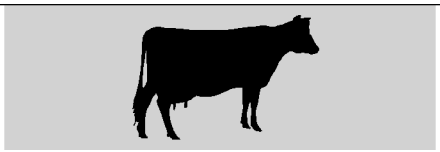
The Professional Animal Scientist 24 (2008):254–259



Case Study: Effect of Propionibacteria Supplementation on Yield of Milk and Milk Components of Dairy Cows

M. B. de Ondarza* and W. M. Seymour,†[1] PAS
*Paradox Nutrition LLC, West Chazy, NY 12992; and †DSM Nutritional Products, Parsippany, NJ 07054

ABSTRACT

A well-managed, high-producing commercial dairy herd was used to test the impact of dietary propionibacteria (Propionibacteria freudenreichii, P169) supplementation (6×10^{10} cfu/d per cow). Inclusion of propionibacteria in the diet increased ($P < 0.05$) milk production (44.31 and 43.06 kg/d for propionibacteria and control diets, respectively), especially in early lactation (0 to 100 DIM) and in older cows (3rd lactation and greater). The production of 3.5% fat-corrected milk and milk protein percentage were not affected by treatment. Overall milk true protein production (kg/d) was not affected by propionibacteria supplementation. Propionibacteria supplementation did, however, positively impact ($P = 0.01$) milk true protein (kg/d) in late lactation cows (>201 DIM). Daily DMI were excellent considering the summer heat and humidity during the study (23.16 vs. 22.39 kg/d per cow consumed by propionibacteria and control cows, respectively). Pregnancy rate was numerically greater ($P = 0.12$) with propionibacteria supplementation (15 vs. 23% for control vs. treatment). Feeding a specific propionibacteria (P169) at 6×10^{10} cfu/d per cow had beneficial effects on high producing dairy cows. Further research is needed to better understand the complete mode of action.

Key words: propionibacteria, milk yield, milk components, reproduction

INTRODUCTION

High grain diets and abrupt increases in grain intake encourage the growth of lactate producing bacteria such as *Streptococcus bovis* and *Lactobacilli* (Owens et al., 1998). Lactate is a reducer of rumen pH. Supplemental direct-fed microbials such as propionibacteria may reduce rumen acidosis and increase gluconeogenesis. Propionibacteria convert lactate primarily to propionate (Krehbiel et al., 2003). *Propionibacterium shermanii* (10^6 cfu/ml) increased molar proportions of propionate in batch cultures with mixed rumen microorganisms (Kung et al., 1991).

Some in vivo studies have shown limited response to propionibacteria supplementation. When *P. acidipropionici* strain DH42 was fed to steers on a high concentrate diet at 10^7 cfu/d, rumen propionate concentrations increased, rumen acetate concentrations decreased, and rumen pH was unaffected (Kim et al., 2000). Ghorbani et al. (2002) supplemented feedlot cattle on a high concentrate diet with *Propionibacterium* P15 (10 g at 10^9 cfu/g), which had no effect on ruminal pH but increased rumen protozoal concentrations. Francisco et al. (2002) supplemented 17 g/d of propionibacteria culture from –2 to 12 wk postcalving, which did not affect milk production, percentage milk fat, and reproduction. When *P. freudenreichii* (2×10^9 cfu/d) were supplemented with other direct-fed microbials to mid-lactation Holstein dairy cows, production and rumen parameters were not affected (Raeth-Knight et al., 2007).

Others have shown positive responses to propionibacteria supplementation with a limited number of cows. Stein et al. (2006) fed Holstein cows a control diet (n = 13), a diet with a low dose (6×10^{10} cfu/d) of propionibacteria strain 169 (**P169**; n = 14), or a diet containing a high dose (6×10^{11} cfu/d) of P169 (n = 11) from –2 to 30 wk postpartum. The supplementation increased 4% fat-corrected milk (**FCM**; 29.9, 32.7, and

[1]Corresponding author: william-m.seymour@dsm.com

32.2 kg/d for control, low dose, and high dose, respectively). Multiparous cows fed the high dose of P169 had greater molar percentages of rumen propionate, reduced rumen pH, and reduced milk fat percentage. In a similar study, Lehloenya et al. (2007) found that P169 (6×10^{11} cfu/d) tended to improve solids-corrected milk production but reduced milk fat percentage. The objective of this trial was to determine the effect of supplementing P169 on milk production and milk components of a greater number of high-producing cows.

