

# Donaghys ProDairy lactation study #2 (2008). Draft report August 2008.

## Effect of oral supplementation of ProDairy® or 'New Strains' on lactating dairy cows in late lactation

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### Abstract

Objectives: This study was performed to determine the comparative effect different lactobacillus organisms have on milk production in late lactation dairy cows.

Methods: Cows were stratified according to age and pregnancy status and then randomly assigned to one of three groups: control group (no treatment); T1 group (10mls of ProDairy® daily *per os*) or T2 group (16mls of 'NewStrains' daily *per os*). All cows were also orally drenched daily with a routine dose (60g) of magnesium oxide suspension. Herd tests were performed prior to the start of treatment, midway through treatment, and at the end of treatment. Body condition was assessed prior to the start and at the end of treatment.

Results: Daily yield of MS, MP and MF fell to a lesser extent among cows treated with ProDairy than control cows, although this was not a statistically significant effect. Cows treated with NewStrains, however, had significantly reduced cumulative MS and MF compared to those treated with ProDairy or control cows. They also had significantly greater reductions in daily yield of MF, MP and MS compared to the other two groups. Drought conditions impacted on both production and body score over the course of the trial. Body score dropped less in both the probiotic treatments but this was not statistically significant.

### Abbreviations

BCS- Body Condition Score

DIM- Days in Milk

DMI- Dry Matter Intake

EMM- Estimated Marginal means

GLM- Generalised Linear Model

MF- Milk Fat

MP- Milk Protein

MS- Milk Solids

SCC- Somatic Cell Count

## Introduction

ProDairy® (ACVM regn A008265) is a liquid probiotic digestion enhancer. It is manufactured for use in dairy cattle as an oral compound and contains live cultures of *Lactobacillus acidophilus*, *Lactobacillus thermophilus*, *Lactobacillus casei* and *Bifidobacter bifidus*. NewStrains is a probiotic containing *Lactobacillus plantarum* (two strains) and *L. rhamnosus* (one strain). Work has recently been performed (AE 11243) on the effects of these organisms in young calves.

Previous smaller scale studies have indicated that feeding ProDairy® to lactating cows has a beneficial effect on milk production. Last year, a large study performed on the same property (AE11119, M Bryan) demonstrated that ProDairy® significantly lessened the drop in both daily MS and particularly daily MF production during the period of administration. These data were used for the initial power analysis to determine the size and scope of the study.

Probiotics are naturally occurring bacteria that have been shown to enhance the rumen flora. This can lead to increased efficiency. If a ruminant is able to more efficiently convert feed to milk or meat then this reduces feed requirements and in turn could increase productivity. Moreover, improved feed utilisation could reduce methane output.

The principal goal of this study was to follow on from last year's study and determine if feeding different varieties of *Lactobacillus spp* to late lactation dairy cows had any effect on production or milk composition; or on body condition score. The trial was a randomised blinded controlled trial.

## Materials and Methods

The same herd of cows used in the previous trial in Southland was selected on the basis that they performed daily oral drenching and kept good records, and to provide comparative data if necessary. The herd comprised 409 Jersey and Jersey cross cows. Cows were in late lactation, and were fed predominantly from pasture. A small amount of supplement (baleage) was introduced mid way through the study.

Cows were stratified according to age and pregnancy status (early, mid, late, empty) and randomly assigned to either of 3 groups. The distribution of allocation was varied to achieve a

roughly 40:60 split of control versus treatment. (This was achieved using a skewed ZRAND function in Excel, specifically, a ZBernoulli function taking the probability of treatment as 0.6.) From this treatment group a subset of cows was randomly chosen to become the T2 group.

10mls of ProDairy® was administered orally combined in the normal drench (60g Magnesium Oxide suspension) to the T1group, 16mls of NewStrains was administered to the T2 group, and the control group received only standard drench. Treatment groups were identified by a red (T1) or green (T2) ear tag in the ear for ease of drenching. Control cows received only the normal drench at each milking.

