calendar-based approach was adopted, whereby cows were restricted from accessing paddocks for 13 hours every night from mid-September until late October.

How long should cows be stood off wet pastures?

Some grazing of wet soils, in combination with limited use of the off-paddock facility, will be more economical than no grazing at all. For example, 8 hours grazing/day and 13 hours standoff/day, leaving 3 hours for milking and laneway time allows cows sufficient time to harvest a large percentage of their daily feed requirement. To maximise pasture utilisation, two short grazing events per day will be more effective than a single grazing once per day (e.g. use two periods of 4 hours grazing rather than one of 8 hours grazing)[6, 10].

Where cows are held off for one entire grazing bout (i.e. during the day or during the night), the quantity of supplementary feed required and therefore costs increase. Frequent removal of cows from wet pastures will also pose difficulty in managing average pasture cover and pasture quality, promoting increased pasture surplus and cost for conservation.

What is the benefit of implementing duration controlled grazing?

Two farmlet systems were implemented at Telford, each with a herd size of 110 cows.

The treatments included:

1. a Control where cows were grazed on paddocks continuously between milkings regardless of soil water content
2. a DCG treatment where grazing on wet soils was minimised. The effect of this was that during spring, cows in the DCG grazing herd were removed from pasture for 13 hours overnight on days when the soil water deficit was less than 3mm[1]. During spring, the DCG herd spent approximately 10% less time on pasture compared with the control herd. In autumn cows were removed from pasture for 13 hours per day for 6 weeks from mid-April to dry off and all cows were wintered off paddock in the off-paddock facility.

Fertiliser N inputs to the Control and DCG farmlets averaged 100 and 75 kg N/ha/yr, respectively. Average annual pasture growth measured during 2012-13 (Year 1) and 2014-15 (Year 3) seasons was 0.4 T DM/ha higher on the DCG farmlet (Table 1). When adjusted for the different N fertiliser inputs, based on an assumed N response rate of 12 kg DM per unit of N applied, the difference was 0.7 T DM/ha. Data for year 2 of the study is not available due to issues with regular pasture assessments on the control farmlet.

Milk solids production did not differ between the farmlets over the 3 years of the trial. The control farmlet conserved less pasture silage, fed more total supplement during lactation and therefore imported more supplement (Table 1).

The cost of using the off-paddock facility at Telford for DCG in spring, autumn and winter was $298/ha/annum ($105/cow/annum). This cost included bedding material, daily maintenance of the barn, seasonal cleaning and replenishment of the bedding and the additional costs associated with effluent and manure management. It does not include the capital or interest costs associated with building and operating the infrastructure.

Total labour inputs did not differ between the systems except for approximately 20 minutes per day additional time required for moving effluent equipment when cows were in the barn. However, there were differences in the timing and nature of the tasks, particularly those associated with winter feed management and collecting cows for milking. Additional costs were incurred for silage making on the DCG farmlet but this was offset by higher imported supplement costs for the control herd.

Before investing in infrastructure for DCG, a full farm systems analysis, including detailed whole farm systems budgets, should be completed.

The costs and benefits for individual farms will vary depending on farm specific factors (climate, soil type, current system), how the infrastructure will be used and the type and cost of the infrastructure (which range from approximately $800 to $3000 per cow).

The economic benefits will be dependent on how well the infrastructure can be integrated into the system without adding significant cost, the pasture growth and milk production benefits that are achieved and the milk price.

There will also be other non-financial drivers e.g. farmer goals, peace of mind, environmental regulation that should be

Table 1. Physical performance (mean SEM) of the control and DCG farmlets at Telford and associated differences in operating costs averaged across 3 years of the trial unless stated otherwise. SEM is the standard error of the mean.

Physical performance	Control mean (SEM)	DCG mean (SEM)
Pasture growth (T DM/ha/annum) – actual*	13.1 (0.14)	13.5 (0.69)
Pasture growth (T DM/ha/annum) – adjusted for fertiliser N*	11.8 (0.17)	12.5 (0.74)
Nitrogen fertiliser (kg N/ha)	109 (0.9)	74 (6.1)
Stocking rate (cows/ha)	3.0 (0.09)	2.8 (0.03)
Milk solids (kg/cow)	329 (9.2)	335 (8.2)
Milk solids (kg/ha)	963 (31.9)	947 (21.3)
Days in milk	246 (3.3)	253 (4.3)
% of farm conserved	20 (7.0)	47 (13.5)
Pasture silage conserved (T DM/ha)	0.33 (0.08)	0.91 (0.34)
Supplement fed lactation (T DM/ha)	1.79 (0.17)	1.37 (0.31)
Other supplement grown on farm (T DM/ha)	0.43	0
Net imported supplement – lactation (T DM/ha)	1.03 (0.42)	0.46 (0.63)
Winter feed (T DM/ha)	3.07 (0.15)	2.68 (0.06)
Operating costs that differed between farmlets		
Barn maintenance & manure management ($/ha)	-	$298
Pasture silage making ($/ha)	$47	$128
Imported supplement - lactation ($/ha)	$299	$168

