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# Lactation curves for milk, fat and protein in dairy cows under regenerative versus conventional farming practices

Yaliska Moreno-González <sup>a,b</sup>, Nicolas López-Villalobos <sup>a</sup>, Danny Donaghy<sup>a</sup>, Ignacio F. López<sup>a</sup>, Alastair MacGibbon<sup>c</sup> and Stephen E. Holroyd <sup>c</sup>

<sup>a</sup>School of Agriculture and Environment, Massey University, Palmerston North, New Zealand; <sup>b</sup>Universidad de Panamá, Facultad de Ciencias Agropecuarias, Panamá, Panamá; <sup>c</sup>Fonterra Research & Development Centre, Palmerston North, New Zealand

## ABSTRACT

Regenerative agriculture aims to utilise more diverse pasture species and enhance animal performance through sustainable soil management and pasture quality. This study evaluated the influence of regenerative and conventional farming on dairy cow performance and milk production under different pasture mixes and management strategies. Monthly herd test records were used to model individual lactation curves for daily milk, fat, and protein yield for the 2022–2023 season using random regression with third-order orthogonal polynomials. Total yields were calculated from predicted daily yield. Treatments were SPCM: Standard pasture under conventional management, DPCM: Diverse pasture mix under conventional management, and DPRM: Diverse pasture mix under regenerative management. Total milk yield was similar across treatments, averaging 3370 kg (SPCM), 3649 kg (DPCM), and 3626 kg (DPRM) for the 2022–2023 season. No significant differences were observed in fat, protein, milk solids yield, or milk composition. Cows on diverse pastures, regardless of management approach, showed heavier liveweights than those on standard pastures. DPCM and DPRM cows averaged 474 kg, significantly greater than SPCM cows at 464 kg ( $P < 0.0001$ ), likely due to longer grazing rotation and higher post-graze mass. These findings suggest that pasture species diversity, regardless the management, enhances liveweight without affecting milk composition.

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## Introduction

The sustainability of our global food production systems has become an increasingly critical issue. As agriculture continues to push planetary boundaries, there is a pressing need to explore innovative farming practices that not only sustain but regenerate our ecosystems (Campbell et al. 2017; Broom 2019). In response to the growing demand for sustainable agriculture, regenerative farming practices are emerging as a feasible possibility.

**CONTACT** Yaliska Moreno-González  ygonzale@massey.ac.nz

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Introduced by the Rodale Institute in the 1980s and rejuvenated in recent years, regenerative agriculture practices aim to enhance soil health, increase biodiversity, and reduce environmental footprints (Grelet et al. 2021). This system aims to minimise synthetic inputs, e.g. synthetic fertilisers, pesticides, growth regulators, and feed additives to enhance agricultural productivity (Botinggo et al. 2021) and promote diverse pasture regimes that benefit both the soil and the grazing animals (Rowarth et al. 2021). In contrast, conventional farming practices, while efficient, use high-input methods that may not be sustainable long-term in some regions and typically rely on limited number of pasture species (Distel et al. 2020; Rowarth et al. 2021).

The New Zealand dairy industry predominantly operates on grass-based systems, where pasture management and farming systems are integral to dairy performance with effective pasture management that not only lowers costs but also boosts farm profitability by maximising grassland utilisation (Finneran et al. 2010; Dillon et al. 2005). Therefore, with an increasing demand for sustainable practices, regenerative farming has recently gained attention among farmers and scientists (Grelet et al. 2021; Giller et al. 2021). The utilisation of a high number of pasture species diversity is a regenerative practice that not only improves soil ecosystems but also can provide cows with a variety of nutrients, which probably enhance their health and productivity (Rowarth et al. 2021). Recent studies have shown significant improvements in milk yield and quality when cows graze on diverse pastures compared to standard pastures (Wilson et al. 2020; Loza et al. 2021). However, there remains a gap in the scientific consensus both within New Zealand and globally, regarding the benefits that drive regenerative practices, particularly concerning animal performance and productivity.

The nutritional quality of milk is influenced by intrinsic animal factors and external environmental conditions, including breed, lactation stage, management practices, dietary regime, and seasonal feed availability (Auldist et al. 1998; O'Brien and Guinee 2011; Alothman et al. 2019; MacGibbon 2020). Furthermore, the interaction of these factors plays a critical role in determining the nutritional profile of the produced milk. To better understand how these factors influence milk production, lactation curve provides a display of the daily milk production pattern throughout a dairy cow lactation period (Wood 1967). Generally, this curve is segmented into three phases: an initial increase from calving to the peak milk yield, a stable phase where this peak yield is maintained, and a decline phase where milk production gradually decreases after reaching the peak (Gengler 1996). Analysing the lactation curves offers valuable insights into milk production patterns and the health of dairy cows under different farming conditions.

This study aims to bridge a significant gap in comprehensive comparative data by examining how different farming systems, specifically regenerative versus conventional practices, influence overall milk production and its nutritional composition. It hypothesises that the diversity of pasture species in regenerative farming systems may lead to changes in intake and nutrient supply, which could enhance both milk yield nutritional quality. These benefits are expected likely due to the combined effects of diverse dietary intake and regenerative practices. The objective of this study was to model the lactation curves for daily yields of milk and milk composition, in dairy cows across both farming systems in New Zealand, covering an entire lactation period.

