Final Project Report – the contracted report to farmers

SFF #404979 Are there long-term advantages in giving probiotic supplements to neo-natal calves?

A Sustainable Farming Fund project supported by BioBrew Ltd and Otago farmers.

Project development and work by the Clutha Agricultural Development Board Inc.

Executive Summary

- This project was a follow up to the 2012-13 SFF project L12-083 – "The effects of a probiotic supplement on growth, feed conversion and general health of dairy calves."
- This 2016-17 project collected available data on the three trial farms relating to weight, milk yield, mortality and general health of the 2012 calves, now production cows.
- The project considered whether there was any difference in the data sets for the supplement-fed calves compared to the control groups.
- We collected data for almost all the 283 heifer calves from the spring 2012 trial; and with culls, sales and deaths there were 174 (62%) of these calves left in herds on their original three farms by the end of the 2015-16 season.
- The answer to the project question "Are there long-term advantages in giving probiotic supplements to neo-natal calves?" is that we don't know. Instead of firmly answering the question we have been able to make observations and seen trends that should be of interest to the industry.
- There is a possible trend of slightly higher production seen in the MS kgs figures for the 2015-16 season across the three farms, but analysis of these production figures show no statistically significant difference.
- Data collected from two farms appears to show the neo-natal probiotic treated cows being retained in their herds in greater numbers. For 6 of the 7 paired groups of calves a higher % of the control groups were culled, sold or had died. This was not the case for Farm 1.
- 38% more control group cows died or were culled (taking out sales) over all three farms than those from the probiotic groups – 42 v 29 animals.
- The probiotic supplement had little or no effect on animal live weights (beyond the initial 2012 trial period) or on days in milk.
- Milk Efficiency data was available from one farm and showed a 10.2% increase in milk efficiency by the probiotic cows over the control group that was statistically close to the 95% probability threshold ($p<0.058$).
- Neo-natal probiotic supplement treatment may have beneficial long-term effects but our study may have been insufficiently sensitive to determine this, given the environmental and management differences amongst the farms.
- This project found trends that could potentially benefit the industry and further research on the use of probiotic and similar animal supplements is recommended.
- This project was only possible through great support from our trial farmers, the project team and is much appreciated.
- The raw data is available with this report.

SFF #404979 Are there long-term advantages in giving probiotic supplements to neo-natal calves?

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This project has been a follow up to our 2012-13 SFF project L12-083 – "The effects of a probiotic supplement on growth, feed conversion and general health of dairy calves." This was a trial of approx. 300 calves with 20 pens of calves (10 replications) over three farms in the spring of 2012. Half the calves were fed a fresh probiotic supplement and half were a control group.

This 2016-17 project was to go back to these three trial farms (near Balclutha, Clinton and in West Otago) and collect relevant data relating to weight, milk yield, mortality and general health. We were interested in whether there was any difference (statistically significant or otherwise) in the data sets for the supplement-fed calves compared to the control groups now that they were production cows on farm. The collected data related to 3 and 4 years after their trial situation.

The AgBoard's work here has always recognised that the conditions under which these 2012 calves (that became production cows) were quite different across the three trial farms. There has been no attempt to monitor or control their farming systems. Feed management, wintering arrangements, climatic conditions, for example, will all be affecting the data collected.

What we can say, however, is that there were initially 6, 8, and 6 paired groups of calves respectively in Farms 1, 2 and 3, and that each pair of supplement and control calves would have experienced the same farming systems on those individual farms over the years involved. We believe therefore that comparisons between the pairs (matched for birth weight and dates), and each farm's calves can have some validity, but any averages across farms is debateable because of the many variables involved.

The project did not aim to prove any causal effects, but rather to give researchers some indications that may prove worthy of further investigations. Would any aspect of the data collected show a significant difference between the group of calves given a neo-natal fresh probiotic and the control group of calves?

Before looking at the current 2016-17 project data, it is worth remembering the project design and conclusions from the original 2012-13 project – so these are included in this report.

2012-13 Trial: Average growth per day in pens from Farm 1, 2 and 3

	Probiotic treatment calves	Control calves	Difference	P value
Farm 1	0.662	0.644	+18g/day	Not significant[2]
Farm 2	0.634	0.577	+57g/day	P > 0.01[3]
Farm 3	0.573	0.534	+39g/day	P > 0.02

[2] 'Significance' here is a statistical term. It does not mean 'important'; it means that the P value is greater than 0.05

[3] The P value indicates the probability that the results are consistent with being due to chance.

2012-13 Trial Design (arranged by the project team in association with DairyNZ):

- 3 Farms
- 20 pens (10 control; 10 treatment)
- Approx 10-20 calves in each pen

Treated calves had a multi-strain probiotic (lacto-bacillus & yeasts) added to the milk replacement from approx. day three (after initial colostrum period) when appropriate numbers of calves were available to be assembled and penned.