**Data for years 1 and 3 only.*

considered when determining the value proposition of off-paddock infrastructure for your farm. For example, in drier regions, the impact of treading damage across the whole farm may be low and so production and financial gains will often be less than the costs of implementing a DCG strategy [5,7]. However, there may be valuable environmental gains from reduced surface runoff or leaching of nutrients.

standard required for lactating cows, especially following winter use; complicated daily decision making for the farm team regarding when to move cows off pasture into the off-paddock facility during busy periods; and an inability to maintain milk production when using the off-paddock facility for extended periods due to insufficient high quality supplements being available to maintain early lactation production and large

changes in the proportion of silage in the diet from day to day.

Grass silage was offered to the DCG herd at a rate of 5.2 kg DM/cow/day when cows were stood off wet paddocks for 13 hours during spring. As grass silage tends to have a lower metabolisable energy content compared with fresh pasture, a greater DM intake (or higher quality supplement) may be required to maintain milk production in early lactation when standing cows off pasture as they cannot consume sufficient pasture during the grazing period to meet their energy demands. This cost must be factored in as an implication of off-paddock facility use.

The use of DCG also results in an increase in the volume of solid and liquid effluents captured, and which must be applied back to land. The collected effluent(s) will typically require effluent-treated areas of the farm to be enlarged, or alternative effluent spreading options adopted e.g. slurry wagon to spread barn generated effluent to other areas of the farm, to account for the additional nutrients that are collected at the off-paddock facility.

What are the implications of taking cows off?

When grazing hours are reduced during calving and late spring, managing average farm covers is important to prevent loss of pasture quality and subsequent reductions in net pasture growth rates.

For the farmlet study at Telford, a single grazing of 8 hours per day between the morning and afternoon milkings did not provide sufficient time for cows to achieve target pasture intakes for that period despite there being sufficient pasture on offer within a pre-graze herbage mass range of 2800-3200 kg DM/ha.

As a result, the farm failed to achieve the spring rotation planner area targets i.e. less area was offered through spring to allow post grazing residuals of 1500-1600 kg DM/ha to be achieved within the grazing period. This change resulted in slower rotations and reduced pasture utilisation, despite conserving 68% of the farm as silage. Achieving lower pasture covers on the farm at drying-off, or strategic grazing of paddocks with high pasture mass during winter, would help to keep pasture covers at a more manageable level during spring.

Other challenges faced in the Telford farmlet included: difficulties in maintaining a clean, dry barn surface to the

Animal welfare and health issues

Standing cows off-paddock for long periods may increase the risk of mastitis and lameness. This is particularly important where the off-paddock facility has been used for calving and the bedding material not replaced prior to use with lactating cows. However, despite high usage of the barn during spring at Telford, the risk of mastitis did not increase in the DCG farmlet because the bedding surface was refreshed before being used for milking cows in spring.

You can minimise cow energy expenditure and lameness risk by reducing the walking distances between paddocks and the off-paddock facility (approximately 1.8 MJ ME/cow/d is required per 1 km walked). There may also be productivity gains by housing cows indoors during wet/cold weather events to achieve better feed utilisation.

Duration-controlled grazing for environmental benefits

The tactical removal of animals from pasture during autumn and winter is an effective strategy for reducing nitrogen loss. This benefit is achieved due to the reductions in urinary N returns to soil at a time when pasture N uptake is limited by declining soil temperatures and the onset of drainage is likely.

Farmlet experimentation at Telford indicated that overnight use of the barn (13 hours/day) during April and May led to a 10% decrease in N leaching risk when compared with keeping cows in the paddock.

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Pasture species mixtures to reduce nitrogen leaching

The Forages for Reduced Nitrate Leaching (FRNL) Programme is a six-year study investigating how any of a wide variety of forages can be used within modern grazing systems to both improve animal production and reduce nitrate leaching

Key findings

- Mixed-species pastures (herb species added to grasses and white clover), can increase herbage production and reduce the risk of nitrogen leaching.
- Milk production from mixed-species has sometimes been shown to be greater, but is no less than from cows grazing ryegrass/white clover.
- Plantain is a key species for increasing dry matter production and reducing urine nitrogen concentration. A critical threshold of plantain in the animal diet should be clearly established.