## Materials and methods

### Experimental site, animals, and treatment

This study is part of the Whenua Haumanu project, led by Massey University. The data analysis included herd test records from 72 dairy cows at Massey University Dairy Farm No. 1 in the lower North Island of New Zealand. The group was composed of 23 Friesians (F), 25 Jerseys (J), and 24 Friesian-Jersey crosses (F $\times$ J) cows, all calving in the spring dairy season of 2022–2023.

The farm operates a low-input system, characterised by a pasture-based feeding approach with minimal supplementary feed, reduced fertiliser use, and lower stocking rates, with cows milked once-a-day (OAD) throughout the season. The study took place on 36 hectares divided into three 12-hectare farmlets, each comprising 24 cows with a stocking rate of 2.0 cows ha<sup>-1</sup>. Each farmlet had distinct sown multi-species pasture mixes and management strategies: **SPCM**: standard pasture under conventional management, consisting of a traditional mix of perennial ryegrass (*Lolium perenne*), Italian ryegrass (*Lolium multiflorum*), red clover (*Trifolium pratense*), and white clover (*Trifolium repens*); **DPCM**: diverse pasture mix under conventional management, and **DPRM**: diverse pasture mix under regenerative management.

The diverse pasture treatment included the standard pasture species along with additional grasses such as tall fescue (*Lolium arundinaceum*), cocksfoot (*Dactylis glomerata*), phalaris (*Phalaris aquatica*), and prairie grass (*Bromus willdenowii*); herbs like chicory (*Chicorium intybus*) and plantain (*Plantago lanceolata*); a variety of clovers: red, white, crimson (*Trifolium incarnatum*), balansa (*Trifolium michelianum*), persian (*Trifolium resupinatum*), subterranean (*Trifolium subterraneum*); and other legumes like lucerne (*Medicago sativa*).

The soil profile consisted of alluvial soils, loamy gravels, sandy loam, and loamy sands. Milking occurred daily at 8:30 am, followed by the allocation of fresh pasture at a daily rate of approximately 17 kg DM cow day<sup>-1</sup>. Supplementary feeding during early lactation (< 90 days in milk) included maize silage (an average of 1.6 kg DM cow day<sup>-1</sup>). During mid lactation (90–180 days in milk), a summer crop was provided (4.3 kg DM cow day<sup>-1</sup> on average; see Table 1 for crop details). Baleage was offered in April and early May (an average of 4.4 kg DM cow day<sup>-1</sup>), and haylage (an average of 3.5 kg DM cow day<sup>-1</sup>) during late lactation (>181 days in milk; Table 1). For the 2022–2023 season, the median calving dates across different treatments were August 2<sup>nd</sup> for SPCM, August 7<sup>th</sup> for DPCM, and August 4<sup>th</sup> for DPRM, with the dry-off date for all treatments being May 24<sup>th</sup>.

### Apparent DM intake and pasture nutritive value

Group herbage dry matter intake (DMI) was estimated by determining pre- and post-grazing pasture mass using random quadrat samples (0.1 m<sup>2</sup>) collected with an electric shearing handpiece. The calculations were made as per Rivero et al. (2013); Ström (2012) and Wilson et al. (2020):

$$\text{DMI} = \frac{[\text{Pre mass (kg DM ha}^{-1}\text{)} - \text{Post mass (kg DM ha}^{-1}\text{)}] \cdot \text{Area (ha)}}{\text{No. of animals}}$$

**Table 1.** Dietary and chemical composition (Mean  $\pm$  SD) of feed offered during the 2022–2023 production season.

Item	Dietary treatments <sup>1</sup>			Silage	Baleage	Haylage
	SPCM	DPCM	DPRM			
Ingredient, kg DM cow <sup>-1</sup> day <sup>-1</sup>						
Pasture <sup>2</sup>	14.8	16.2	16.6			
Maize silage <sup>3</sup>	1.6	1.6	1.6			
Crop <sup>4</sup>	4.3	4.3	4.3			
Baleage <sup>5</sup>	4.4	4.4	4.4			
Haylage <sup>6</sup>	3.5	3.5	3.5			
Chemical composition						
n =	22	21	22	4	2	2
CP, % of DM	21.8	21.7 $\pm$ 2.5	21.2 $\pm$ 4.1	14.1 $\pm$ 4.5	14.9 $\pm$ 2.2	10.8 $\pm$ 3.1
NDF, % of DM	39.7	37.4 $\pm$ 3.5	38.3 $\pm$ 5.5	44.3 $\pm$ 5.3	50.2 $\pm$ 4.5	60.0 $\pm$ 20.4
ADF, % of DM	22.4 $\pm$ 2.5	21.8 $\pm$ 1.7	22.7 $\pm$ 2.7	30.7 $\pm$ 6.3	37.6 $\pm$ 3.1	36.1 $\pm$ 11.4
SS, % of DM	9.3 $\pm$ 2.2	9.3 $\pm$ 2.4	9.0 $\pm$ 2.8	2.1 $\pm$ 1.2	3.7 $\pm$ 0.0	4.4 $\pm$ 2.1
CF, % of DM	3.6 $\pm$ 0.4	3.4 $\pm$ 0.3	3.3 $\pm$ 0.4	3.1 $\pm$ 0.9	2.2 $\pm$ 0.0	1.3 $\pm$ 0.9
NSC, % of DM	24.8 $\pm$ 6.3	26.3 $\pm$ 3.4	26.2 $\pm$ 3.4	25.3 $\pm$ 14.0	15.8 $\pm$ 8.6	18.2 $\pm$ 9.4
DOMD, %	73.3 $\pm$ 4.9	73.2 $\pm$ 3.7	72.7 $\pm$ 5.1	59.3 $\pm$ 6.6	51.8 $\pm$ 4.6	46.7 $\pm$ 19.8
ME, MJ/kg of DM	11.7 $\pm$ 0.8	11.7 $\pm$ 0.6	11.6 $\pm$ 0.8	9.5 $\pm$ 1.1	8.3 $\pm$ 0.7	7.4 $\pm$ 3.2