A herd test was performed at the beginning of the study (18<sup>th</sup> Feb), midway through the study (14<sup>th</sup> April), and at the end of the study (8<sup>th</sup> May). Body condition score (BCS) was measured at the start and end of the study. Outcomes of interest were changes in milk production (milk fat, milk protein, total milk solids (MS)) and quality (SCC), and changes in BCS during the period of treatment. The total production of MS, MF, and MP produced in the period between herd tests was measured; as was the total daily production of MS, MP, MF produced at both herd tests; and finally the difference between the daily production of MS, MF and MP at each herd test was measured.

In calf status was dichotomised into early in calf, and the remainder ('Incalf2'). The hypothesis being that any effect of pregnancy on milk production is largely confined to the latter trimester and so unlikely to be an issue for this study, when the earliest calving cows were still 90 days away from calving at the end of the study.

BCS measurements were performed by 2 independent veterinarians. The same veterinarians were used for both the first and second BCS measurements, and these were blinded to groups..

Data was collated from all herd tests, from the BCS measurements and from herd health records, and transferred into Excel (Microsoft.com) for manipulation and into SPSS (SPSS.com) for statistical analysis. This study was performed under the auspices of the Invermay AEC (AE11436).

All postulated risk factors were analysed at the univariate level prior to incorporation in the final multivariate model. All factors significant at the  $p < 0.20$  level were included in the final model. Age was dichotomised around 2-3yo, and those older. Data were analysed using the generalised linear model procedure, with significant univariate factors (Age and Treatment

group) as fixed effects. Days in milk (DIM) and the appropriate production at the start of the study (eg MS for MS outcomes, MF for MF outcomes, etc) were used as covariates for the analyses.

Outcome variables analysed were the total cumulative difference in production between the first and last herd test (MS, MF and MP); and the daily difference in production between the first and last herd tests (MS, MF and MP). These figures were calculated from the herd test data. We also investigated any effect on SCC and BCS.