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Background

One of the major aims of the FRNL programme is to determine whether novel pasture species mixtures can contribute to reduced nitrate leaching to see whether they reduce urinary nitrogen (UN) output from cows, rather than looking at individual forage species in monocultures.

When plant attributes are combined in a multi-species pasture, the mixtures typically have greater annual herbage production than conventional grass-clover pastures. These mixtures can achieve greater total annual N uptake due to their complementary root systems and seasonal growth patterns[1]. In New Zealand, the early interest in using diverse pasture mixtures arose from a need to maintain animal production in environments where conventional perennial ryegrass/white clover pastures lacked persistence because of summer drought and pest damage. Herbs such as chicory and plantain were used as they had already shown promise in monoculture, good quality seed was available and their management was understood.

Several experiments comparing the animal feeding benefits of diverse pasture mixtures with conventional ryegrass/clover pastures have been conducted in the last decade (Table 1). These have shown similar or greater milk production and consistently reduced urinary nitrogen concentration. An indoor feeding experiment with forage mixtures including chicory, plantain and lucerne demonstrated greater milksolids (MS) production and N intake allocated to milk[2]. A concurrent grazing experiment in the Waikato with similar diverse pastures supported the expectation of better summer DM production but this did not translate into greater milk production[3]. A series of grazing trials in Canterbury demonstrated the benefits of diverse pasture mixtures (chicory, plantain, red clover and lotus added to the conventional ryegrass/clover sward) [4,5,6]. They showed similar or better MS yield and lower urine N concentration compared with the ryegrass/clover pasture. The milk yield advantage was more closely linked to the legume content, but reductions in UN concentration were influenced by the herb component.

It is important to know whether the benefits can be attributed to the greater diversity of species in the pasture mixture, or to the presence of individual species. Vibart et al.[1] suggested that the presence of specific well-adapted species is more important than the number of species in driving herbage production and N dynamics. A Lincoln grazing study showed an 11% greater MS production per day in late lactation combined with a 56% reduction in UN concentration from cows grazing pure plantain in comparison with irrigated perennial ryegrass and white clover pastures [7].

ryegrass vs. tall fescue as the base pasture grasses, and grass/legume pastures with or without plantain. The pastures were sown at the DairyNZ Scott Farm in spring 2015, and rotationally grazed by dairy cows throughout the year. Paddocks were grazed when pasture cover reached 2800-3200 kg DM/ha to a residual of 1500-1600 kg DM/ha. Sample plots were harvested prior to each grazing with a mower to 4 cm height to determine the component species and dry matter yield. The milk production and UN concentration of small herds were measured during two 10-day periods in February 2016 (mid-lactation) and November 2016 (early lactation).

Table 1: Summary of recent New Zealand published experiments examining the effect of various pasture mixes in comparison with conventional ryegrass/white clover swards, on dairy cow milk production (MS) and urinary nitrogen (UN) concentration. Statistically significant effects are quantified.

Pasture type compared with ryegrass/white clover	Season	Change in MS yield (kg MS/cow/day)	Change in UN concentration (g N/L)
Ryegrass +white clover +chicory +plantain[4]	Autumn	Nil	-40% (5.7 v 3.4)
Ryegrass +white clover +chicory +plantain +lucerne[5]	Spring	Nil	-20% (6.1 v 4.9)
Ryegrass + white clover +prairie grass +chicory +plantain +red clover[6]	Spring Summer Autumn	Nil +17% (1.47 v 1.72) Nil	-30% (4.2 v 2.9) Nil Nil
Ryegrass* + white clover +prairie grass +chicory +plantain +red clover[6]	Spring Summer Autumn	Nil Nil Nil	-28% (4.6 v 3.3) -28% (4.4 v 3.1) -22% (6.8 v 5.3)
Plantain[7]	Autumn	+11% (1.50 v 1.67)	-56% (5.4 v 2.4)
Ryegrass + white clover +plantain[7]	Autumn	+7% (1.50 v 1.60)	-33% (5.4 v 3.6)

** comparison using high sugar ryegrass*

Waikato grazing trial

In a recent grazing trial in the Waikato, the milk production and UN output were measured for cows grazing four pasture mixtures. These mixtures were chosen to provide greater MS production and to reduce UN excretion relative to a conventional ryegrass/white clover pasture. The species were selected based on simulations with the MOLLY animal model, using data on chemical composition and metabolisable energy for a range of pasture species[8]. The modelling tested perennial ryegrass, tall fescue, cocksfoot, kikuyu, prairie grass, white clover, lucerne, red clover, lotus, chicory and plantain, in 72 simulated combinations. Combinations of tall fescue, lucerne and plantain were predicted to maximise the ratio of MS:UN. Therefore, the species combinations chosen for the grazing experiment were:

Ceres One50 perennial ryegrass, Grasslands® Torlesse lucerne, Grasslands® Hummer tall fescue and Ceres Tonic plantain.