The amount of added probiotic was:
i 20ml/ calf drenched twice a day for two days after penning,
ii then, 20ml/ calf added to the milk in the calf feeder once a day for the remainder of the trial.

The aim was to have each pair of pens balanced for weight and for all other feeding, housing and management conditions the same, so that the probiotic treatment is the only variable.

Data Collection Design:

All calves were weighed weekly for the seven weeks of the trial. Other health records were also kept. Calf meal was weighed at entry into each pen and was aggregated over the life of the trial.

2012-13 SFF Project Report Summary

1. The fresh probiotic supplement improved the weight gain of treated calves by up to 57 grams per day over the seven weeks of the trial.

2. The impact of the treatment was shown to vary considerably from farm to farm and is affected by the particular farm's system, calving procedures and rearing conditions.

3. Future work is required to identify the conditions in under which a probiotic has a positive effect and how they can be best used to benefit New Zealand farmers.

The 2016-17 collection of data

The plan was to collect data relating to weight, milk yield, mortality and general health of the 2012 calves. We had great support from the farmers involved, but, of course it was difficult to collect all this data from each farm, and to collect the same data from each farm.

Each of the three farms used a different data collection system: one farm used Milk Hub (MiHub), one Minda and one AlPro. Two farms were adding to their systems for the 2016-17 season, and there were only a few herd tests recorded via Minda across the three farms. One farm weighed monthly, one had few weight records for the four years, and one had gaps in their records because they had moved farms and built a new shed. All three farms had reasonable to very good fate records.

Removing the steers/ bulls from the original 307 calves meant there were 283 heifer calves in the spring 2012 trial. Of these, there were 174 production cows in the current herds at the end of the 2015-16 season across the 3 farms – 61.5%. (*note this is a correction from our interim milestone one report).

The data collected is available in raw form with this report.

Analysis of the 2016-17 collection of data

The analysis of the data collected provided many challenges:

- Different data was available from each farm
- This '4 years later' collection was not envisaged when we set up the original 2012-13 project
- At times, small numbers were left in pairs for comparison purposes after deaths/ culls/ sales
- Calf weights in each pair were generally well matched, but there was a small bias in favour of the control group. The control calves were an average 0.8 kgs heavier over the three farms and 1.9 kgs (6%) heavier across the three pairs on Farm 1. This may mean that any effect of the probiotic treatment has been under-estimated, and might explain the often different results for Farm 1.

Some analysis was undertaken by four people with a variety of experience and expertise. Although two of these people have statistical training and experience, useful formal analysis of the data was limited by the variety of confounding factors in the collection.

Note: In this report we have decided it is more useful to highlight some aspects of the collected data that may prove interesting for farmers on the one hand, and that may prove useful for further research on the other. We do not make any particular claims as to cause and effect. There are too many variables and confounding factors in the data. However, there are indications worth consideration.

Probiotic group or control group – who was left standing?

An analysis of the losses from the respective herds via deaths/ culls/ sales has proved an interesting aspect of our data collection project.

Table A: % Cows lost to herds via died/ culled/ sold as at the end of the 2015-16 season

		Probiotic		Retained in herd	Control		Retained in herd
Farm 1	Pair 1	4/13	31%		3/10	30%	
	Pair 2	3/7	43%		2/10	20%	
	Pair 3	7/14	50%		4/13	31%	
	Average Farm 1		41%	59%		27%	73%
Farm 2	Pair 4	3/12	25%		7/12	58%	
	Pair 5	4/12	33%		5/12	42%	
	Pair 6	0/12	0%		4/12	33%	
	Pair 7	4/12	33%		4/12	33%	
	Average Farm 2		23%	77%		42%	58%
Farm 3	Pair 8	6/20	33%		9/20	45%	
	Pair 9	11/25	44%		12/24	50%	
	Pair 10	4/16	25%		10/16	31%	
	Average Farm 3		34%	66%		42%	58%

Working on the assumption that cows being retained in the herd, rather than having to be culled, dying or having to be sold, is best for the farmers, then the data collected from Farms 2 and 3 consistently appears to show the neo-natal probiotic treated cows being retained in their herds in greater numbers. For 6 of the

7 paired groups of calves a higher % of the control groups were culled, sold or had died (the seventh was equal). Does this indicate a lasting robustness is given to calves via early probiotic treatment encouraging good gut health?

This tends to match our 2012-13 trial result that showed 3 deaths during the trial from the supplement-fed calves and 8 deaths amongst the control groups across all three farms.

Of the 4 deaths since the 2012 trial, 3 have been from the control groups, but these are not statistically significant numbers.