<sup>1</sup>Dietary treatments = Standard pasture under conventional management (SPCM); Diverse pasture under conventional management (DPCM); Diverse pasture under regenerative management (DPRM).

<sup>2</sup>Values represent apparent dry matter intake.

<sup>3</sup>Silage = maize silage offered in winter.

<sup>4</sup>Summer crops = Turnips for SPCM; forage brassica, leafy turnip, diakon, chicory, jap millet, crimson clover, red clover for DPCM and DPRM.

<sup>5</sup>Baleage = baleage offered in autumn ( $\approx$  2 days for SPCM and DPCM;  $\approx$  12 days for DPRM). Baleage is partly dried forage, with 45–55% moisture and is baled and wrapped with at least 6 layers of 0.025 mm plastic.

<sup>6</sup>Haylage = hay offered in early winter ( $\approx$  10 days).

CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; SS = soluble sugars; CF = crude fat; NSC = non-structural carbohydrate; ME = metabolizable energy.

Nutritive value was analysed by a commercial laboratory using near-infrared (NIR) spectroscopy, following standard protocols (Hill Lab 2021).

### **Milk production and composition**

Herd test records (n = 638) of daily milk yield and composition, including total fat, total protein, and somatic cell count (SCC, cells/ml) were used. The yield of milk solids components (fat and protein) on each test day was calculated by multiplying the milk volume by the concentration of milk solid components. The somatic cell score (SCS) was calculated as  $SCS = \log_2(SCC)$ . Milk composition was determined using Milkoscan FT1 (Foss Analytical, Hillerød, Denmark).

### **Liveweight and body condition score**

The average liveweight records (LW; n = 5,710), along with individual body condition score (BCS; n = 814), were recorded monthly after morning milking and before the cows were allocated to fresh pasture, from calving to the dry-off date during the 2022–2023 production season. The BCS was assessed by a single research technician using a 1–10-point scale (DairyNZ 2022).

## Modelling lactation curve in dairy cow

Lactation curves were modelled using a random regression with a third-order orthogonal polynomial (Kirkpatrick et al. 1990) for each cow-lactation combination. For the cow-lactation 'i' in days in milk 't' the equation is re-written as follow:

$$yti = (\beta_0 P_0 + \beta_1 P_1 + \beta_2 P_2 + \beta_3 P_3) + (\alpha_{0i} P_0 + \alpha_{1i} P_1 + \alpha_{2i} P_2 + \alpha_{3i} P_3) + e_{ti}$$

where  $\beta$  values are the fixed regression coefficients of the lactation curve of the population,  $\alpha$  values are the random regression coefficients describing the lactation curve for animal i, and  $e_{ti}$  is the random residual error for animal i at time t. The Legendre polynomials were chosen to standardise values to the interval  $[-1, \dots, 1]$ , and the coefficients  $P_j$  were then calculated as (Spiegel 1971):

$$P_{0(t)} = 1, P_{1(t)} = x, P_{2(t)} = \frac{1}{2}(3x^2 - 1) \text{ and } P_{3(t)} = \frac{1}{2}(5x^3 - 3x),$$

where  $x = -1 + 2 \left( \frac{(t - t_{\min})}{(t_{\max} - t_{\min})} \right)$ , with  $t_{\min} = 1$  and  $t_{\max} = 300$

Total lactation yields for milk production variables for each cow-lactation were estimated using the polynomial equation, as the sum from day 1 to maximum days in milk (300 days). Similarly, average of SCC, LW and BCS were computed as the mean of these variables across lactation, using the same polynomial model.

## Statistical analysis

Regression coefficients of the third-order Legendre polynomial for each cow in each lactation, total lactation yields of milk, fat, protein, mean SCC, LW, and BCS were analysed using the MIXED procedure of SAS version 9.4 (SAS Institute Inc.). The mixed linear model included fixed effects of treatment, breed, and lactation number, as well as the interactions between treatment and breed, treatment and lactation number, and breed and lactation number, and the linear effect of days from the median calving date. Least-squares means, and standard errors were obtained and used for multiple mean comparisons using Fisher's least significant difference test.

## Results

### Intake, pasture quality, and supplements

The apparent dry matter intakes are detailed in Table 1, with the DPRM treatment showing a slightly greater pasture dry matter intake compared to the other treatments. Average chemical composition of pastures and supplementary feeds is summarised in Table 1. Pasture treatments (SPCM, DPCM, DPRM) had similar metabolizable energy (ME) and DOMD values, indicative of high-quality pastures. Supplementary feeds such as maize silage, baleage, and haylage had varying levels of CP and DOMD, aligning with typical nutritional ranges. Specifically, maize silage exhibited expected ME and CP levels, while baleage and haylage had CP and DOMD values within or slightly below normal ranges.