1. Perennial ryegrass + Lucerne **PR+L**
2. Perennial ryegrass + Lucerne + Plantain **PR+L+P**
3. Tall fescue + Lucerne **TF+L**
4. Tall fescue + Lucerne + Plantain **TF+L+P**

This field study allowed two main comparisons; perennial

Over the 2016 calendar year of pasture harvests, there was little difference in the total herbage accumulation of the ryegrass and fescue-based pastures. However, the paddocks with plantain grew on average 2.6 t DM/ha (for ryegrass-based pastures) or 1.6 t DM/ha (for fescue-based pastures) more forage in total than those without plantain. During the short-term measurement period in summer the pastures comprised 25% perennial ryegrass and 34% lucerne in the PR+L pastures; and 24% tall fescue and 43% lucerne in the TF+L pastures. Where plantain was included, this made up 51% (PR+L+P) and 38% (TF+L+P) of the herbage, reducing both the grass and lucerne content by more than half. During the short-term measurement period in spring the pastures comprised 60% perennial ryegrass and 24% lucerne in the PR+L pastures; and 48% tall fescue and 31% lucerne in the TF+L pastures. Where plantain was included, this made up 51% (PR+L+P) and 36% (TF+L+P) of the herbage, again reducing the grass and lucerne content by more than half.

In both summer and spring there were few differences in milk production and percent fat or protein between the treatments. There were never any differences between ryegrass and fescue-based pastures, so the results in Table 2 show only the effects of

Table 2: Milk production from cows grazing pastures with and without plantain in the Waikato, in summer 2016 (mid-lactation) and spring 2016 (early lactation). The results show similar milk production across all four treatments but a lower Fat% from the cows grazing pastures that include plantain. Different letters following the data indicate significant differences between pasture types. MS = milksolids.

Milk production measurement		Grass+ Lucerne	Grass+ Lucerne+ Plantain
Summer 2016 Mid-lactation grazing trial	Milk volume (L/cow/d)	12.2	13.8
	Fat (%)	5.04 a	4.45 b
	Protein (%)	3.80	3.74
	MS (kg/cow/d)	1.09	1.11
Spring 2016 Early lactation grazing trial	Milk volume (L/cow/d)	19.3	19.5
	Fat (%)	4.24 a	4.12 b
	Protein (%)	3.54	3.59
	MS (kg/cow/d)	1.47	1.48

the inclusion of plantain. The daily milk volume of cows grazing the TF+L+P in summer was 26% greater than the other three treatments, although this did not translate into greater daily MS production. The only significant pasture treatment effect was a 12% decrease in milk fat content for the cows grazing pastures with plantain during summer and a 3% decrease during spring.

The UN concentration from cows grazing plantain pastures was significantly less than from those grazing non-plantain pastures in both summer (38% lower) and spring (21% lower, Figure 1). When the data are adjusted for urine volume (i.e. creatinine-corrected) to produce a proxy for daily N excretion, this was also 38% lower in summer, indicating that the cows were excreting less N in total (Figure 2). In spring, the creatinine-corrected N concentration differences were not significant, indicating that total daily N excretion was similar, but diluted by greater water intake when grazing plantain. This seasonal difference may be an important consideration, depending on the period of high N leaching risk.

During early lactation, two herds of 10 cows spent 5 days break-grazing a single paddock of either the PR+L or PR+L+P treatments, while wearing urine sensors (AgResearch MkII[9]). While each herd had a similar mean daily UN output, in the PR+L+P treatment this was spread across a greater urine volume (more urination events and greater volumes), resulting in a 44% lower UN concentration (Table 3). The increase in urine volume may be a result of the diuretic effect of plantain, which has been reported in sheep[10]. Dilution of UN should decrease the risk of N leaching, given that the number of urine patches with very high concentrations of soil nitrate will be reduced, allowing plants to take up a greater proportion of excreted nitrogen[11]. A similar grazing trial with urine sensors at Lincoln, where the proportion of plantain in the pastures was 16-21%, did not detect a significant difference in UN output or concentration (Bryant et al., unpublished report). Since the latest Waikato trial showed an effect at 26% plantain in the pastures, it appears that cows must consume pastures with >25% plantain content to achieve the desired effect of lower UN concentration.