## Results

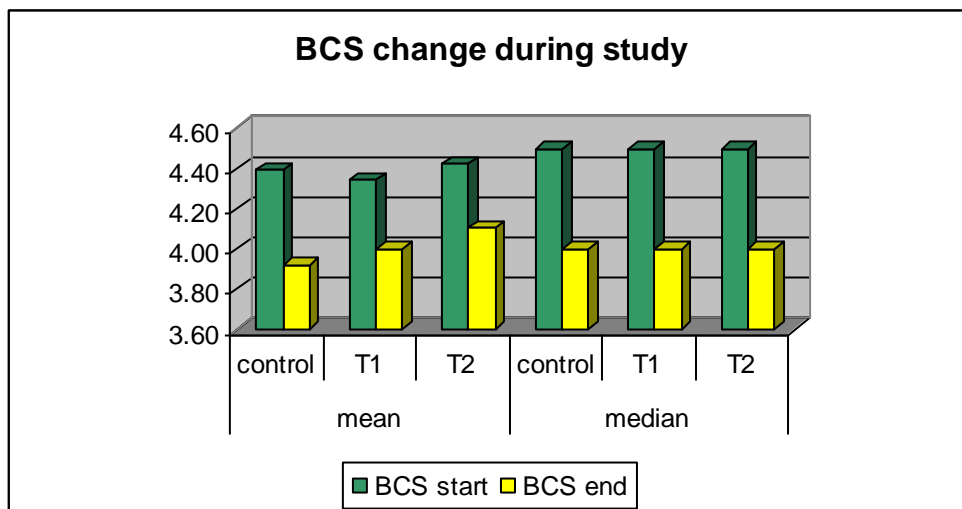
### 1. Descriptive Analysis

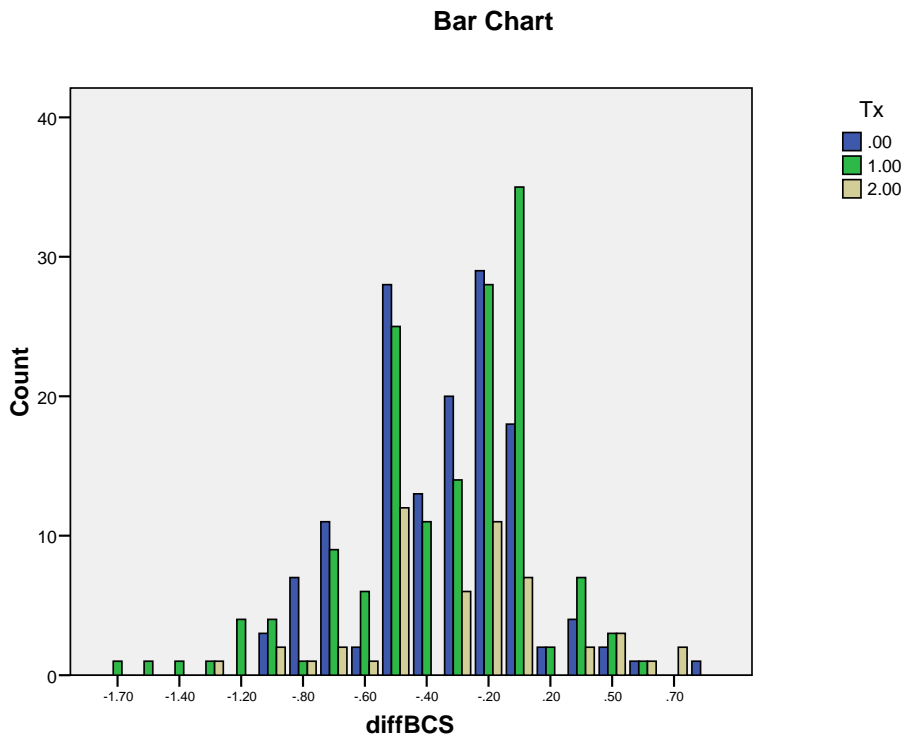
A total of 184 T1, 58 T2, and 167 control cows were enrolled. A final total of 156 T1, 52 T3, and 165 Control cows had full datasets and were used in the final analysis (n = 373).

Analysis of the initial groups showed there were no significant differences between them with regard to Age, DIM, initial daily MF/MP/MS; cumulative production MF/MP/MS, BCS1, Scan data, SCC, and lnSCC.

All groups showed a decrease in BCS during the trial period (of around 0.4 of a condition score) but there was no significant difference between the groups.

Figures 1a and 1b: BCS change





There was a strong Age effect on production, and this could be dichotomized around 3yos into 2 & 3 yos, and older.