## Average production

Descriptive statistics of total milk yield and milk components analysed in this study are detailed in Table 2. On average, cows yielded 3,596 kg of milk, with a fat yield of 199 kg and a protein yield of 155 kg per cow during the whole lactation period (300 days).

## Regression coefficients and factors influencing lactation curves

Significant differences in the regression coefficients of the third-order Legendre polynomial were observed among treatments (Table 3), indicating distinct lactation curve shapes for cows under different pasture and management strategies. The ANOVA for these coefficients shows significant differences in the lactation curves for milk yield, fat yield, protein yield (Figure 1), LW, and BCS (Figure 2). The regression coefficients not only indicate differences in the intercept ( $\alpha_0$ ) but also in the other coefficients ( $\alpha_1, \alpha_2, \alpha_3$ ). The lactation curves for milk yield indicate that cows under SPCM start at a lower production level and has a flatter curve with less steep increases and decreases over time.

In contrast, cows under DPCM and DPRM showed greater fluctuations in their lactation curve shapes. Similarly, the fat yield and protein yield curves follow the same trend, with SPCM showing lower initial values. Cows under SPCM were 8 and 9 kg lighter than those under DPCM and DPRM, respectively, which exhibited greater dynamic fluctuations over time (Figure 2).

Factors influencing milk production and composition traits (Table 4) indicate significant effects of breed and lactation stage. Breed significantly affected milk yield and fat content, while the lactation number strongly influenced fat yield and milk solids yield.

Total milk production and composition variables for different treatment groups are presented in Table 5. Milk production was similar across treatments, with total yields for SPCM, DPCM, DPRM recorded at 3,370, 3,649, and 3,626 kg, respectively. There were no significant differences in fat yield, protein yield, and milk solids yield across

**Table 2.** Descriptive statistics for the total lactation records of milk production variables of dairy cows grazing on standard pasture under conventional management, diverse pasture mix under conventional management, and diverse pasture mix under regenerative management during the 2022–2023 production season.

Trait	Mean	SD <sup>1</sup>	Min	Max	CV <sup>2</sup> (%)
Milk production					
Milk yield, kg	3,596	809	2058	5259	22.5
Fat yield, kg	199.2	37.5	125.0	284.0	18.8
Protein yield, kg	155.4	33.9	86.0	240.0	21.8
Milk solids yield, kg	354.6	70.3	215.0	524.0	19.8
Components					
Fat, %	5.61	0.58	4.15	6.90	10.3
Protein, %	4.33	0.3	3.8	4.9	6.1
Average SCS <sup>3</sup>	6.10	1.35	1.58	13.04	22.2
Average LW <sup>4</sup> , kg	471	54.2	332	672	11.5
Average BCS <sup>5</sup>	4.67	0.4	3.5	6.0	9.5

Note: The values presented in this table are based on raw data, not from fitted lactation curves.

<sup>1</sup>SD = standard deviation.

<sup>2</sup>CV = coefficient of variation.

<sup>3</sup>SCS = somatic cell score, calculated as  $SCS = \text{Log}_2(\text{somatic cell count/ml})$ .

<sup>4</sup>LW = liveweight.

<sup>5</sup>BCS = body condition score on a 1–10 scale.

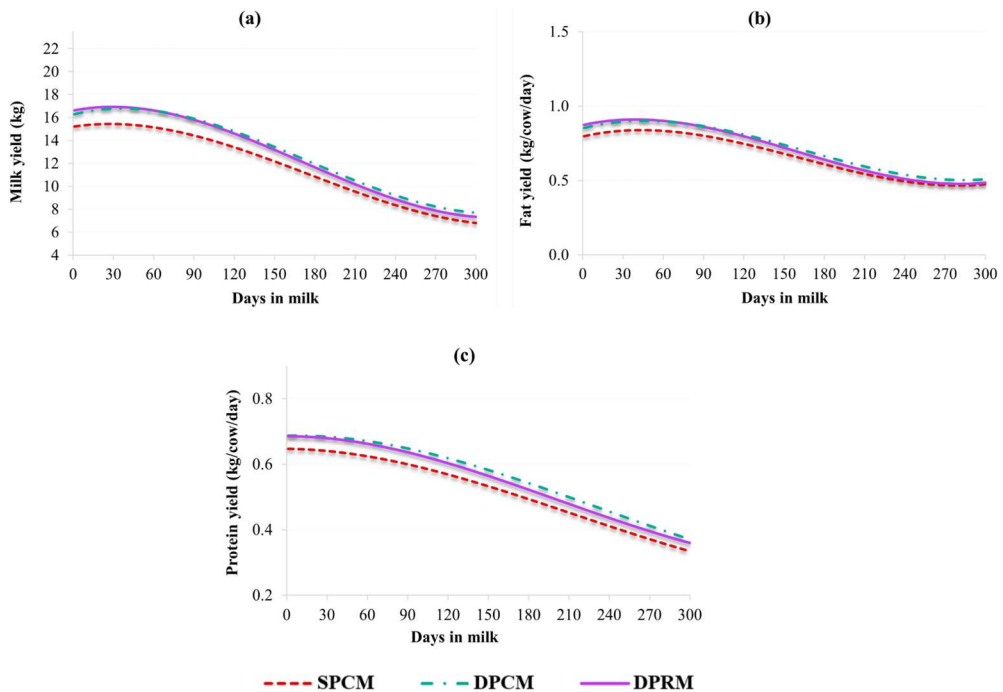
**Table 3.** Least squares means (Mean) and standard errors (SE) of estimates of regression coefficients of the lactation curves of productive parameters, modelled with a third-order orthogonal polynomial fitted to the milk production data from dairy cows grazing standard pasture under conventional management (SPCM), diverse pasture mix under conventional management (DPCM), and diverse pasture mix under regenerative management (DPRM) in the production season of 2022-2023.