MATERIALS AND METHODS

Animals

Treated and control cows were housed in a freestall barn in separate pens in a side-by-side study. The study was 8 wk in length, beginning July 17, 2006, and ending September 10, 2006. Cows were fed TMR once per day and had ad libitum access to TMR and water. Cows were milked 3 times per day. All procedures were in accordance with the guidelines presented in *Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999).

Experimental Treatments

Half of the cows received P169 (6×10^{10} cfu/d per cow) and half did not. The basal ration consisted of corn silage, alfalfa-grass haylage, cornmeal, whole cottonseed, and a commercial feed blend. Cows were fed 266 mg/d per cow monensin (Rumensin 80, Elanco Animal Health, Greenfield, IN). Daily feeding of the cows began with the control group; then the treated pen was fed one TMR batch without P169, followed immediately by a second TMR batch containing the P169 so that the treated TMR was in the top portion of the daily ration in front of the cows.

Measurements and Analytical Methods

Daily pen DMI were monitored and recorded. The nutrient analyses of the diets consumed were calculated using CPM Dairy (1998). Prior to beginning the study and every 2 wk throughout the study, all forages were sampled and analyzed via chemical analysis at Cumberland Valley Analytical Services, Hagerstown, MD. Samples were analyzed for DM [forages: 105°C for 3 h per National Forage Testing Association recommendations (NFTA, 2002); grains: Method 930.15, AOAC, 2000], ash (Method 942.05; AOAC, 2000 modified with 0.5 g sample weight and 535°C furnace temperature), ether extract (Method 920.39; AOAC, 1990, modified with anhydrous ether extraction, boiled 20 min, rinsed 20 min), CP (Method 990.03; AOAC, 2000), soluble CP (Krishnamoorthy et al., 1982), ADF (Method 973.18: AOAC 2000, modified with Whatman 934-AH glass micro-fiber filters with 1.5μm particle retention used in place of fritted glass crucible), NDF (Goering and Van Soest, 1970, modified without sodium sulfite and using Whatman 934-AH glass micro-fiber filters with 1.5μm particle retention used in place of fritted glass crucible), lignin (Goering and Van Soest, 1970), starch (Holm et al., 1986), sugar (Dubois et al., 1956). Non fiber carbohydrate was calculated as the difference between 100 and the sum of CP (minus NDF bound CP), NDF, ether extract, and ash. Analyses of Ca, P, Mg, K, Na, Fe, Zn, Cu, and Mn were conducted by atomic spectroscopy (Perkin Elmer 3300 XL ICP, Perkin Elmer, Shelton, CT) according to the AOAC (2000) 985.01 method. Sulfur was analyzed using a Leco S-144DR sulfur combustion analyzer (Leco, St. Joseph, MI). Chloride ion was extracted with 1% nitric acid and analyzed using a Corning 925 chloride analyzer (CIBA-Corning, Medfield, MA).

Daily milk production of individual cows was recorded using the AFI2000 V1.28 system (S.A.E. Afikim, Kibbutz Afikim, Israel). Milk was sampled on wk 5 and 6 of the study. Milk samples were preserved with Bronolab-W II (D&F Control Systems Inc., Dublin, CA), a liquid preservative containing the active ingredient Bronopol, and sent to Dairy One Milk Laboratories (Ithaca, NY). Milk was analyzed for fat, true protein, SCC, and milk urea nitrogen (**MUN**) using an infrared analyzer (Milk-O-Scan 4000; Foss Electric, Hillerod, Denmark).

Table 1. Overview of the cow characteristics by treatment

Item	Control	Propionibacteria
Total cows	119	115
Total observations	4,998	4,830
Cows – 2nd lactation	57	50
Cows – 3rd lactation	32	42
Cows – 4th lactation	9	10
Cows – 5th lactation	9	10
Cows – 6th lactation	6	2
Cows – 7th lactation	5	1
Cows – 8th lactation	1	0
Mean lactation number[1]	3.11 ± 1.48	2.91 ± 1.08
Mean previous ME 305-d milk[2]	25,298 ± 5,337	26,385 ± 5,389
Mean DIM	144 ± 61	140 ± 64
Mean conductivity (millimho)	11.67 ± 1.14	11.36 ± 1.02
Mean activity (steps/h)	125 ± 57	121 ± 54

[1]Mean ± SE.

