Ruminant response to non-live probiotic microorganism extracts

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Abstract

Live probiotic microorganisms can improve ruminant productivity but results are variable and modes of action uncertain. Non-live microbial extracts provide insight into probiotic modes of action and their efficacy challenges the definition of probiotics. Twenty-two trials on lambs and ten on dairy cows were undertaken with non-live probiotic microorganism extract products RumenZyme Plus (RZP) and ProDairy (PD) respectively. Lambs treated with single or double doses of RZP tended to gain extra live weight relative to control with a 445 g median treatment response (P=0.001). The patterns of RZP results did not fit with an anti-inflammatory response alone but rather general productivity improvement. Cows receiving daily doses of PD had higher daily MS production with mean treatment response of 49.7 g/cow/day (P=0.008, 95% confidence interval of 16.3 to 83.2 g/cow/day). There was no evidence of benefit from the addition of live probiotic lactic acid bacteria in these trials. Live lactic acid bacteria supplementation actually reduced response relative to the non-live probiotic extract in one dairy and two lamb trials (P<0.05). The results in this study demonstrated efficacy of non-live probiotic extracts for ruminant productivity.

Keywords: lamb; dairy cow; probiotic extracts; ProDairy; RumenZyme Plus

Introduction

Supplementation with probiotic microorganisms can increase dairy cow milk production and livestock weight gain, but results in studies are variable and mechanisms of action uncertain (Yoon & Stern 1995). Much recent probiotic research in ruminants has focussed on yeasts, with a meta-analysis (Desnoyers et al. 2009) reporting overall positive effects on milk production. The success with yeast in ruminants is in conflict with a classical inoculant view of probiotic action since yeasts are not considered to remain for a long period of time nor regenerate within the rumen or intestine (Fuller 1992; Newbold et al. 1990). Non-live cultures of yeast and *Aspergillus* fungi, have been established as effective in increasing ruminant productivity albeit variably (Yoon & Stern 1995). In fact the study of Desnoyers et al. (2009), aimed at live yeast supplements, inadvertently included several positive results from a non-live yeast product (Diamond-V). A meta-analysis of Diamond-V yeast-culture dairy trials (Poppy et al. 2012) indeed demonstrated an overall positive effect of the product on milk yield. Moreover, it has been established in human research that non-live preparations of lactic acid bacteria (LAB) can show probiotic effect (Ouwehand & Salminen 1998) while offering storage and potentially safety advantages over live probiotic organisms (Kataria et al. 2009).

Probiotics are now commonly defined in scientific literature as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (a working party definition from FAO/WHO, 2001). Probiotics were originally defined as "growth promoting factors produced by micro-organisms" by Lilly and Stillwell (1965). The definition was narrowed to "organisms and substances which contribute to intestinal microbial balance" by Parker (1974). "Substances" was considered imprecise by Fuller (1989) who changed the definition to "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance".

The term "non-viable probiotics" was soon introduced by Fuller (1992) reflecting the efficaciousness of non-live microbial products. Several reviews suggested definitions of probiotics that include non-live microorganisms and/or their products (e.g., Salminen et al. 1999).

Modes of action of non-live probiotic microorganisms may include anti-inflammatory effects (Adams 2010) or, as has been shown for yeast cultures, there may be modification of rumen ecology and fermentation efficiency (Poppy et al. 2012).

RumenZyme Plus (RZP, ACVM Licence A8217) and ProDairy (PD, ACVM Licence A8265) are non-live products from fermentations of probiotic lactic acid bacteria and yeasts (produced by Donaghys Industries). There have been 22 RZP and 10 PD randomised trials. Some trials included live probiotic lactic acid bacteria (LAB) with the product treatments. The current combined trial analysis investigated the effect of these non-live probiotic extracts on lamb growth and dairy cow milk production, and assessed evidence for any benefit from adding live LAB to the products.

Materials and Methods

Model description

Trial reports are available from Donaghys (2014). All RZP and PD trials were included in the analysis as long as treatment allocation was

Table 1 RumenZyme Plus (RZP) trial s daily live weight gain s (LWG). For P value of non-live-RZP effect, bold text indicates significance at P<0.05, italics indicates P value between P=0.05 and P=0.10, 'nt' indicates not tested (no individual lamb ID). Trials 11 to 17 had live lactic acid bacteria (LAB1 at c. 10^8 cfu mL^{-1}) added to RZP as a further treatment; response is presented in last column. FB refers to Farming Beyond 2000 farmer project. Superscript 'Co' on tr ial numbers 10 to 21 indicates cobalt sulphate (at 0.8 mg Co mL^{-1}) added to the RZP ; superscript 'B' indicates lambs fed on brassicas after treatment.