Implications

While the inclusion of plantain in pasture mixtures has been shown to have a positive effect on DM production and urinary N concentration, it appears the content of plantain in the pasture must be above a critical level. This level will be verified in future animal feeding trials in the FRNL programme. Another key information gap that remains is how to establish and manage pastures containing plantain to achieve and maintain this desired level, and whether this applies to the whole year or just the

Figure 1: Urinary nitrogen concentration (UN%) of cows grazing four pasture types in summer 2016 (mid-lactation) and spring 2016 (early lactation). The results show no effect of the base pasture grass but a consistent effect of including plantain on reduced UN%. Bars indicate standard errors. PR = perennial ryegrass; TF = tall fescue; L = lucerne; P = plantain.

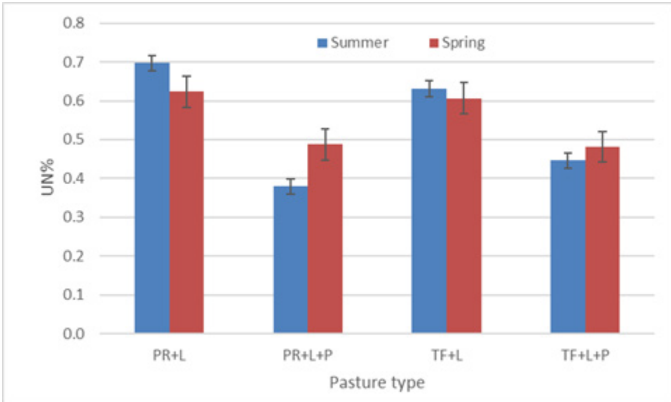

Figure 2: Creatinine-corrected urinary nitrogen excretion (a proxy for daily N excretion) of cows grazing four pasture types in summer 2016 (mid-lactation) and spring 2016 (early lactation). The results show no effect of the base pasture grass but a significant effect of including plantain on reduced total N excretion in summer only. Bars indicate standard errors. PR = perennial ryegrass; TF = tall fescue; L = lucerne; P = plantain.

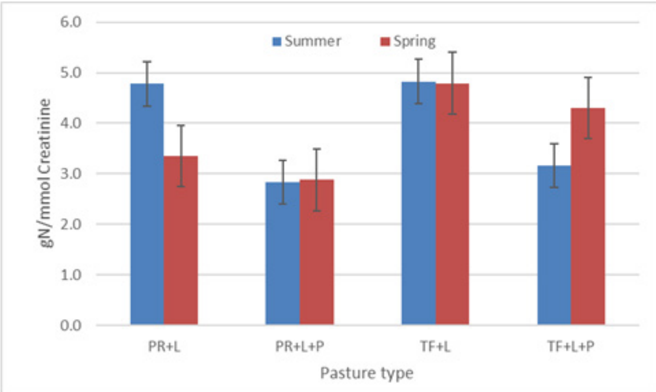

Table 3: Urine sensor measurements on two herds of lactating cows grazing two pasture types in the Waikato, November 2016. The results show greater urine volumes and urination frequency on the plantain pastures, leading to reduced UN concentration. Different letters following the data indicate significant differences between pasture types. PR = perennial ryegrass; L = Lucerne; P = plantain.

Urine measurement	PR+L	PR+L+P
Mean daily N excretion (g/d)	183	178
Mean urine volume (L/d)	31 b	51 a
Mean number of urination events (#/d)	11 b	15 a
Mean UN concentration (%)	0.62 a	0.35 b

period immediately preceding and during periods of high risk of nitrogen leaching.

The benefits of mixed-species pastures for milk production are less clear. Although some studies have demonstrated significantly greater MS production from diverse pastures that have included plantain and chicory[4,7], others have not[3,5,6]. The results from the latest Waikato research did not show any difference in MS production when cows grazed pastures including plantain.

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Nutrition for the transition cow's immune system

Treating, or attempting to prevent disease, is fact-of-life for dairy farmers, whether dealing with their cows or themselves. Many diseases are infectious, meaning they are caused by pathogenic organisms that can be transferred between individuals of the same species and, in some instances, may be transferrable between species including from cow to staff or staff to cow.

Key findings

- Around calving the cow's innate and adaptive immune system undergo changes that increase her risk to get an infectious disease.
- Nutrition and BCS management can help to support the immune system and mitigate the risk to contract infectious disease after calving:
- A BCS 5 at calving is optimal for immune health.
- Feeding 75% of requirements pre-calving in adequately conditioned cows (BCS 5) is optimal for immune health.