[2]ME = mature equivalent.

Daily milk conductivity and daily activity were measured and recorded using AFI2000 V1.28 system (S.A.E. Afikim). Milk conductivity (measured in millimho) indicates the onset of mastitis (Jones et al., 1994; Chagunda et al., 2006). An increase in total ion concentration of milk increases the milk conductivity. Activity was measured using a pedometer that recorded the number of steps per hour for each animal in the trial. An increase in activity indicates a cow may be exhibiting estrus.

Pregnancy rate (the percentage of cows who were eligible to become pregnant and became pregnant) was evaluated for six 21-d periods from the beginning of the trial (July 17, 2006) using Dairy Comp 305 (Valley Ag Software, Tulare, CA).

Statistical Analysis

Prior to beginning the study, a balanced cow subset was compiled based on lactation number, DIM, and milk production during the week before the start of the study (Table 1). At the end of the study, 119 cows in the control subset and 115 cows in the treatment subset remained in the groups for the entire study. These cows were used for data analysis. Data were analyzed using JMP statistical software (SAS Inst. Inc., Cary, NC) to determine if milk production and milk component production were affected by treatment. Milk yield, percent milk fat, kilograms milk fat, percent protein, kilograms protein, 3.5% FCM, MUN, and SCC were analyzed using JMP REML model fitting protocol (SAS Inst. Inc.), and subsequent multiple 2-sample comparisons were performed with Tukey's honest significant differences test. The model fit used DIM (by category), lactation number (by category), and pen (treatment vs. control) as independent factors. Milk conductivity, previous milk (average from 2 wk before start of study), previous 3.5% FCM, previous milk fat (% or kg), previous true protein (% or kg), and previous MUN were included as covariates in the statistical models.

Pair-wise comparisons between control and treatment within lactation category (parity 2 or 3+) and within DIM category (0 to 100 DIM, 101 to 200 DIM, and 201+ DIM) were subsequently recalculated using JMP contrast analysis, which utilizes Student's *t*-test. The Student's *t*-test does not adjust for multiple comparisons and is less conservative than Tukey's honest significant differences test that did not detect any significant differences in this study. Significant differences from the contrast analysis indicate trends in the data. The effect of treatment on pregnancy rate was evaluated using a Chi-square approximation (Wilcoxon/Kruskal-Wallis Test; JMP software; SAS Inst. Inc.).

RESULTS AND DISCUSSION

Animals

The 2 groups of cows used for the study were very similar in lactation number (3.11 and 2.91 for control and treatment, respectively), DIM (144 and 140 for control and treatment, respectively), conductivity, and activity (Table 1).

Experimental Diets

The diet was typical of high production diets fed in the northeast United States. Diet nutrient analyses and diet ingredient compositions were calculated from means of daily ingredients

Table 2. Ingredient composition of diets by treatment (% DM)

Item	Control	Propionibacteria
Corn silage, brown midrib	26.93	26.88
Mixed alfalfa and grass haylage	25.19	25.15
Corn meal	19.49	19.52
Soybean meal 48% CP	6.86	6.85
Whole cottonseed	6.66	6.78
Soy hulls	2.61	2.60
Canola meal	2.25	2.25
Amino Plus[1]	1.99	1.99
Corn distillers grains	1.19	1.19
Calcium carbonate	1.11	1.10
Dextrose	0.73	0.73
Blood meal	0.74	0.74
Energy Booster[2]	0.64	0.63
Sodium bicarbonate	0.57	0.56
Molasses	0.58	0.58
Rumen bypass fat[3]	0.44	0.44
Fat mixer	0.47	0.47
Salt	0.38	0.38
Dicalcium phosphate	0.25	0.25
Yeast culture[4]	0.23	0.23
Whey permeate	0.21	0.21
Urea	0.15	0.15
Magnesium oxide	0.11	0.11
Calcium sulfate	0.06	0.06
Trace minerals and vitamins	0.11	0.11
Alimet[5]	0.01	0.01

[1]Ag Processing Inc., Omaha, NE.
[2]MS Specialty Nutrition, Dundee, IL.
[3]Cargill, Minneapolis, MN.
[4]Diamond V XP, Diamond V Corp., Cedar Rapids, IA.
[5]Novus Int. Inc., St. Louis, MO.