Trial Code	Year	Region	Research Group	Lambs per Treatment	Duration (days)	RZP Dose (mL)	LWG Control (g/day)	LWG RZP (g/day)	RZP Effect (g/day) ± SED	P value	RZP (4 mL) + LAB1 (4mL) Effect (g/day)
1	2000	Canterbury	Donaghys	20	62	5 + 5	107	149	42	nt	
2	2001	Southland	Donaghys	192	19	3	218	239	21	nt	
3	2001	Canterbury	Donaghys	50	28	3	136	170	34 ± 19	*0.082*	
4^{B}	2002	Canterbury	AgResearch/FB	100	35	3	189	278	89	nt	
5^{B}	2002	Canterbury	AgResearch/FB	100	35	3	285	346	61	nt	
6	2002	Canterbury	AgResearch/FB	350	42	3	220	229	9	nt	
7	2002	Canterbury	AgResearch/FB	50	42	3	265	248	-17	nt	
8	2002	Canterbury	AgResearch	100	42	3	273	278	5 ± 14	0.727	
9	2002	Canterbury	AgResearch	100	42	3	279	295	16 ± 12	0.182	
$10^{Co,B}$	2006	Canterbury	Donaghys	100	28	3	61	93	32 ± 12	**0.010**	
11^{Co}	2008	Southland	Donaghys	70	24	4	179	180	1 ± 19	0.957	0
12^{Co}	2008	Southland	Donaghys	75	41	4	225	232	7 ± 11	0.272	8
13^{Co}	2008	Southland	Donaghys	70	42	4	115	116	1 ± 12	0.930	-6
14^{Co}	2008	Wellington	Donaghys	70	42	4	94	97	3 ± 11	0.775	10
15^{Co}	2008	Canterbury	Donaghys	70	42	4	87	126	40 ± 16	**0.011**	37
$16^{Co,B}$	2008	Canterbury	Donaghys	70	43	4	157	162	5 ± 11	0.691	-2
$17^{Co,B}$	2008	Canterbury	Donaghys	70	82	4	30	27	-3 ± 6	0.569	2
$18^{Co,B}$	2008	Gisborne	AgResearch	70	42	4	155	167	12 ± 9	0.169	
$19^{Co,B}$	2008	Rangitikei	AgResearch	70	25	4	164	157	-6 ± 13	0.619	
20^{Co}	2008	Canterbury	AgResearch	70	42	4	160	172	12 ± 10	0.258	
21^{Co}	2008	Canterbury	AgResearch	70	42	4	190	227	37 ± 10	**<0.001**	
22^{Co}	2012	Canterbury	Donaghys/ Lincoln University	60	42	4 + 4	75	98	23 ± 11	**0.030**	
Mean									19.8 ± 5.1	**0.001**	

randomised and a control (untreated) group was present. RZP trials involved weighing lambs at the start, drenching treated lambs with a single dose of RZP (or in some cases the dose was repeated later) and weighing lambs at the end of the trial. Seven RZP trials involved a feed change to brassicas at commencement. Cobalt sulphate was added to the RZP formulation (at 0.8 mg Co mL^{-1}) in the 13 latest trials and some trials included live lactic acid bacteria. The trials affected by these factors are indicated in Tables 1 and 2. There were no other RZP formulation changes during the course of the trial programme.

PD was either drenched daily or dispensed through drinking water (Trial 9) or feed (Trial 11). Milk solids (MS) production per cow was assessed prior to treatment and at completion of the trial. Assessment frequency during the trial is detailed in Table 3. The average yield during the trial was compared with the starting yield. Only cows present at the beginning and end of the trial period were assessed.

Tables 1 to 3 detail levels of live LAB inclusion and extra methodology. Two groups of LAB strains were trialled, LAB1 comprising *Lactobacillus acidophilus*, *Bifidobacterium* sp., *L. casei*, *Streptococcus thermophilus* (at either c. 10^5 colony forming units cfu mL^{-1} or c. 10^8 cfu mL^{-1}) and LAB2 comprising *L. plantarum* and *L. rhamnosus* (at c. 10^8 cfu mL^{-1}). Apart from addition of live LAB bacteria, PD formulation remained constant over the trial programme.