And yet it is also possible that if a farm is geared partly to selling cows, then the percentages in Table A will mean something different. Farm 1 reported that after the 2012 calves were born they were overstocked and had to move out a larger-than-usual number. This is likely to be the reason for the high number of "sold or culled?" notes in our data for Farm 1, where the 'losses' to the herd were greater from the probiotic groups in each pair by a considerable margin. This was not the same situation as in Farms 2 and 3.

Which of the probiotic and control groups had the most deaths and culls?

To take out the effects of sales (which may be seen as positive or negative depending on the farm's situation, Table B shows a compilation of the deaths and culls from the first weighing to the end of the 2015-16 season.

Table B: Deaths and culls only
From first weighing to the end of the 2015-16 season

		Probiotic		Control	
Farm 1	Pair 1	4/13	31%	2/10	20%
	Pair 2	1/7	14%	1/10	10%
	Pair 3	1/14	7%	1/13	8%
	Average Farm 1		17%		13%
Farm 2	Pair 4	1/12	8%	4/12	33%
	Pair 5	4/12	33%	5/12	42%
	Pair 6	0/12	0%	3/12	25%
	Pair 7	4/12	33%	4/12	33%
	Average Farm 2		19%		33%
Farm 3	Pair 8	4/20	20%	5/20	25%
	Pair 9	8/25	32%	9/24	37%
	Pair 10	2/16	12%	8/16	50%
	Average Farm 3		21%		37%

Again the data from Farms 2 and 3 is very consistent in that for 6 of the 7 pairs fewer probiotic treated calves have been lost to the herd in a way that may show their vulnerability compared to their respective control groups. The seventh pair was equal. The average across the paired groups in Farms 2 and 3 was a 35% loss to the control groups and only a 20% loss to the probiotic groups – 38 v 23 animals.

Overall 38% more control group cows died or were culled (taking out sales) than those from the probiotic groups – 42 v 29 animals.

It was not within the brief of this small-scale project, but it would be interesting to quantify these losses to farmers in dollar terms.

We also note, as we did in the original 2012-13 project, that farms do not respond in a consistent way to the probiotic treatment, as is shown for Farm 1 in the Table B data. This difference may be accounted for by the very small numbers involved in all groups of Farm 1 (just 2 calves more overall in the deaths/culls column – 6 v 4 animals), the bias towards the control groups in the birth weights, or in the explanation around overstocking and sales above. However, it may be (as in 2012) that this farm's practices and systems did not need the additional advantages of the neo-natal probiotic treatment that can be seen to be beneficial in Farms 2 and 3.

The big question – did the probiotic group or the control group put more milk solids in the vat?

The 2015-16 season data (second lactation) was the most complete for the milk production of our trial cows. The analysis was to consider any differences between these weight and birth date matched pairs of calves for their average yield and the yield of each farm in turn .

The different farming systems and feeding regimes of the three farms will confound the analysis between farms so we have not included an overall average across the three farms.

Also causing problems for the analysis was the fact that Farms 1 & 2 collected yield in MS kgs, and Farm 3 had figures for litres of milk. For consistency, we have translated Farm 3 to MS kgs by assuming a constant milk solid percentage of 8.84.

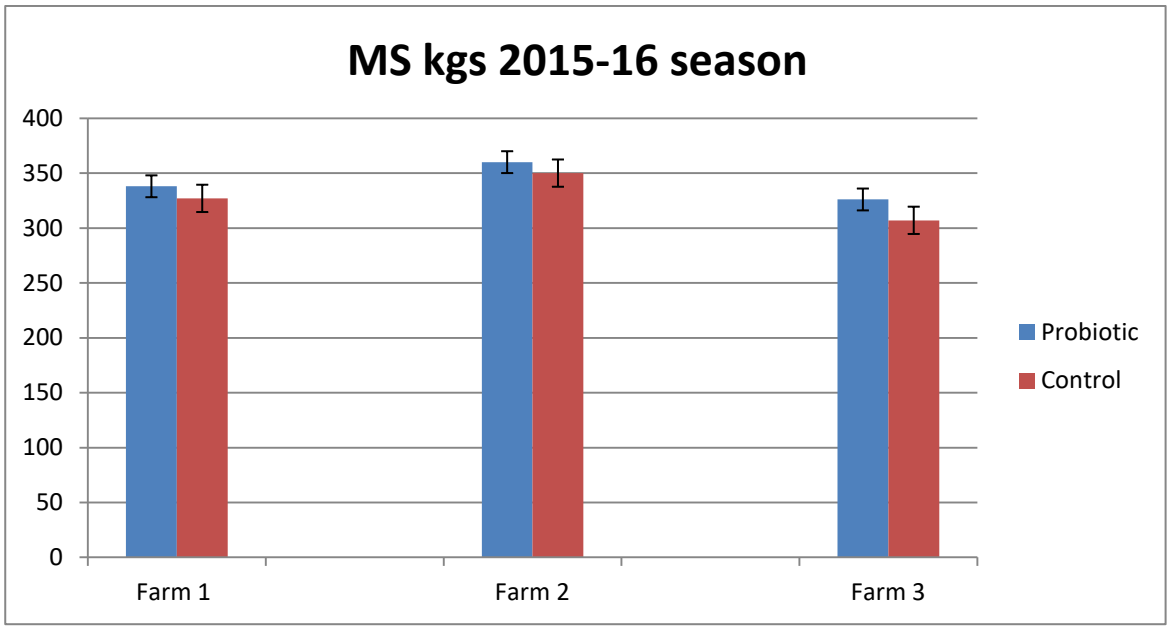
The raw numbers show inconsistent results across the ten pairs but the average across the pairs on all farms showed a small difference in favour of the probiotic group. This advantage was more pronounced for Farm 3.