Figure 2: Age effect on production

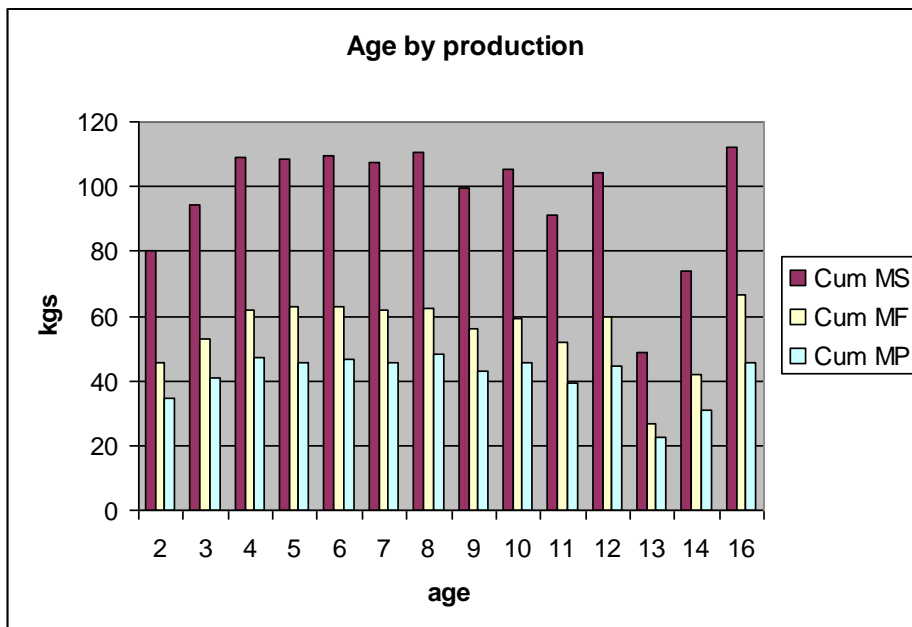
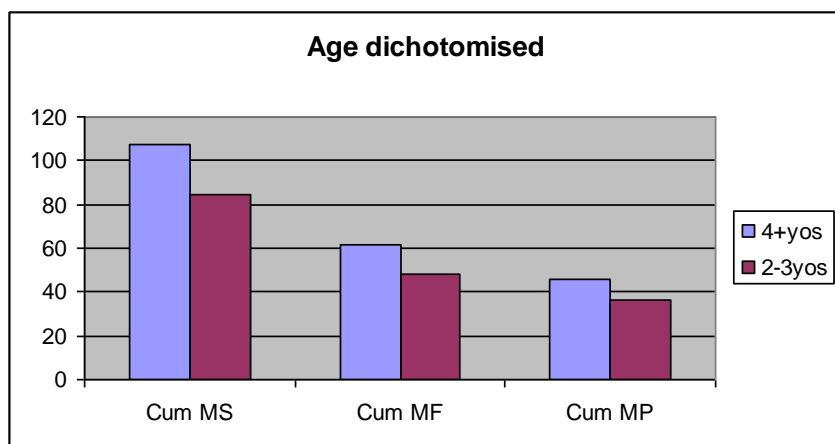
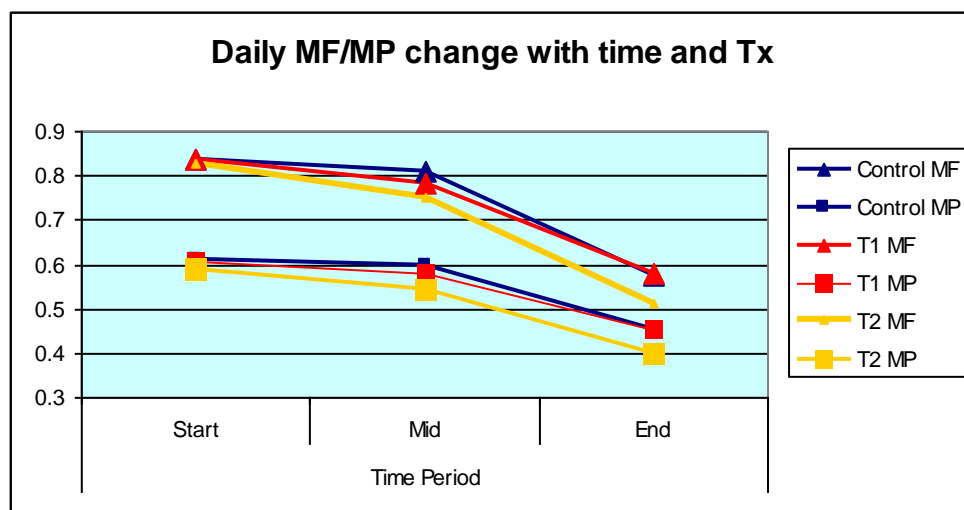
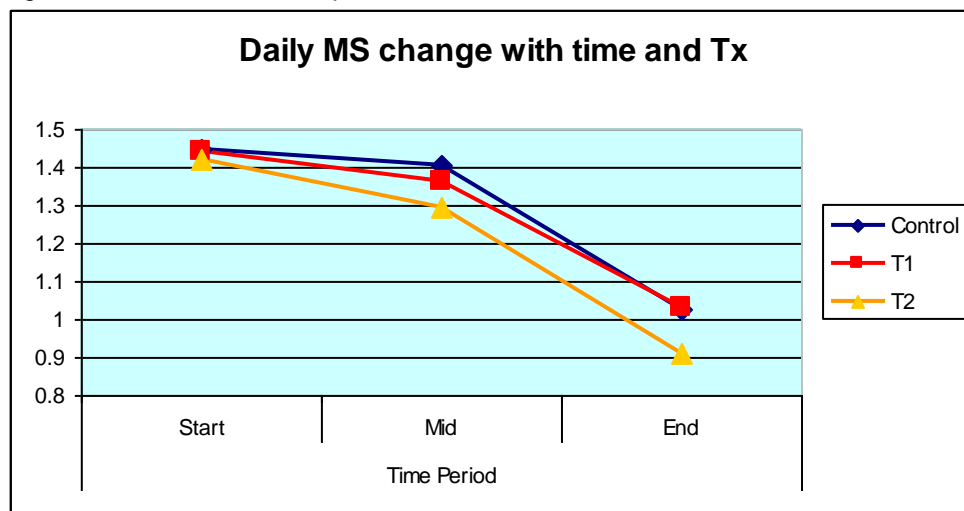