Trait	Treatment	$\alpha_0$		$\alpha_1$		$\alpha_2$		$\alpha_3$	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Milk yield	SPCM	11.77 <sup>a</sup>	0.11	-5.11 <sup>a</sup>	0.11	-0.76 <sup>a</sup>	0.05	0.90 <sup>a</sup>	0.04
	DPCM	12.77 <sup>b</sup>	0.11	-5.46 <sup>b</sup>	0.11	-0.94 <sup>b</sup>	0.05	1.17 <sup>b</sup>	0.04
	DPRM	12.93 <sup>b</sup>	0.11	-5.77 <sup>c</sup>	0.11	-0.78 <sup>a</sup>	0.05	1.13 <sup>b</sup>	0.04
Fat yield	SPCM	0.664 <sup>a</sup>	0.01	-0.237 <sup>a</sup>	0.01	-0.028 <sup>a</sup>	0.002	0.076	0.003
	DPCM	0.718 <sup>b</sup>	0.01	-0.251 <sup>a</sup>	0.005	-0.038 <sup>b</sup>	0.002	0.079	0.003
	DPRM	0.706 <sup>b</sup>	0.01	-0.277 <sup>b</sup>	0.01	-0.026 <sup>a</sup>	0.002	0.082	0.003
Protein yield	SPCM	0.518 <sup>a</sup>	0.005	-0.169	0.005	-0.028	0.003	0.013	0.002
	DPCM	0.564 <sup>b</sup>	0.005	-0.171	0.005	-0.035	0.003	0.013	0.002
	DPRM	0.550 <sup>b</sup>	0.005	-0.179	0.005	-0.027	0.003	0.016	0.002
LW <sup>1</sup>	SPCM	457.85 <sup>a</sup>	1.87	33.31 <sup>a</sup>	1.20	21.80 <sup>a</sup>	1.36	-7.88 <sup>a</sup>	1.13
	DPCM	470.09 <sup>b</sup>	1.86	27.03 <sup>b</sup>	1.19	23.19 <sup>a</sup>	1.35	-7.87 <sup>a</sup>	1.12
	DPRM	468.05 <sup>b</sup>	1.90	27.79 <sup>b</sup>	1.22	28.39 <sup>b</sup>	1.39	-1.17 <sup>b</sup>	1.15
BCS <sup>2</sup>	SPCM	4.686	0.02	0.105 <sup>a</sup>	0.01	0.162 <sup>a</sup>	0.01	-0.070 <sup>a</sup>	0.003
	DPCM	4.668	0.02	0.075 <sup>b</sup>	0.01	0.198 <sup>b</sup>	0.01	-0.057 <sup>b</sup>	0.003
	DPRM	4.648	0.02	0.046 <sup>c</sup>	0.01	0.277 <sup>c</sup>	0.01	-0.029 <sup>c</sup>	0.003

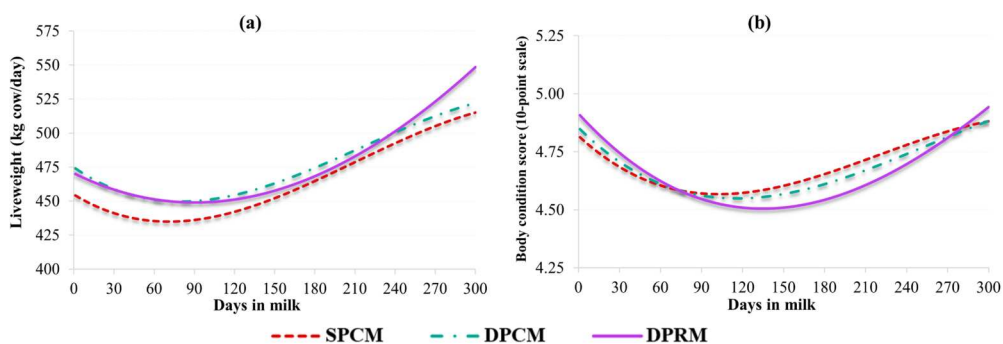
<sup>1</sup>LW = liveweight.

<sup>2</sup>BCS = body condition score on a 1–10 scale.

<sup>a,b,c</sup>Least-squares means with different superscripts within treatment are significantly different ( $P < 0.05$ ).



**Figure 1.** Lactation curves of daily yields of (a) milk, (b) fat, and (c) protein from dairy cows grazing standard pasture under conventional management (SPCM), diverse pasture mix under conventional management (DPCM), and diverse pasture mix under regenerative management (DPRM) during the 2022–2023 production season.



