# A field study to evaluate a direct-fed microbial/enzyme product[1]

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Objective – This project was initiated to test a feed supplement in commercial dairy herds feeding Total Mixed Rations. The supplement contained a combination of yeast, enzymes, and microbials. The goal was to overcome the inability to divide each herd into treatment and control groups by designing an experiment whereby the same cows were measured before, during, and after consuming the supplement. We felt that with an appropriate statistical analysis, there would be sufficient "power" to detect responses in daily milk of half a kilogram per cow (about 1 lb). This presentation will focus on two things; 1) the unique design and analysis of the field study, and 2) the response to the supplement.

Description of Supplement - *Bacillus subtilis* (primarily viable spores, $1x10^8$ CFU/g and 60 BAU/g), *Aspergillus oryzae* (attenuated culture, 55 SKBU/g), *Lactobacillus acidophilus* (viable, but no specification). Enzymes were primarily *alpha*-amylase with lesser amounts of *beta*-glucanase, hemi-cellulase and cellulase. Yeast culture was viable *Saccaromyces cerevisiae* (minimum $1 \times 10^9$ CFU/g). (COMBO®, American Biosystems, Inc., Roanoke, VA)

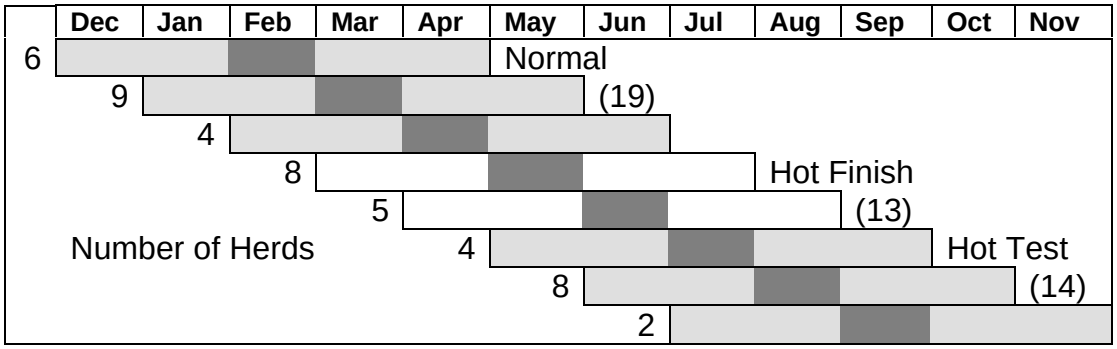
Special Challenges

- Unable to feed a control diet to other cows at the same time in the same herd.
- Unknown as to how many cows and herds were needed to "prove" anything.
- Problems in interpreting results based on feeding and withdrawing the supplement.
  - Lactation curve (days in milk) can hide individual cow response.
  - Season of the year masks individual herd response.
  - Age of cow may or may not have an effect.
  - Other supplements being fed may reduce response.

Overview of study – DHI records were used from all cows for 5 months from 46 herds (originally 50). The product was fed in the TMR at 21 grams/cow/day (7.5 oz) for 6 weeks in the midst of the 5 months. The shading indicates the 6 treatment weeks. Cows were restricted to those past 60 days in milk and having all 5 tests above 5 kilograms/d (11 lbs). The 60-day restriction is a critical point, as response to the product assumes there would have been a natural and consistent decline in production without the treatment. In the first of a two-part analysis, 5 averages were found for the cows on the study in each herd, one for each month. These averages were weighted in a second analysis to obtain 5 monthly averages for the entire study. A contrast of the 3rd test against the average of the 1st and 5th determined the response. **If the 3rd test was exactly equal to the average of the 1st and 5th tests, then the contrast will be zero and the supplement will have had no effect.**

[1] Presented at Dairy Nutrition Cow College, Blacksburg, VA, January 7, 1998.

Time Line – Herds entered the project staggered over 8 months to spread them across seasons. The first 19 herds completed the study before July. The second group finished in July or August, and the third group was feeding the product in July or August. Four herds did not test all 5 months and were discarded, hence 46 herds.




Record Counts – After restrictions for 60 days in milk (maximum of 365) and 5 consecutive DHI tests, 3,400 cows were used. This was 43% of all milking cows, and averaged 74/herd. Original herd sizes averaged 173 milking cows.

Distribution of Records – One third of the cows used were in 1st lactation and 1/6 were between 60 and 90 days in milk at Test 1.