Table 2 RumenZyme Plus (RZP) trials with partial replacement of RZP with live LAB culture (c. 10^8 cfu mL^{-1}; either LAB1 or LAB2) and feed change to brassicas. Daily live weight gain (LWG) results presented for 10 days after treatment and for total trial duration. Trials as numbered in Table 1. Treatments in a single column sharing the same letter are not significantly different from each other based on least significant difference (LSD, 5%). Last column is total kg LWG for the mean of all trials.

	Trial 18		Trial 19		Trial 20		Trial 21		Overall	
Treatment	Ten Day LWG (g/day)	Total 42 day LWG (g/day)	Ten Day LWG (g/day)	Total 25 day LWG (g/day)	Ten Day LWG (g/day)	Total 42 day LWG (g/day)	Ten Day LWG (g/day)	Total 42 day LWG (g/day)	Mean Ten Day LWG (g/day)	Mean Total LWG (kg)
Control	30[a]	155[a]	209[a]	164[a]	82[b]	160[ab]	44[b]	190[c]	52[ab]	7.08[a]
RZP (4mL)	10[a]	167[a]	204[a]	157[a]	135[a]	172[a]	143[a]	227[a]	96[a]	7.93[a]
RZP (2mL) + LAB1 (2mL)	-11[a]	157[a]	225[a]	162[a]	51[b]	153[ab]	75[b]	211[ab]	38[ab]	7.11[a]
RZP (2mL) + LAB2 (2mL)	-1[a]	170[a]	212[a]	179[a]	44[b]	147[b]	46[b]	191[bc]	30[b]	7.29[a]
LSD 5%	46	17	41	15	52	20	39	20	56	0.87

Table 3 ProDairy (PD) trial information and milk solid (MS) responses. PD was non -live in most trials but had LAB1 (c. 10^5cfu mL^{-1}) added where indicated by superscript 'L'. Trials 6 and 8 had LAB2 (c. 10^8cfu mL^{-1}) and LAB1 (c. 10^5cfu mL^{-1}) respectively added to PD as a further treatment (response indicated in brackets). Trials 9 to 11 were feedlot cows on TMR (total mixed rations); all other trials were pasture fed. In the overseas trials (9 to 11) data for calculating SED (standard error of difference) were not available (indicated by 'na').

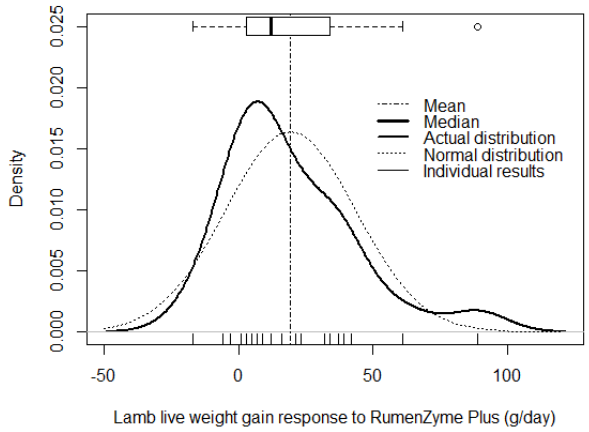
Trial Code	Year	Region	Research Group	Cows per Trial	Duration (days)	Daily Dose (mL)	Herd Tests After Start	Adjusted Control MS (g/day)	Adjusted ProDairy MS (g/day)	ProDairy Response MS (g/day)	SED (g/day)
1	2001	Waikato	Donaghys	60	29	5.5	2	58	121	63^L	35
2	2001	Southland	Donaghys	300	35	10	2	-5	56	61	43
3	2001	Manawatu	Feilding Ag High	48	28	7	2	-65	-7	58	57
4	2002	Canterbury	Donaghys	40	44	6	3	-303	-260	43^L	52
5	2007	Southland	VetSouth	178	75	10	1	-144	-104	40^L	21
6	2008	Southland	VetSouth	124	80	10	2	-432	-420	12^L,(-98^{LAB2})	37
7	2011	Waikato	EOS Consulting	144	70	10	2	-247	-225	22^L	35
8	2011	Bay of Plenty	EOS Consulting	288	68	10	2	-465	-502	-37,(-58^L)	49
9	2011	California, US	UC Davis	1022	70	10	5	-28	2	30	na
10	2011	Newcastle, UK	Newcastle Univ.	40	49	10	Daily	-301	-206	95	na
11	2011	Georgia, US	Georgia State Univ.	36	56	10	Daily	-210	-50	160	na

Statistical analysis was with R (Version 3.0.2) including kernel density plots (see Faraway 2006 for discussion of superiority over histograms), normality testing including Shapiro Wilk test and SAS G2 method for skewness (Joanes & Gill 1998), t-tests and the non-parametric Wilcoxon rank sum test. Individual trials were analysed with restricted maximum likelihood (REML) mixed model analysis or analysis of covariance using starting weight (lambs) or starting daily MS production (cows) as