**Figure 2.** Lactation curves for (a) liveweight (LW), and (b) body condition score (BCS) of dairy cows grazing standard pasture under conventional management (SPCM), diverse pasture mix under conventional management (DPCM), and diverse pasture mix under regenerative management (DPRM) during the production season 2022–2023.

the treatment groups. Percentages of fat and protein were similar across treatments. Cows on diverse pastures (DPCM and DPRM) were 2% heavier compared to those on standard pastures (SPCM) ( $P < 0.0001$ ) (Figure 2). Averages of BCS were similar across grazing treatments (Figure 2). There were significant differences in the SCS trait among treatments ( $P < 0.05$ ). SPCM had higher SCS values compared to both DPCM and DPRM. The SCS value for SPCM was 5–6% higher than DPCM and DPRM. The difference between DPCM and DPRM was not significant.

## Discussion

### *Dairy cow performance and milk composition*

The current study examined the relative influence of regenerative agriculture practices, such as diverse pasture mixes on dairy cow productivity in New Zealand. Research on regenerative management systems for milk production remain scarce. Our findings show no significant differences in total milk yield among the treatment groups ( $P = 0.19$ ), consistent with studies by Ström (2012) and Carmona-Flores et al. (2020) who found no significant difference in overall milk yield between diverse and simple pastures. On the contrary, Wilson et al. (2020) observed greater yields for cows grazing on forb and legume pastures compared to grass ( $P = 0.01$ ), and Loza et al. (2021) reported increased milk yields for cows on diverse pastures compared to binary mixtures in two different periods (+4%,  $P < 0.01$ ).

Regarding milk composition, our study found no significant differences in total fat yield, protein yield, and milk solids yield across treatments. This contrasts with Wilson et al. (2020), who found greater milk fat and protein concentration in cows on legume and forb pastures. Carmona-Flores et al. (2020) reported 4.2% less fat and lactose content on average, and 1.7% less protein content, when pasture diversity increased. Loza et al. (2021) found greater milk solids (228 g/d,  $P < 0.05$ ) and protein yield (105 g/d;  $P < 0.01$ ) in cows grazing diverse pastures.

In our study, the lack of significant differences in total milk yield and composition may be partly due to the use of an OAD milking system, which typically results in lower yields

**Table 4.** F- and p-values of factors influencing milk production and composition traits in New Zealand dairy cows grazing standard pasture under conventional management, diverse pasture mix under conventional management, and diverse pasture mix under regenerative management in the production season of 2022–2023.

Trait	Factor													
	Treatment		Breed		Lactation		Treatment×Breed		Treatment×Lactation		Breed×Lactation		DCD <sup>1</sup>	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Milk production														
Milk yield, kg	1.5	0.2219	14.6	<0.001	31.2	<0.001	0.4	0.8338	1.4	0.2500	1.8	0.1676	0.2	0.6889
Fat yield, kg	0.9	0.3967	2.8	0.0681	23.2	<0.001	0.2	0.9293	1.2	0.3163	0.6	0.5421	0.2	0.6642
Protein yield, kg	0.8	0.4544	6.3	0.0033	25.7	<0.001	0.2	0.9246	1.1	0.3427	1.2	0.3044	0.9	0.3499
Milk solids yield, kg	0.9	0.4042	4.4	0.0163	25.3	<0.001	0.2	0.9210	1.2	0.3118	0.9	0.4211	0.5	0.4921
Components														
Fat, %	0.7	0.4696	19.9	<0.001	3.3	0.0757	0.2	0.9278	0.3	0.7325	1.4	0.2487	0.01	0.9137
Protein, %	0.7	0.4750	12.5	<0.001	0.04	0.8488	0.4	0.7688	0.2	0.8424	0.3	0.7389	3.9	0.0518
Average SCS <sup>2</sup>	4.95	0.0074	7.92	0.0004	5.77	0.0166	9.96	<0.001	6.48	0.0016	5.27	0.0054	5.15	0.0236
Average LW <sup>3</sup> , kg	25.9	<0.001	1840.3	<0.001	100.6	<0.001	51.9	<0.001	4.6	0.0099	16.33	<0.001	28.9	<0.0001
Average BCS <sup>4</sup>	0.2	0.8352	5.3	0.0051	89.8	<0.001	6.7	<0.001	6.7	0.0013	1.1	0.3273	11.9	0.0006

<sup>1</sup>Deviation from the median calving date.

<sup>2</sup>SCS = somatic cell score, calculated as  $SCS = \text{Log}_2$  (somatic cell count/ml).

<sup>3</sup>LW = live weight.

<sup>4</sup>BCS = body condition score on a 1–10 scale.

**Table 5.** Least squares means (Mean) and standard errors (SE) of total milk production and composition of dairy cows grazing standard pasture under conventional management (SPCM), diverse pasture mix under conventional management (DPCM), and diverse pasture mix under regenerative management (DPRM) during the 2022–2023 production season.