Raw Means – Here are the raw means and their standard deviations for the cows in the study. Two-thirds of the cows will be between the mean +/- 1 SD. Days in milk averaged 200, or about 140 at 1st test. For pounds, multiply by 2.2 (29.8 kg = 66 lb).

Table 1. Means and standard deviations.

| Variable | Mean | SD |
|---|---|---|
| Milk, kg/d | 29.8 | 8.3 |
| Fat, kg/d | 1.03 | 0.31 |
| Fat, % | 3.51 | 0.76 |
| Protein, kg/d | 0.94 | 0.23 |
| Protein, % | 3.19 | 0.31 |
| Parity | 2.5 | 1.5 |
| BW, kg | 611 | 72 |
| DIM at mo 3 | 200 | 48 |

Individual Herd Analyses – The first analysis was applied to records from cows in individual herds, one herd at a time. Five averages were generated for each herd, one for each test day. A contrast of the 3rd minus the average of the 1st and 5th measured the response to supplement. This, however, is not a good indicator of overall product performance because an individual herd is subject to conditions on each test day, such as weather. Here is an example of the means from two herds. Herd 2 will count more in the overall analysis because it either had more cows or they produced more uniformly (lower SE).

Table 2. Example output from two herds.

| Herd | Test | Mean (kg) | SE | $1/SE^2$ |
|---|---|---|---|---|
| 1 | 1 | 28.4 | 0.31 | 10.54 |
| 1 | 2 | 27.9 | 0.31 | 10.54 |
| 1 | 3 | 25.5 | 0.31 | 10.54 |
| 1 | 4 | 25.5 | 0.31 | 10.54 |
| 1 | 5 | 24.5 | 0.31 | 10.54 |
| 2 | 1 | 33.8 | 0.56 | 3.21 |
| 2 | 2 | 31.2 | 0.56 | 3.21 |
| 2 | 3 | 30.4 | 0.56 | 3.21 |
| 2 | 4 | 27.2 | 0.56 | 3.21 |
| 2 | 5 | 25.1 | 0.56 | 3.21 |

All-Herds Analysis – These 46 sets of 5 averages were analyzed together to achieve an over-all average for each of the 5 test months. Again, a contrast of test 3 minus the mean of 1 and 5 provided an overall response under the hypothesis that, with no treatment, the 3rd test would be midway between the 1st and 5th.

Milk Analysis – Below are the resultant means from the all-herd analysis for milk (column labeled "All"). The contrast indicates a response of +0.64 kg/d (+1.4 lb), with the chance of getting a response this large by accident being only 3%. We had decided before the experiment to exclude test 2 from the contrast because the product had been fed for 2 weeks at that point, and to exclude test 4 because of potential carryover effects, particularly if someone continued to feed left-over product..

Table 3. Response at each test day (kg/cow per day) [Multiply by 2.2 for lbs]

| | | Lactation Number | | DIM at Test mo 1 | | |
|---|---|---|---|---|---|---|
| Variable | All | First | Older | <120 | 120 to 180 | >180 |
| Records, % | 100 | 33 | 67 | 39 | 38 | 23 |
| Test mo 1 | 33.1 | 29.0 | 35.1 | 35.8 | 33.0 | 29.7 |
| Test mo 2 | 31.7 | 28.4 | 33.3 | 34.7 | 31.4 | 28.1 |
| Test mo 3[1] | 29.9 | 27.7 | 30.9 | 32.8 | 29.4 | 26.6 |
| Test mo 4 | 28.4 | 26.8 | 29.0 | 31.4 | 27.8 | 25.1 |
| Test mo 5 | 25.4 | 25.0 | 25.5 | 28.5 | 24.7 | 22.2 |
| SE | 0.25 | 0.32 | 0.23 | 0.26 | 0.26 | 0.34 |
| Mo 3 vs. mo 1 and 5[2] | +0.64* | +0.73* | +0.56* | +0.65* | +0.59* | +0.65 |

[1] After 6 wk of supplementation, withdrawn after test mo 3.
[2] Milk production during mo 3 versus mean milk production during mo 1 and mo 5.
* Significant difference ($P<0.05$).

Individual Herd Response – Though results from individual herds are fairly meaningless due to environmental influences, a composite of herd results may be of interest. Twice as many herds responded to the supplement as did not respond.