Several classes of infectious organisms affect dairy cows, including. Viruses, such as bovine viral diarrhoea virus causing BVD, a common contributor to poor fertility, and the much feared FMD (Foot and Mouth Disease)

- Bacteria, such as Staphylococcus aureus, often a cause of clinical mastitis as well as skin infections
- Fungi, such as Trichophyton verrucosum causing Ringworm
- Protozoa, such as Theileria orientalis, which caused the major anaemia problem in the north of the North Island a couple of years ago
- Nematodes, such as Dictyocaulus viviparus the lungworm that affects calves

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It is important to distinguish between infectious diseases that are contagious, and diseases cause by pathogens that may be sourced from the environment and are not 'transmitted'.

Fortunately, evolution has provided animals with defences against both types of infection. The skin as well as mucosal surfaces, such as those inside the lung, the gut, the udder and the uterus, provide physical barriers to stop invasion. If these barriers are damaged and pathogens invade tissues, the first of two parts of the immune system is activated. This is the innate immune response, which is mostly localised and non-specific. It is inflammation, usually seen as redness, pain, and swelling. Later, when the body has 'recognised' the cause of the problem in some way, the adaptive immune response takes over (more later; Figure 1).

By far, most diseases in dairy cows are seen around calving time, also known as the transition period. At calving, cows transition from dry and then into lactation. During this period 30 to 50% of dairy cattle can be affected by of some form of infectious and/or metabolic disease[1]. The transition period is a high-risk time, mostly because of extra exposure to pathogens, and because of the huge hiatus in bodily function at calving; nutrition is being changed, and the uterus and mammary gland undergo huge changes. No part of the cow is stressed more than the immune system. How nutrition influences these changes has been the focus of recent study in New Zealand that aimed to:

- Understand the changes in function of the immune system over the calving period and thus how to help manage infection.
- Determine if nutritional management (pre-calving feeding level and calving body condition score [BCS]) can improve immune function and give a better response to the initial infection.

Innate immunity and inflammation

The 'innate' arm of the immune system is non-specific, targeting any foreign intruder. When it works properly, initial infections should not establish and go on to cause disease.

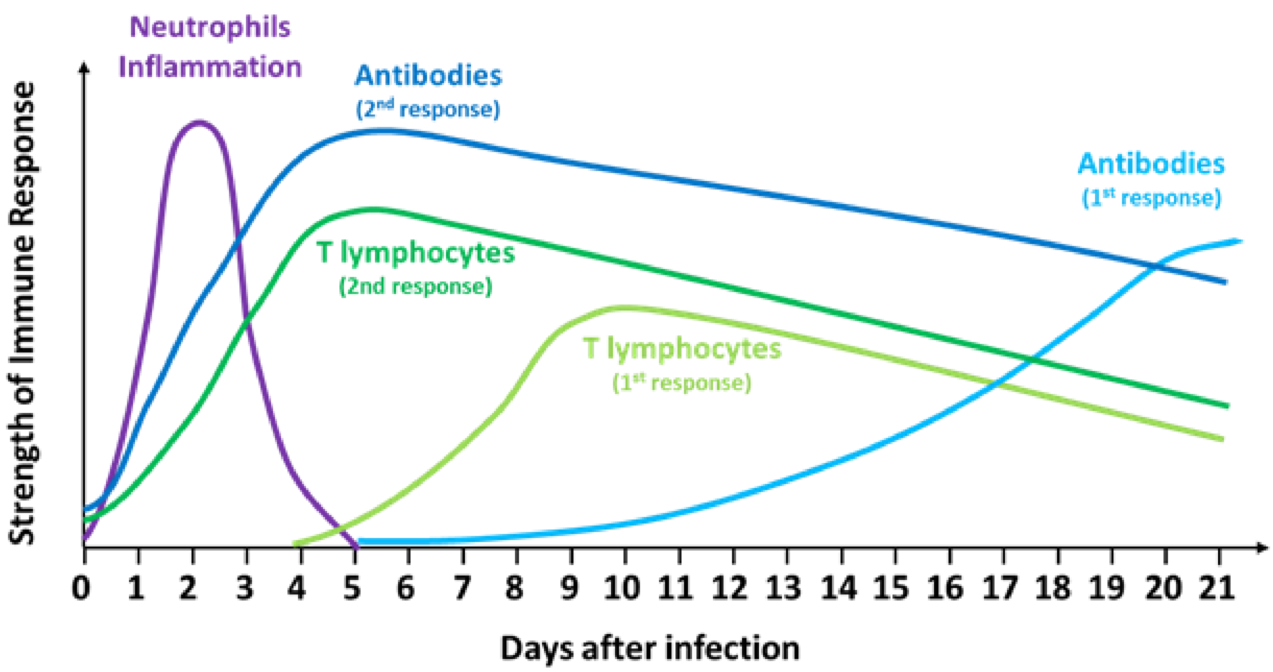
The innate immune response works as a rapid first defence against the invading pathogen(s), using 'weapons' such as antimicrobial substances. Inflammation occurs, which can isolate the affected area and result in poor growth conditions for the pathogens and results in rapid accumulation of 'killer' cells, from the blood to the infection site (Figure 1).