Figure 3: dichotomized age effect



All groups dropped in daily production during the period of the study. The drop in production was varied across groups.

Figures 4a and 4b: effect of production with time



The drop in all production indices was much greater than in the previous study as shown below:

**Table 1: Change in milk production, both studies**

		diff daily MS	diff daily MF	diff daily MP
<b>07 trial</b>	<b>T</b>	-0.0998	-0.0382	-0.0617
	<b>C</b>	-0.1442	-0.0676	-0.0716
<b>08 trial</b>	<b>C</b>	-0.4299	-0.2713	-0.1603
	<b>T1</b>	-0.4183	-0.2619	-0.1579
	<b>T2</b>	-0.5289	-0.3248	-0.2051

## 2. Statistical Analysis

Factors significant at the univariate level at  $p < 0.2$  were included in a final GLM. Covariates used were DIM and appropriate production level at the start of the study. Estimated Marginal Means (EMM) were calculated for the treatment groups for each outcome variable.

Outcome variables analysed were:

- a) Cumulative production – MS, MP, MF from beginning to end of the study. Calculated by the difference between the respective herd tests at the start and end of the study.
- b) Difference in daily production – MS, MP, MF from the beginning compared to the end of the study.
- c) Change in lnSCC and change in BCS from the beginning compared to the end of the study.

a) In the final GLM model, the factors affecting cumulative MF production were:

- Treatment group ( $p = 0.025$ )
- Age3 ( $p = 0.017$ ); DIM ( $p = 0.002$ )
- MF production at the start ( $p = 0.000$ )

The factors affecting cumulative MS production were:

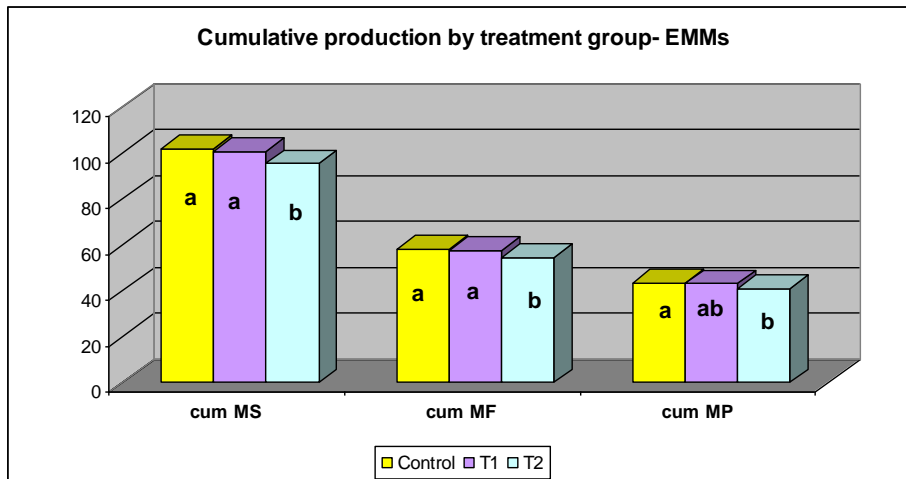
- Treatment group ( $p = 0.012$ )
- Age3 ( $p = 0.011$ )
- DIM ( $p = 0.001$ )
- MS production at the start ( $p = 0.000$ )