Table 4. Responses of individual herds.

| Response | Herds | Note |
|---|---|---|
| Positive and significant | 17 | 9 normal and 8 finishing in summer |
| Positive | 14 | Spread evenly through all seasons |
| Negative | 8 | 3 normal and 5 testing in summer |
| Negative and significant | 7 | 3 normal and 4 testing in summer |

Milk by lact – In Table 3 above one can observe a slightly higher response from 1st calf heifers (+0.73 kg/d, +1.6 lb) than from older cows (+0.56 k/d, +1.2 lb).

Milk by Days in Milk – Again, from Table 3 above, the response was similar for different stages of lactation for cows when they entered the study.

Milk by Season – Season had a major influence on the results of this study. Fortunately, herds began the study in different months of the year, enabling us to determine the seasonal effects. Herds that finished before July had a response of +0.70 kg/day (+1.5 lb), close to the +0.64 (+1.4 lb) response overall. Herds that finished the study in the summer, July and August, appeared to have a larger response, but it was due to heat stress at the 5th test, making test 3 look especially good. Finally, those herds feeding the product in the summer showed a decline (-0.80 kg/d, -1.8 lb), though not significant, and due largely to heat stress at test 3.

Table 5. Herd responses in different seasons of the year. [Multiply by 2.2 for lbs]

| | Season[1] | | |
|---|---|---|---|
| Variable | Cool | Hot at mo 5 | Hot at mo 3 |
| Test mo 1 | 33.8 | 34.0 | 32.5 |
| Test mo 2 | 33.0 | 33.1 | 28.9 |
| Test mo 3[2] | 31.2 | 31.0 | 27.3 |
| Test mo 4 | 30.1 | 28.9 | 25.1 |
| Test mo 5 | 27.2 | 23.6 | 23.6 |
| SE | 0.28 | 0.51 | 0.48 |
| Mo 3 vs mo 1 and mo 5[3] | +0.70* | +2.21* | -.80 |

[1] Cool = Herds that finished the study before July, hot at mo 5 = herds that finished the study in July or August, and hot at mo 3 = herds that completed supplementation in July or August.
[2] After 6 wk of supplementation, withdrawn after test mo 3.
[3] Milk production during mo 3 versus mean milk production during mo 1 and mo 5.
* Significant response (P<0.05).

Other Responses – Fat % declined 0.10% during supplementation. Fat-corrected milk increased, but not significantly. Protein showed no significant change. Herds feeding another yeast product before or during this study responded similarly to the other herds.

A follow-up analysis on herds in the summer indicated that the supplement helped alleviate production decline in hot weather (1.8 lb less decline), but not statistically significant.

Probability of Detecting Responses – One of the important issues of this field study design is determining the number of herds necessary to detect significant responses. The most important factor is the number of herds, not the number of cows per herd. If the cows in each herd responded uniformly to a product, then fewer herds would be needed. But, cows are cows, and we know from studies such as this how they will naturally vary. Using the records from this study, and applying sample size techniques, we conclude that if one wishes to call significant a difference of 0.5 kg/d (1.1 lb), 50 herds will provide less than a 50:50 chance, whereas 100 herds will give a 75% chance. Moving to a 1 kg/d (2.2 lb) difference, there will be an 80% chance with 50 herds. Twenty-five herds will be adequate only for detecting responses of 1.25 kg (2.7 lb) or larger.

Profitability – Cost of supplement is 7.5 cents per cow per day. At $12/cwt milk price and the 1.4 lb response measured in this study, an investment of 7.5 cents will yield 16.5 cents more income ignoring additional feed costs, or 10.5 cents after theoretical feed cost to produce that response.

Table 6. Expected profitability from feeding the supplement (ignoring fat%).

| Milk Price ($/cwt) | Break-Even Response (lb/cow/d) | Net Cash at +1.4 lb/d Response ($/cow/d) | Net Cash minus Feed Costs ($/cow/d) |
|---|---|---|---|
| $ 10 | 0.75 lb | $ 0.07 | $ 0.01 |
| $ 12 | 0.62 lb | $ 0.09 | $ 0.03 |
| $ 14 | 0.53 lb | $ 0.12 | $ 0.06 |
| $ 16 | 0.46 lb | $ 0.15 | $ 0.09 |

Summary – This study has focused on the combined response of herds rather than individual cows. Cows were restricted to all 5 tests and at least 60 days in milk. Seasonal influences played an important role and cannot be ignored. This study detected a response of +0.64 kg/d (+1.4 lb) with a P value of 0.03 using 46 herds. Twice as many herds responded positively as negatively, and most negative responses occurred in the summer. We were able to calculate from these data appropriate numbers of herds required for future field studies of this nature.