A rapid mold and yeast enumeration technique is comparable to a conventional technique for animal feedstuffs

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Introduction:

- Feedstuff and TMR yeast and mold enumerations (colony-forming units per grams of feed, CFU/g) have grown in popularity to diagnose forage and diet stability and opportunities on dairy and beef farms
  - Mold and yeast enumerations can indicate spoiled feed and forage
  - Epiphytic yeast species (such as *Issatchenkia orientalis*) have further been implicated in negatively altering rumen function (Santos et al., 2015)
- Conventional enumeration techniques, such as that described by Adesogan et al. (2004), solubilize a sample and then serially dilute the sample prior to plating on an agar substrate
  - Mold and yeast spores and colonies are then allowed to grow for up to a week prior to reading colony counts (CFU) by direct microscopy and the most probable number technique
- Turnaround time with conventional enumeration (CON) limits utility
  - The CON technique requires a five-day, or longer, plate incubation prior to microscopy
  - The extended incubation time then equates to seven days, or more, from time of sampling to reporting results
- Recently a rapid yeast and mold enumeration technique, 3M Petrifilm Rapid Yeast and Mold Count (RAP), has been developed and validated upon human food-grade matrixes (accepted; AOAC 2014.05) .
  - The RAP technique offers faster turnaround and may have utility for animal agriculture

Objective:

The objective was to determine if RAP, tested under two incubation lengths at similar temperatures, is equivalent to CON.

Materials and Methods:

- Commercial farm corn silage (n=17), TMR (n=3), alfalfa silage (n=15), high moisture corn or snaplage (n=6), small grain silage (n=6), and concentrate (n=6) samples submitted for routine analysis by CON in late February, 2015 were further assayed using RAP
- When samples arrived, roughly 5g of feed was blended using a Waring laboratory blender (Conair Corporation, Stamford, CT) and stored at 1°C for later plating
- At plating, 1g of wet, blended feed was subsampled and diluted to 100ml in sterile Butterfield's phosphate buffer, shaken, then serially diluted to 1:1000, 1:10,000, and 1:100,000 for most probable number enumeration by both CON and RAP

For CON:

- Subsamples of each dilution were taken with sterile glass pipettes and plated on potato-dextrose agar (PDA) using the spread-plate method
- The glass spreader was sterilized by a momentary rinse, sequentially, in 50% HCl solution, followed by 100% acetone solution, followed by deionized water
- Plates were spread according to dilution 3, followed by dilution 2, followed by dilution 1 to avoid contamination
- PDA plates containing sample solutions were placed in an aerobic incubator set at 28°C (+/- 2°) and allowed to incubate for 5 days

For RAP:

- Subsamples of each dilution taken with an electronic pipette (3M, St. Paul, MN) and were plated on Petrifilm using a Petrifilm flat spreader (6425, 3M, St. Paul, MN)
- Plates were aerobically incubated at 28°C for both 48 hours and 5 days

Data Analysis:

- Post incubation, CFU counts were enumerated by direct microscopy for both CON and RAP
- Raw and log-transformed data were determined to be not normally distributed, hence data were fit using one-way analysis option of SAS JMP v11.0
- Technique (CON, RAP-48h, and RAP-5d) and feed main effects were compared using non-parametric Wilcoxon/Kruskal-Wallis test (Wilcoxon, 1945)
- Significance was declared if resulting Chi-square statistic p-value was <0.05

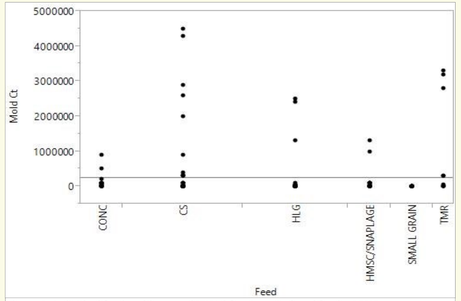

Figure A: Individual feed mold counts (CFU/g) for six agricultural feeds. CONC = concentrate feed, CS = corn silage, HLG = alfalfa silage, HMSC/Snaplage = high moisture shelled corn or snaplage, TMR = total mixed ration.

Table A: Mold count nonparametric comparisons for individual feeds using Wilcoxon (1945) method. Feed is measured against compared feed, with Feed being the greater measure, and p-Value < 0.05 suggests a significant difference between the pair.

Feed Type	Compared Feed	Score Mean Difference	St. Err. Diff	Z	p-Value
TMR	HLG	21.47	5.59	3.84	0.0001
TMR	CS	19.22	5.86	3.28	0.001
TMR	SMALL GRAIN	13.00	3.01	4.33	<.0001
HMSC/SNAPLAGE	HLG	11.08	4.96	2.24	0.0254
HMSC/SNAPLAGE	CS	10.22	5.13	1.99	0.0461
TMR	HMSC/SNAPLAGE	7.17	3.23	2.22	0.0264
TMR	CONC	5.75	3.22	1.78	0.0745
HLG	CS	2.84	5.25	0.54	0.5879
HMSC/SNAPLAGE	CONC	-2.11	3.46	-0.61	0.5421
SMALL GRAIN	CS	-8.04	4.67	-1.72	0.0854
CS	CONC	-11.20	5.12	-2.19	0.0288
SMALL GRAIN	HLG	-11.82	4.68	-2.53	0.0116
SMALL GRAIN	HMSC/SNAPLAGE	-12.17	3.28	-3.71	0.0002
SMALL GRAIN	CONC	-12.67	3.28	-3.86	0.0001
HLG	CONC	-14.00	4.96	-2.82	0.0048