Table 3. Nutrient analysis of diets by treatment

Item	Control	Propionibacteria
Ration DM, % as-fed	49.81	49.84
CP, % DM	18.34	18.34
Soluble CP, % CP	39.32	39.31
RUP, % CP	34.05	34.42
Met (% metabolizable protein)	1.97	1.96
Lys (% metabolizable protein)	6.88	6.86
NE_l, Mcal/kg	1.74	1.74
ADF, % DM	21.97	21.98
NDF, % DM	32.61	32.62
Forage NDF, % DM	22.92	22.89
Lignin, % DM	4.15	4.16
Non fiber carbohydrate, % DM	37.30	37.29
Sugar, % DM	3.81	3.82
Starch, % DM	22.65	22.66
Soluble fiber, % DM	6.55	6.54
Ether extract, % DM	6.18	6.18
Long-chain fatty acids, % DM	5.03	5.04
Duodenal C18:1 *trans* FA (g/d)	113.44	119.75
Ash, % DM	7.92	7.91
Ca, % DM	1.00	1.00
P, % DM	0.40	0.40
Mg, % DM	0.30	0.30
K, % DM	1.39	1.39
S, % DM	0.24	0.24
Cl, % DM	0.51	0.51
Zn, ppm	101.30	101.21
Cu, ppm	21.68	21.66
Mn, ppm	58.89	58.82
Se, ppm	0.37	0.37
Co, ppm	1.14	1.14
I, ppm	0.79	0.79
Vitamin A, kIU/kg	6.25	6.23
Vitamin D, kIU/kg	1.14	1.14
Vitamin E, IU/kg	26.97	26.93

offered in the TMR and adjusted for refusals (Tables 2 and 3). Daily pen DMI were excellent throughout the study for both groups especially considering the summer heat and humidity, with 23.16 kg/d per cow consumed by propionibacteria-supplemented cows vs. 22.39 kg/d per cow consumed by control cows.

Milk Yield and Milk Components

Overall milk production was excellent throughout the study with means of 44.31 kg/d and 43.06 kg/d for the propionibacteria supplemented and control diets, respectively. Milk production was positively affected ($P = 0.04$) by propionibacteria supplementation (Table 4). Early lactation cows (0 to 100 DIM) and older cows (3rd lactation and greater) had the most positive response ($P < 0.03$) of all animal categories (Table 5).

Milk fat percentage was numerically reduced ($P = 0.14$) for cows fed propionibacteria with means of 3.45% for cows fed propionibacteria and 3.57% for cows on the control diet (Table 4). This trend was especially true for early lactation cows (0 to 100 DIM) and cows in their second lactation ($P < 0.12$; Table 5). The 3.5% FCM was not affected by propionibacteria supplementation (Table 4). Stein et al. (2006) and Lehloenya et al. (2007) had improvements in milk production with propionibacteria supplementation, yet had some negative effects on percent milk fat. This makes sense if the mode of action of propionibacteria is primarily increasing production of propionate for gluconeogenesis without increasing ruminal pH.

Overall milk fat production (kg/d) was unaffected by treatment (Table 4). When cows were separated according to stage of lactation and lactation number, cows between 0 and 100 DIM supplemented with propionibacteria produced 1.54 kg/d milk fat and control cows produced 1.60 kg/d ($P = 0.14$) (Table 5).