Table C: Comparison between probiotic supplement calves and control group
Kgs MS yield for the 2015-16 season
Average for each pair of cows still in the herd

		Probiotic	Control
Farm 1	Pair 1	369	317
	Pair 2	338	350
	Pair 3	309	315
	Average Farm 1	338	327
Farm 2	Pair 4	345	354
	Pair 5	354	336
	Pair 6	374	330
	Pair 7	368	381
	Average Farm 2	360	350
Farm 3	Pair 8	333	327
	Pair 9	324	319
	Pair 10	322	276
	Average Farm 3	326	307

In addition, if the raw numbers for MS kgs or litres of milk were to be totalled for the season for each initial pair of calves, then due to the advantages shown in table C above and the deaths, culls and sales explained earlier, then there is an advantage to the probiotic treated calves groups. This is a layman's observation and is not statistically valid.

So there is a possible trend of slightly higher production from the probiotic addition in their first seven weeks of life, but statistical analysis of these production figures three seasons on show no statistically significant difference.



When the 2015-16 production figures have margin for standard error bars added (via Excel spreadsheet) they overlap. As they overlap, this indicates that the difference between the two means in each set of data is not statistically significant (P>0.05).

Because of the trends here which could potentially benefit the industry, more extensive work on the effects of probiotic and similar supplements is recommended.

Other observations

The **initial weights of the calf pairs**, although matched as successfully as general farming practice would allow, and also more so than any other known similar trial, showed that the control cohort were on average 0.9kgs heavier than the probiotic supplement calves. This means that there was a bias introduced against the probiotic treated calves. Most of this bias is seen in the pairs on Farm 1, and might explain somewhat several differences in conclusions between Farm 1 and Farms 2 and 3.

It was not possible to collect **cow weights** on all farms. Farm 1 provided extensive records but the bias explained above meant that further analysis may not be valid. The analysis that was done showed no statistically significant difference between probiotic and control groups (beyond the initial 2012 trial period).

MS kgs yield data as at February 2017 on Farms 1 and 2 was analysed but showed no statistically significant difference (p=0.05) overall between probiotic and control cows. The averages across the pairs were inconsistent.

Also looking at the **Days in Season** data collected, averages across the pairs were inconsistent. We decided that in depth analysis of this features was not necessary. Days in season is more likely an aspect of the practice on each farm. Also, it was not clear whether more or fewer days would be an advantage to a farmer. Kgs of milk solids or litres of milk is a much more relevant measure of success or otherwise of a treatment.

Farm 1 provided data in relation to **Milk Efficiency** in February 2017. This is a measure of a cow's ability to turn dry matter into milk. On this limited data the increase in milk efficiency from the probiotic cows over the control group was statistically close to the 95% probability threshold ($p<0.058$).

Discussion and conclusion

We believe we have completed our brief to consider the data collected and then to highlight interesting aspects of the data.

A summary of these aspects has been compiled at the start of this report.

It is acknowledged that this data collection is something of an 'afterthought' to the initial trial of calf weights in the spring of 2012. Of course, outcomes may have been more reliable had we had an on-going data collection in mind as we began this work then.

However, for a very small cash investment and lots of voluntary in-kind contributions, we believe we have provided a set of data and some highlights that are worthy of follow-up work. This is real, on-farm data and indications that are not easily obtained and the powers that be in the dairy industry should take note.

The original 2012 trial on neo-natal calf weights from weeks 1-7, the bias in the birth weights against the probiotic calves, the positive retention and survival of the treated calves shown in this 2016-17 project, and the suggestion that probiotic treatment as calves may put more milk in the vats of New Zealand farmers is all positive evidence to suggest further work in the area of probiotic supplements is required.

It is recommended that this work take into account cow genetics, feed quality and management, and milking arrangements to further objectively consider the long term impact of fresh probiotic supplementation (as in our projects discussed here) and also other calf supplements.

Thanks to MPI's Sustainable Farming Fund, industry partner BioBrew Ltd, Dr Peter Espie, Project Manager Murray Harris, the project team and especially the farmers on our three trial farms.

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