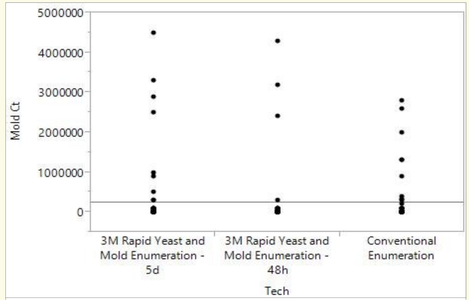

Figure B: Individual feed mold counts (CFU/g) for three mold enumeration techniques, replicated across feeds. Techniques were not different.

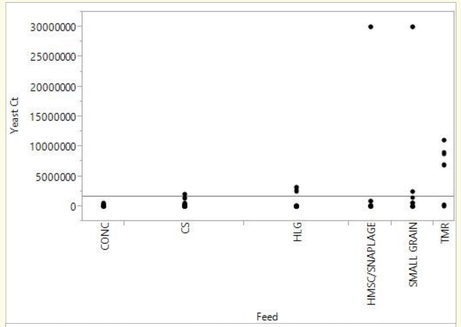

Figure C: Individual feed yeast counts (CFU/g) for six agricultural feeds. CONC = concentrate feed, CS = corn silage, HLG = alfalfa silage, HMSC/Snaplage = high moisture shelled corn or snaplage, TMR = total mixed ration.

Table B: Yeast count nonparametric comparisons for individual feeds using Wilcoxon (1945) method. Feed is measured against compared feed, with Feed being the greater measure, and p-Value < 0.05 suggests a significant difference between the pair.

Feed Type	Compared Feed	Score Mean Difference	St. Err. Diff	Z	p-Value
TMR	HLG	25.00	5.69	4.39	<.0001
TMR	CS	24.58	6.13	4.01	<.0001
HMSC/SNAPLAGE	HLG	13.96	5.07	2.76	0.0059
HMSC/SNAPLAGE	CS	13.42	5.36	2.50	0.0124
SMALL GRAIN	CS	12.18	5.33	2.28	0.0223
TMR	CONC	10.75	3.23	3.33	0.0009
SMALL GRAIN	HLG	7.54	5.05	1.49	0.1352
TMR	HMSC/SNAPLAGE	6.42	3.22	1.99	0.0464
TMR	SMALL GRAIN	5.92	3.23	1.83	0.0669
HMSC/SNAPLAGE	CONC	1.50	3.49	0.43	0.6677
HLG	CS	0.73	5.54	0.13	0.8949
SMALL GRAIN	CONC	0.50	3.50	0.14	0.8865
SMALL GRAIN	HMSC/SNAPLAGE	-1.22	3.48	-0.35	0.7254
CS	CONC	-11.73	5.39	-2.17	0.0297
HLG	CONC	-15.21	5.08	-2.99	0.0027

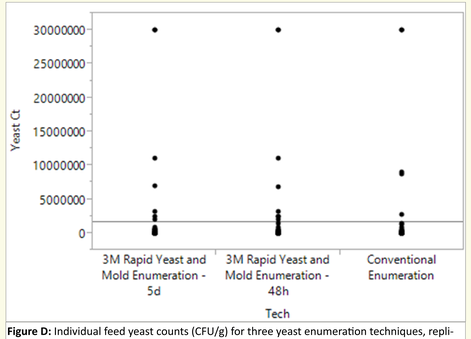

Figure D: Individual feed yeast counts (CFU/g) for three yeast enumeration techniques, replicated across feeds. Techniques were not different.

Results and Discussion:

- Yeast and mold count mean and median across feeds and techniques were:
  - Yeast count: mean of 1.69×10^6 and median of 1×10^3
  - Mold count: mean of 2.53×10^5 and median of 1×10^4
- For mold and yeast enumeration, techniques did not differ (P>0.05) while feed types differed (P<0.01)
  - Tested samples represent random samples submitted by commercial dairy and feedlot consultants
  - TMR samples were greater in both yeast and mold counts than many individual respective feed types (Tables A and B)

Conclusions

- Results suggest both yeast and mold enumeration results are comparable for the agricultural feeds assessed among the techniques tested here
- Future work is warranted to further evaluate the technique performance across a wider array of agricultural feedstuffs
- The 3M Perifilm Rapid Yeast and Mold enumeration technique appears to offer faster sample turnaround with comparable results to the conventional enumeration technique.

References:

Adesogan, A.T., N. Krueger, M.B. Salawu, D.B. Dead and C.R. Staples. 2004. The influence of treatment with dual purpose bacterial inoculants or soluble carbohydrates on the fermentation and aerobic stability of bermudagrass. J Dairy Sci. 87:3407-3416.

Santos, M.C., A.L. Lock, G.D. Mechor, and L. Kung. 2015. Effects of a spoilage yeast from silage on in vitro ruminal fermentation. J Dairy Sci. 98:2603-2610.

Wilcoxon, F. 1945. Individual Comparisons by Ranking Methods. Biometrics Bulletin. Vol 1 (6): 80-83.