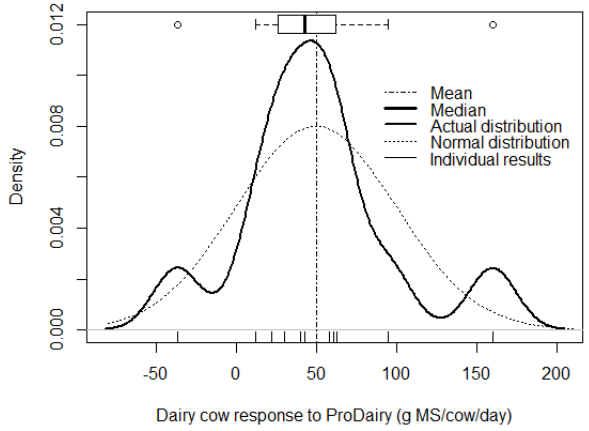
covariates when available. Generalised linear model (GLM) analysis was employed to test combined trial effects of treatments, year, region, live bacteria, cobalt and brassica feed change.

Figure 1 Distribution of mean treatment responses for each trial highlighting the positively skewed distribution of RumenZyme Plus live weight gain responses (a) and the approximately normal symmetrical distribution of ProDairy milk solids responses (b). The kernel density plot (bold curve) provides a visual test for normal distribution and is supplemented with a rug plot (small vertical lines at the base) to show individual trial means. A theoretical normal distribution is shown in the dotted bell curve based on the overall mean (vertical dashed line) and standard deviation of the trial means. A boxplot is added to indicate the overall median response (thick line near centre of rectangle) and emphasise potential outlier results and skew.

a)



b)



Results

RumenZyme Plus

Live weight gain (LWG) of RZP-treated lambs was higher than control in 19 out of 22 trials (86%). This was statistically significantly positive ($P<0.05$) in four out of 15 trials (27%) where individual lamb ID allowed statistical analysis. Mean RZP responses across trials (Fig. 1(a)) were not distributed normally ($P=0.047$) and had a strong positive skew (skewness +1.25). The unweighted mean RZP response over all trials was 19.2 g/day extra LWG for non-live-RZP-treated lambs. Given the heavy skew, the median RZP response of 12.0 g/day was a better representation of average treatment effect. Instead of a t statistic 95% confidence interval (8.5 to 30.0 g/day), the lower and upper quartiles (3.5 and 33.5 g/day respectively) were a more appropriate estimate of the range that may include the true population average effect.

The non-live-RZP effect was statistically significant over the trial means ($P=0.001$, for both Wilcoxon rank sum test and t-test) providing evidence of a beneficial treatment effect overall. GLM analysis detected no statistically significant differences due to trial year ($P=0.641$), region ($P=0.874$), brassica feed change ($P=0.392$) or cobalt fortification ($P=0.123$).

Distribution of both control and non-live RZP lamb weight gains was skewed in some individual trials. The mean of measured skewness over all 15 statistically analysed trials was -0.085 for control and -0.390 for treatment (not significantly different, $P=0.379$). Individual trial mean RZP effect did not tend to change with higher control lamb LWG; a slight positive trend was not statistically significant ($P=0.953$).

Live LAB culture replaced half the dose of non-live product for two treatments in four trials where feed was changed to brassicas (Table 2). In the combined results of all four trials, non-live RZP lambs grew faster in the first ten days than lambs treated with RZP+LAB2 but the difference in LWG was not significant overall at the end of the trials. In two trials where treatment effects were most clearly seen, non-live-RZP-treated lambs had overall higher LWG than both control and live LAB treatment lambs in the first ten days. By the completion of one of those trials non-live RZP treated lambs had gained statistically significantly more weight than control and LAB2 lambs while in the other trial there was only a statistically significant difference between RZP lambs and control.

Live LAB1 culture was tested as additional to a full dose of RZP in a further seven of the trials (Table 1). Just one of these trials showed a statistically significant treatment response, with both RZP and RZP+LAB1 treated lambs outperforming control. In the seven trials combined, RZP treated lambs mean LWG (over control) was 7.5 g/day compared to 1.7

g/day for RZP+LAB1 lambs (not statistically significantly different, P=0.189).

ProDairy

PD-treated cows generally produced more milk solids (MS) over the course of the trials (Table 3) with an overall unweighted mean of 49.7 g/cow/day above control cows (P=0.008, 95% CI of 16.3 to 83.2) and an approximately normal symmetrical distribution of trial means (Fig. 1(b)).