Trait	Treatment						P-value
	SPCM		DPCM		DPRM		
	Mean	SE	Mean	SE	Mean	SE	
Milk production							
Milk yield, kg	3370	124.7	3649	122.1	3626	127.4	0.2219
Fat yield, kg	188.6	6.8	201.4	6.7	198.3	7.0	0.3967
Protein yield, kg	147.1	5.9	157.3	5.8	154.5	6.0	0.4544
Milk solids yield, kg	335.6	12.5	358.5	12.3	352.9	12.8	0.4042
Components							
Fat, %	5.70	0.1	5.59	0.1	5.53	0.1	0.4696
Protein, %	4.37	0.05	4.32	0.05	4.30	0.05	0.4750
Average SCS <sup>1</sup>	6.36 <sup>a</sup>	0.1	6.05 <sup>b</sup>	0.1	5.97 <sup>b</sup>	0.1	0.0074
Average LW <sup>2</sup> , kg	465 <sup>a</sup>	1.0	473 <sup>b</sup>	0.9	474 <sup>b</sup>	0.9	<.0001
Average BCS <sup>3</sup>	4.71	0.02	4.71	0.02	4.69	0.03	0.8352

<sup>1</sup>SCS = somatic cell score, calculated as  $SCS = \text{Log}_2(\text{somatic cell count/ml})$ .

<sup>2</sup>LW = liveweight.

<sup>3</sup>BCS = body condition score on a 1–10 scale.

<sup>a,b,c</sup>Least-squares means with different superscripts within effect are significantly different ( $P < 0.05$ ).

compared to TAD systems (Lopez-Villalobos et al. 2023). Studies have shown that OAD milking can reduce milk yield by 22–50%, depending on lactation stage and breed (Holmes et al. 1992; Lopez-Villalobos et al. 2023), while often increasing fat and protein percentages due to less frequent milking (O'Brien et al. 2002).

While our study provides insights into regenerative practices under OAD milking, it is important to note the significant methodological differences between our approach and TAD-based studies (e.g. Ström 2012; Wilson et al. 2020; Carmona-Flores et al. 2020; Loza et al. 2021). Twice-a-day milking systems generally produce greater milk yields and involve different nutrient dynamics, which may not align with OAD outcomes. Additionally, differences in pasture management and forage diversity further complicate these comparisons, underscoring the need for caution when comparing studies with different milking frequencies.

The average digestibility of organic matter (DOMD) was 73% in our study, which is higher than the typical digestibility for pasture (>65%; Ravensdown 2020; Hill Lab n.d.), indicating it was of good quality. This could be attributed to the similar nutritional profiles provided by the different pasture mix treatments, ensuring that cows received adequate high-quality diets for maintaining milk production. Soder et al. (2006) found that increasing complexity of forage mixtures (chicory and several species of grasses and legumes), did not increase milk production when compared to simpler mixtures. However, their study involved supplementing the pasture with 40% concentrates, which may have masked the potential benefits of diverse pasture mixtures alone.

### **Regression coefficients and factors influencing lactation curves**

Although no significant differences were observed in the total milk yield accumulated over the whole lactation period (300 days) between treatments (Table 5), the analysis of lactation curves using third-order Legendre polynomials revealed significant

differences in the regression coefficients. These findings suggest that the treatments differentially impact milk production dynamics over time. Specifically, the significant differences in regression coefficients indicated higher initial milk production levels for DPCM and DPRM compared to SPCM (Table 3). This suggests that variations in milk production at different stages of lactation are compensated for when considering the accumulated total yields. Unlike total yield means, the regression coefficients capture the dynamic changes over time, providing detailed information on how each treatment influences the lactation curve.

Although the average LW of the cows were similar at the beginning of this study, cows under SPCM had lower milk production in the intercepts and exhibited flatter lactation curves compared to DPCM and DPRM treatments, indicating more stable but generally lower production. This pattern suggests greater lactation persistency, likely due to the consistency of the standard pasture diet. Before the study began, all cows were on a standard pasture diet at Dairy 1. Cows under SPCM continued this familiar diet, likely resulting in a more stable metabolic state and production baseline. This consistency in diet may have contributed to steadier milk production and minimised metabolic fluctuations, supporting a persistent lactation pattern. In contrast, cows under DPCM and DPRM had greater initial yields and more pronounced variations in milk production traits, likely due to enhanced nutrient utilisation and the cow's productive response to the diverse pastures. These differences can be attributed to the nutritional benefits of greater species diversity, which provides a wider range of bioavailable nutrients, and likely to variations in apparent pasture DMI ( $\text{kg}\cdot\text{day}^{-1}$ ; Table 1). Cows under diverse pastures (DPRM and DPCM) ingested nearly 12% more pasture DM than those in SPCM, likely providing the nutrients needed for their greater milk yields. The DPRM cows produced 7.6% more milk than the SPCM cows and 0.6% less than the DPCM, despite DPCM's slightly higher mean yield. Species diversity in DPRM and DPCM pastures may enhance nutrient intake and absorption, directly contributing to the observed production differences. Additionally, diverse pastures not only increase the ingested herbage mass intake but also offer bioactive compounds (Van Vliet et al. 2021) that can improve digestive efficiency (Provenza et al. 2003; Makkar et al. 2007), overall cow health and grazing behaviours, thereby supporting greater milk production (Distel et al. 2020). These findings are further supported by Carmona-Flores et al. (2020), who observed similar improvements in nutrient utilisation on cows grazing diverse pastures.

The present study also highlights the significant effects of breed and lactation stage in milk production and composition traits (Table 4). These findings underscore the influence of genetic and physiological factors in dairy production supported by Auldist et al. (2004), Palladino et al. (2010), Lembeye et al. (2015) and Samková et al. (2018).