The factors affecting cumulative MP production were:

- Treatment group ( $p = 0.008$ )
- Age3 ( $p = 0.003$ )
- DIM ( $p = 0.001$ )
- MP production at the start ( $p = 0.000$ )

The Estimated marginal means for all 3 variables are shown below.

Figure 5: EMM for all groups for cumulative production.



(Taken from the final multivariate model. Differing subscripts represent differences significant at  $p < 0.05$ )

Table 2: EMM for all groups for cumulative production.

Tx Group	cum MS	cum MF	cum MP
Control	101.8a	58.1a	43.6a
T1	100.7a	57.5a	43.1ab
T2	95.5b	54.6b	40.8b

b) The factors affecting the daily difference in MS production were:

- Treatment group ( $p = 0.005$ )
- DIM ( $p = 0.012$ )
- MS production at the start ( $p = 0.000$ )

Age3 did not have a significant effect ( $p = 0.148$ ).

The factors affecting the daily difference in MP production were:

- Treatment group ( $p = 0.005$ )
- DIM ( $p = 0.013$ )
- MP production at the start ( $p = 0.000$ )



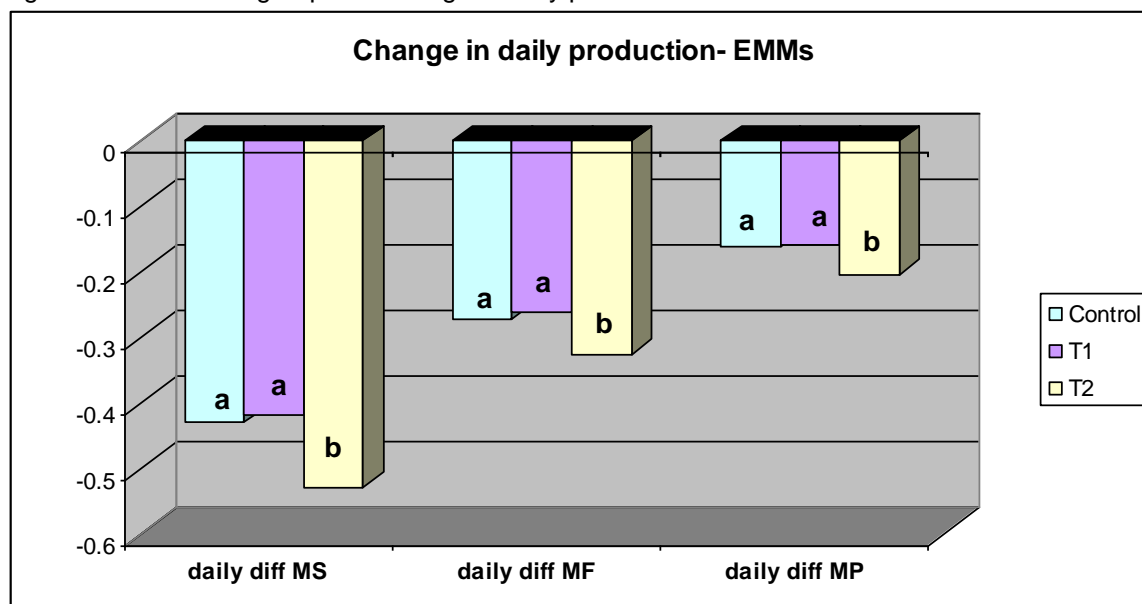
Age3 showed a trend towards having an effect ( $p = 0.062$ ).