The function of inflammation is to eliminate the pathogen, remove damaged and dead tissue cells, and initiate tissue repair. Although inflammation is seen as a localised response, the process is very complex and includes a body-wide alert to make and recruit more white blood cells. The most common white blood cells of innate immunity are neutrophils (also known as polymorphonuclear cells or PMN). Neutrophils are the predominant cell type counted in a somatic cell count (SCC), which gives an indication of the severity of mastitis.

Mastitis is a good example of how the innate response works. Neutrophils exist in the udder naturally, usually no more than 100,000 cells/mL in a healthy udder. When infection occurs the number increases rapidly by recruitment from the blood. The cells release antibacterial signals that inflame the tissue (a bit like the response to an insect bite) and they engulf bacteria. Normally this happens quickly, maybe between milkings or over a very few days. If effective, a 'full blown' infection is avoided and no signs are ever noticed. When the immune system is compromised, as in the transition period, the balance swings to the pathogen and disease results. Neutrophil function is being studied extensively during the transition period due to the important role of neutrophils in fighting infection and previous international research that indicated the number of neutrophils and their activity was reduced during this high risk period[2].

Our work demonstrates that neutrophils from transition cows are impaired such that their capacity to even reach the site of an infection is reduced[3]. Therefore, if an infection occurs around calving, the innate immune response will likely be slower and less effective, increasing the potential for full blown disease to occur.

Figure 1: This figure shows the timing for innate immune responses (neutrophils) and inflammation, primary (1st) adaptive responses and secondary (2nd) 'memory' responses by T lymphocytes and antibodies.



Adaptive immunity

The 'adaptive arm' of the immune system is highly specific utilising specialised cells (T lymphocytes, another type of white blood cell) and antibodies that are 'programmed' to lock onto and destroy the particular pathogens causing the disease. Being so specific makes T lymphocytes and antibodies very effective killers, but this programming takes time, usually one to two weeks (Figure 1), so this part of the response is actually dealing with disease and not simply the primary infection. The benefit of the adaptive immune system, however, is that it has a 'memory' that usually provides some long-term immunity. 'Memory cells' are created and programmed to provide a much faster response within hours of a re-infection or similar new infection (Figure 1). This memory function of the adaptive immune system can be induced by vaccination. Vaccines use an infectious agent that has been rendered ineffective, or something not infectious that resembles the pathogen or maybe a particular set of proteins from a pathogen to teach the immune system what the intruder 'looks' like but without the risk of disease. Should the pathogen later invade the body, the adaptive immune system is armed and ready to respond. Vaccinations are one of the big success stories of science, not only influencing disease prevalence in humans but also in animals. The viral disease, cattle plague known as rinderpest, has been eradicated worldwide thanks to a vaccine. For dairy cattle, leptospirosis, blackleg, BVD, husk and several other diseases, mostly viral, can be controlled effectively with whole herd vaccination programmes.

Transition cows are often considered to be 'immunosuppressed' which means they are unable to mount a full adaptive immune response[4]. This reduces their ability to fight off infections common after calving, such as metritis and mastitis.

Our study confirmed that New Zealand cows are immunosuppressed during the transition period. Their T lymphocytes have a diminished ability to produce some signalling molecules (known as cytokines) shortly after calving[5]. Cytokines aid cell to cell communication during an immune response, stimulating movement of cells towards the affected area. If this function is compromised then the adaptive immune response is slower and less effective, increasing the likelihood of disease.

So what are the probable causes of immunosuppression?

Negative energy balance is associated with metabolic disorders and disease susceptibility.

Both innate and adaptive immunity are tightly regulated and interact with each other. They are influenced by the inflammatory status as well as the metabolic status of the cow. During the transition period, and particularly shortly after calving, dairy cows undergo dramatic physiological changes. The endocrine system needs to change from producing hormones associated with 'pregnant' to a 'non-pregnant' state, then going into heat etc. The cow's metabolism needs to switch from nourishing the foetus to producing milk. At calving, a cow quickly needs more nutrients, especially a large amount of energy. Since the cow simply cannot eat enough to satisfy the energy requirements, transition cows enter into a state of severe negative energy balance in early lactation. Negative energy balance is associated with an increased risk of metabolic disorders such as ketosis and milk fever and higher susceptibility to infectious diseases (i.e., mastitis, metritis). These diseases then lead to reduced reproductive performance and decreased productivity[6].