Lactation curves for daily milk, fat, and protein yield follow typical patterns (Figure 1), with milk yield peaking for the first three to five months of lactation and gradually declining until the end of lactation, in concordance with the literature (Holmes and Wilson 2003). However, the present study indicates that the shapes of these lactation curves vary significantly across different treatments, suggesting that pasture type and management system do influence production dynamics. Lembeye et al. (2016) reported similar early peaks and declines in lactation curves for different breeds in mixed pasture systems. Our study did not focus on breed differences, but the overall yields

were comparable across treatments, suggesting the lactation dynamics regardless of management system and diet.

### ***Liveweight, body condition score and somatic cell count***

A significant difference in LW was observed in cows under diverse pastures (DPCM and DPRM) compared to those on standard (SPCM;  $P < 0.0001$ ) (Figure 2). The heavier LW in our study could be attributed to the diversity in pasture species and grazing management imposed, specifically longer grazing rotations and higher post-graze pasture mass in the regenerative management. Post-graze pasture mass was 2,015 kg DM/ha in DPRM, compared to 1,863 kg DM/ha in DPCM and 1,866 kg DM/ha in SPCM. Despite lower post-graze pasture mass in one diverse pasture treatment (DPCM), cows achieved higher LW gains, possibly due to improved nutrient bioavailability and consistent access to high-quality forage, suggesting a more efficient utilisation of available nutrients.

Moreover, regenerative management practices, such as longer rotation lengths and higher post-graze pasture mass, may enhance soil health and plant growth (Gosnell 2021; Rowarth et al. 2021), resulting in nutrient-dense forages that could support better weight gain. These factors possibly contribute to better grazing behaviours and overall improvement, highlighting the benefits of diverse pastures in supporting cow LW gain. Our results align with Moscovici Joubran et al. (2021), who noted a 2–3% weight increase in cows on pasture-based systems versus total mixed ration systems. However, Loza et al. (2021) reported no significant variations in LW in diverse pastures.

Results of BCS in the present study were uniform across all treatment groups, similar to the findings of Wilson et al. (2020). This suggests that while LW was affected, the overall body condition remained stable, indicating that diverse pastures did not negatively impact the cows body reserves. Despite having lower LW, SPCM cows maintained higher body condition scores, suggesting a different allocation of energy and nutrients after meeting maintenance requirements. These cows may be using diet available resources to prioritise maintain body reserves rather than increasing milk production or LW. Typically, in dairy animals, early lactation is associated with greater nutrient partitioning towards milk synthesis at the expense of body reserves to meet the considerable demands of nutrients by the mammary gland (Bauman and Currie 1980). However, as lactation progresses, cows shift energy towards body tissue stores at the expense of milk production (Koenen et al. 2001; Yan et al. 2006), even when the feed is energy-dense and its composition remains consistent throughout lactation (Friggens and Badsberg 2007). The SPCM cows in this study might be following this metabolic pattern, which, combined with their lower DMI, likely limited the energy available for weight gain and milk production. Although the diet was consistent, the reduced energy intake in the SPCM group could explain why these cows were the lightest among the three groups.

The average SCS values were statistically significant among the pasture treatments in our study, indicating a reduction in SCS in cows grazing on diverse pastures. Carmona-Flores et al. (2020) and Wilson et al. (2020) reported similar results, with lower SCC in milk from cows on diverse pastures. This reduction trend in diverse pastures, although not significantly, remains unclear and probably can be linked to the beneficial secondary metabolites found in species like red clover, chicory, and plantain, such as anthelmintic, antimicrobial, and digestive aid compounds (Flythe et al. 2013; Das et al. 2016; Sahan

et al. 2017). Additionally, these plants species have high levels of micronutrients like  $\beta$ -carotene and vitamin E (Elgersma et al. 2013), which are associated with improved immune function and healthier mammary glands (O'Rourke 2009). In our study, the lower SCC in milk from cows on diverse pasture compared to those on standard pastures is positive, given the negative association between SCC and both milk quality and mammary health (Wollowski et al. 2019).

## Conclusion

The current study found that grazing on diverse pastures, whether under conventional or regenerative management, did not significantly impact detrimentally overall total milk yield or composition compared to standard pastures. However, significant differences were observed in lactation curves and regression coefficients among treatments, indicating that pasture type and management system influence milk production dynamics. Cows under standard pastures with conventional management exhibited flatter lactation curves and greater lactation persistency, likely due to the consistency of the standard pasture diet. In contrast, cows under diverse pastures treatments showed greater initial yields and greater variations in milk production, possibly due to increased dry matter intake and enhanced nutrient utilisation, as well as the cows' adaptive response to the diverse pastures. Additionally, there was a notable positive trend in liveweight gain and a potential reduction in somatic cell score, which can be attributed to a greater pasture species diversity rather than regenerative management practices. These findings align with existing literature, support that diverse pastures can contribute to sustainable dairy farming without negatively compromising milk production or quality.

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## ORCID

Yaliska Moreno-González  <http://orcid.org/0000-0001-6643-7713>

Nicolas López-Villalobos  <http://orcid.org/0000-0001-6611-907X>

Stephen E. Holroyd  <http://orcid.org/0000-0002-2064-5995>

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