The factors affecting the daily difference in MF production were:

- Treatment group ( $p = 0.008$ )
- DIM ( $p = 0.011$ )
- MF production at the start ( $p = 0.000$ )

Age3 did not have a significant effect ( $p = 0.152$ ). The Estimated marginal means for all 3 variables are shown below.

Figure 6: EMM for all groups for change in daily production.



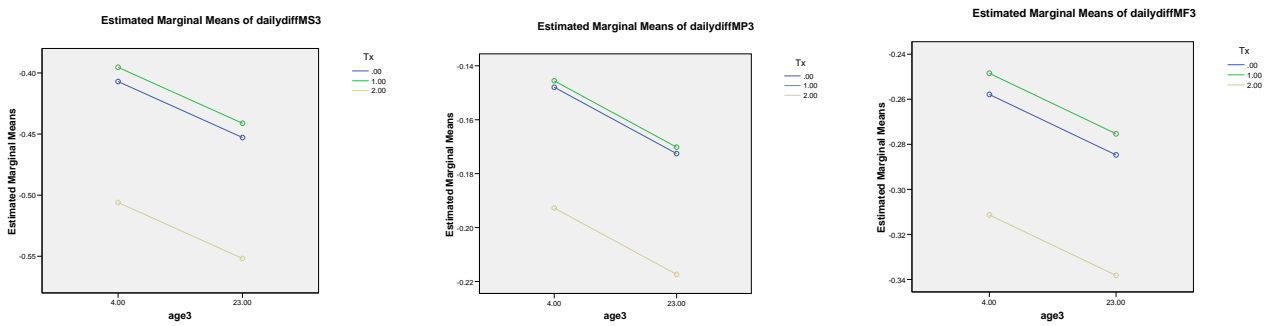
(Taken from the final multivariate model. Differing subscripts represent differences significant at  $p < 0.05$ )

Table 3: EMM for all groups for change in daily production.

Tx Group	daily diff MS	daily diff MF	daily diff MP
Control	-0.430a	-0.271a	-0.160a
T1	-0.418a	-0.262a	-0.158a
T2	-0.529b	-0.325b	-0.205b

The series of graphs below illustrate the EMM for change in daily production for all three products graphically, stratified by the dichotomous age grouping.

Figures 7: EMM for all groups for changes in daily production (3).



c) There were no significant differences between groups with regard to the InSCC ( $p = 0.943$ ) nor the change in BCS ( $p = 0.629$ ) at the end of the study after correcting for Age, DIM and initial production covariates (and initial InSCC for SCC data).

**Table 4: Estimated marginal means of differences in BCS between treatment groups**

Tx	Mean	Std. Error	95% Confidence Interval	
			LCI	UCI
<b>C</b>	-0.335	0.037	-0.407	-0.263
<b>T1</b>	-0.303	0.036	-0.373	-0.232
<b>T2</b>	-0.274	0.058	-0.388	-0.160

## Discussion

The study last year demonstrated a significantly smaller reduction in both MS and MF production among the treated cows compared with the control cows. This study demonstrated a similar effect, but the size of the change was not statistically significant.

Of note in this study is the very much greater overall fall in production at the tail of the season compared with the previous year. This season was uncharacteristically dry for Southland. It is also of note that the cows lost condition during the study, which was also not an effect noted last season.

It was postulated following the last study that the effect of the treatment product may only come from either increased efficiency of food metabolism and conversion; or from increased food (dry matter) intake (DMI). If the latter is indeed the case, this season cows would have struggled to increase DMI in the absence of any significant pasture during the drought conditions

experienced. This observation may be backed up by the loss in condition the cows experienced this season.

Of interest is the negative effect of the 'NewStrains' product. This study suggests that not all Lactobacilli are equal in effect; indeed, not all have a positive effect on lactating cow milk production.

## **Acknowledgements**

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