PD was non-live in six out of the eleven trials. In one of those trials (Trial 8), there was also a comparison PD treatment with a small concentration of live LAB1 bacteria (c. 10^5 cfu mL^{-1}) and that PD treatment was employed in the other five live-PD trials. Mean MS production was similar for non-live PD and live supplemented PD at 61.4 and 29.3 g MS/day respectively (no statistical significance, P=0.328). GLM analysis did not show statistically significant effects of trial year (P=0.965) or region (P=0.615). TMR fed herds tended to have higher PD response means than pasture fed herds (95.0 and 32.8 g MS/day) but this did not reach statistical significance (P=0.060).

In Trial 6, 10 mL/cow PD was trialled with and without the addition of 6 mL live LAB2 bacteria (c. 10^8 cfu mL^{-1}). There was a significant 98 g MS/cow/day reduction in MS production by cows receiving the live bacteria compared to control (P=0.010), while the non-live only treatment group produced 12 g MS/cow/day above control (not statistically significant).

PD Trials 4-11 monitored cow weights and body condition and no significant treatment differences were detected.

Discussion

Trials for both probiotic extract products showed variable results. While random variation alone could account for much of the distribution of results, the non-normal distribution of the RZP trial results indicated that product effect could vary related to farm conditions. Treatment responses may have been adversely influenced in three drought-affected trials, which were RZP Trial 18 (this trial was stopped early due to feed shortage), RZP Trial 16 (with low lamb growth rates) and PD Trial 8.

A feed change to brassicas caused an apparent lag in lamb growth rates in the first ten days in Trials 18 to 21 (Table 2). RZP positive effect on growth rate was clear during that initial period in two trials, indicating that RZP may assist in adjustment to feed change. Over all trials, however, there was insufficient evidence that RZP effect was greater on brassica fed lambs compared to pasture fed lambs.

RZP effect tended to increase slightly for trials that had higher control growth rates though this was not statistically significant. An opposite trend may have been expected if the mode of action of RZP was solely to suppress inflammatory responses in lambs (a postulated mode of action for non-live probiotics). Within a flock, lambs with inflammatory lung or intestine conditions would have generally low growth rates. If RZP treatment worked solely on those lambs then less negative skew of lamb growth rates may result. While RZP Trial 21 showed this trend, overall skewness actually tended to be more negative in RZP treatment groups (not statistically significant) indicating a more general productivity improvement. Although probiotic effect may be hypothesised to be higher in poorer producing animals, such a trend was not detected in this study nor in the Hoyos et al. (1987) study of yeast culture effect on cow milk production.

The approximately normal distribution of PD mean trial responses does not preclude farm conditions affecting treatment response. TMR-fed cows had generally higher PD responses but this was not statistically significant compared to pasture-fed cow results and may be confounded with higher per-cow productivity in TMR-fed herds.

The lower response to live LAB1 and LAB2 treatments (2 mL RZP, 2 mL LAB per lamb) compared to 4 mL RZP in Trials 19 and 20 may be partially due to the lower volume of non-live RZP product in the combined treatment. When RZP dosage was maintained in Trials 10 to 16 there was no significant negative or positive effect of adding LAB1. In PD Trial 6, the significant reduction in MS production in PD+LAB2 treated cows (LAB2 incorporated additional to the same PD dosage) may indicate a negative reaction to the bacteria or a reduction in PD efficacy when the product was mixed with high concentrations of live bacteria. Livestock responses to live bacterial probiotics can be dose variable with reduced responses sometimes reported at high doses in sheep (Soren & Sahoo 2011) and calves (Hutchenson et al. 1980; Orr et al. 1988). The results of the current study suggest that some caution is warranted in the supplementation with high doses of live lactic acid bacteria in some circumstances.

The combined trials approach increased the statistical power of analysis and demonstrated significant overall effects from the use of both RZP (P=0.001) and PD (P=0.008). The estimated economic return on investment based on median RZP effect and mean PD effect would be over 18.5:1 (median 0.445 kg LWG valued at NZ\$2.50 kg^{-1}; \$0.06 per dose) and around 2.7:1 (mean 0.050 kg MS response at NZ\$7.00 kg^{-1}; \$0.13 per dose) respectively. The measured responses of these products compare favourably with reported livestock responses to probiotics and yeast cultures (Poppy et al. 2012; Yoon & Stern, 1995), and provide evidence for the efficacy of non-live probiotic extracts in ruminants.

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