Rate of gain of BCS pre-calving influences the degree of metabolic inflammation

Apart from acute, infection-related inflammation, there is also a chronic low-grade inflammation within the metabolic tissues, often referred to as metabolic inflammation. We now know that in the days around parturition (birth), nearly all cows experience some level of metabolic inflammation in liver and fat tissue, but this is also evident body-wide. The degree of this postpartum inflammation has been linked to increased risk of disease and lower whole-lactation milk production[7]. The strength of this metabolic inflammation and its duration varies significantly. So far, little is known about which organs are key initiators and what signalling molecules are responsible for systemic and tissue-specific inflammation.

Our research has revealed that fat tissue is involved in inflammatory and immune responses[8]. It has demonstrated that cows that gained body condition score (BCS) slowly during the dry period had higher concentrations of non-esterified acids (NEFA) in blood than cows that gained BCS quickly[9]. Excess NEFA can initiate inflammatory signalling and immune cell infiltration and so contribute to this problematic inflammation in metabolic tissues.

Calving BCS determines transition cow health risk

BCS has an influence on reproduction and milk production. DairyNZ science has clearly demonstrated that cows are healthiest if they calve between BCS 4.5 and 5[10,11]. If thinner than BCS 4.5, cows have poorer immune function and are more at risk of a uterine infection. However, fatter cows (i.e., BCS 5.5 or higher) have an increased risk of ketosis and other metabolic diseases, particularly if they were fed high quality feed in the weeks before calving (i.e., consuming more than 100 MJ metabolisable energy/day). These metabolic diseases are also known to increase the risk of uterine infections and mastitis. Therefore, the cow's BCS at calving determines the effect of transition cow nutrition on the incidence of disease.

Nutritional management

Liver function and consequent metabolic and inflammatory status of the transition dairy cow can be influenced by nutrition management pre-calving. From the 1920s, it has often been recommended to begin to 'steam-up' dairy cows several weeks before calving for a successful transition from gestation to lactation[12]. From research conducted in New Zealand we now know that when dry matter intake is intentionally reduced before calving, the resulting slight negative energy balance improves post-calving energy balance, metabolic health, immunological performance, and, in some instances, milk production[11,13,14].

In a recent study, we aimed to improve the function of immune cells using common management techniques. We investigated the function of neutrophils and T lymphocytes between cows:

- With a calving BCS 4 vs BCS 5.
- Offered 75% vs 125% of maintenance requirements (at BCS 5).

The results demonstrate that there is no overt adverse effect on immune function when calving between BCS 4 and 5 and confirms the industry guidelines that calving at BCS 5 is best for cow health (including reproduction and production benefits). Furthermore, a feeding restriction pre-calving (to 75% of maintenance requirement) in adequately conditioned cows (BCS 5) is better for immune health than overfeeding pre-calving.

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Science snapshot

Cells send each other messages to tell each other how they're feeling![1]

Exosomes are very small particles (nanoparticles: one thousand-millionth of a meter) that are produced by every cell in the body; they carry a cargo to inform other cells around the body about the state of the tissue of origin. These proteins then influence how other tissues in the body perform. In this way, exosomes can be both biomarkers of tissue health and function and can be used to influence how tissues act.

Because of these unique features, exosomes have become one of the major areas of research in human medicine; for example, they can inform doctors of a disease state (e.g., Alzheimers), when a tissue is growing uncontrollably (i.e., cancer), and can be used to deliver a cure to treat the disease state (i.e., by providing a protein signal to stop cells dividing).

The major animal health issues in dairy cows happen around calving; 90% of metabolic diseases and 75% of infectious diseases occur during the period from a week before calving to three weeks post-calving. We wanted to determine if exosomes in blood informed us about the health status of the cows.

We measured the concentrations of exosomes and their protein cargo in the blood of dairy cows during the transition between pregnancy and lactation. The results indicate that the exosomal protein cargo is different in cows who are on the verge of having a metabolic disease when compared with cows whose metabolic profile is very healthy. This means that exosomal proteins could potentially be used to diagnose animals that aren't coping with metabolic stress before they are symptomatic, leading to earlier treatment and, thus, improved health and productivity of farm animals.

This area of science is very new for farm animal studies, but we can build on the discoveries being made in human medicine to accelerate our understanding of cow health and to identify solutions for the benefit of dairy farmers.

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