Milk protein percentage and overall milk protein production (kg/d) were

Table 4. Production and component least-squares means and SE of all cows by treatment

	Control		Propionibacteria		
Item	LS Mean	SE	LS Mean	SE	*P*-value
Milk, kg/d	43.06	0.42	44.31	0.43	0.04
3.5% FCM,[1] kg/d	43.54	0.57	43.85	0.58	0.70
Fat, %	3.57	0.06	3.45	0.06	0.14
Fat, kg/d	1.53	0.03	1.52	0.03	0.92
Protein, %	2.79	0.02	2.82	0.02	0.33
Protein, kg/d	1.21	0.01	1.24	0.01	0.20
MUN,[2] mg/dL	13.88	0.13	14.01	0.13	0.49
SCC × 1,000	242.89	52.74	161.19	54.26	0.30

[1]FCM = fat-corrected milk.

[2]MUN = milk urea nitrogen.

Table 5. Production and component least squares means according to parity and DIM by treatment

	Control		Propionibacteria		
Item	LS Mean	SE	LS Mean	SE	*P*-value[1]
Milk,[2] kg/d					
Parity 2	42.92	0.61	43.51	0.65	0.50
Parity 3+	43.21	0.58	45.10	0.57	0.02
0–100 DIM	44.68	0.47	46.17	0.48	0.03
101–200 DIM	43.41	0.44	44.53	0.45	0.07
201+ DIM	41.10	0.49	42.24	0.51	0.11
3.5% FCM,[2,3] kg/d					
Parity 2	42.79	0.82	42.25	0.87	0.65
Parity 3+	44.28	0.79	45.46	0.77	0.28
0–100 DIM	45.48	0.63	44.73	0.64	0.40
101–200 DIM	43.30	0.58	44.04	0.60	0.37
201+ DIM	41.83	0.65	42.79	0.67	0.30
Fat,[3] %					
Parity 2	3.51	0.08	3.32	0.08	0.10
Parity 3+	3.63	0.08	3.58	0.07	0.68
0–100 DIM	3.56	0.10	3.34	0.10	0.11
101–200 DIM	3.43	0.07	3.49	0.08	0.59
201+ DIM	3.72	0.12	3.53	0.11	0.23
Fat,[2,3] kg/d					
Parity 2	1.48	0.04	1.44	0.04	0.45
Parity 3+	1.57	0.04	1.61	0.04	0.51
0–100 DIM	1.60	0.03	1.54	0.03	0.14
101–200 DIM	1.51	0.03	1.53	0.03	0.62
201+ DIM	1.48	0.03	1.51	0.03	0.48
Protein,[2] %					
Parity 2	2.79	0.03	2.83	0.03	0.30
Parity 3+	2.80	0.03	2.81	0.03	0.72
0–100 DIM	2.73	0.03	2.72	0.03	0.73
101–200 DIM	2.81	0.03	2.86	0.03	0.20
201+ DIM	2.84	0.04	2.88	0.04	0.39
Protein,[2] kg/d					
Parity 2	1.20	0.02	1.22	0.02	0.37
Parity 3+	1.23	0.02	1.25	0.02	0.34
0–100 DIM	1.30	0.02	1.28	0.02	0.50
101–200 DIM	1.21	0.01	1.25	0.01	0.17
201+ DIM	1.13	0.02	1.18	0.02	0.01
MUN,[4] mg/dL					
Parity 2	13.90	0.18	14.21	0.19	0.24
Parity 3+	13.86	0.17	13.81	0.17	0.82
0–100 DIM	13.91	0.23	13.82	0.22	0.79
101–200 DIM	14.01	0.17	14.20	0.19	0.45
201+ DIM	13.73	0.26	14.00	0.26	0.45

[1]Student's *t*-test contrast analysis does not adjust for multiple comparisons. Significant differences indicate likely trends in the data.
[2]DIM category interactions with treatment were significant ($P < 0.05$).
[3]Lactation category interactions with treatment were significant ($P < 0.05$).
[4]MUN = milk urea nitrogen.

not affected by propionibacteria supplementation (Table 4). Propionibacteria supplementation did, however, positively impact ($P = 0.01$) milk protein (kg/d) in late-lactation cows (201 DIM and greater; Table 5), suggesting a possible improvement in protein supply in late lactation.

There was no difference among treatments in overall MUN (mg/dL) levels (14.01 and 13.88 mg/dL for propionibacteria supplemented and control cows, respectively; Table 4), regardless of DIM or lactation number (Table 5), suggesting propionibacteria

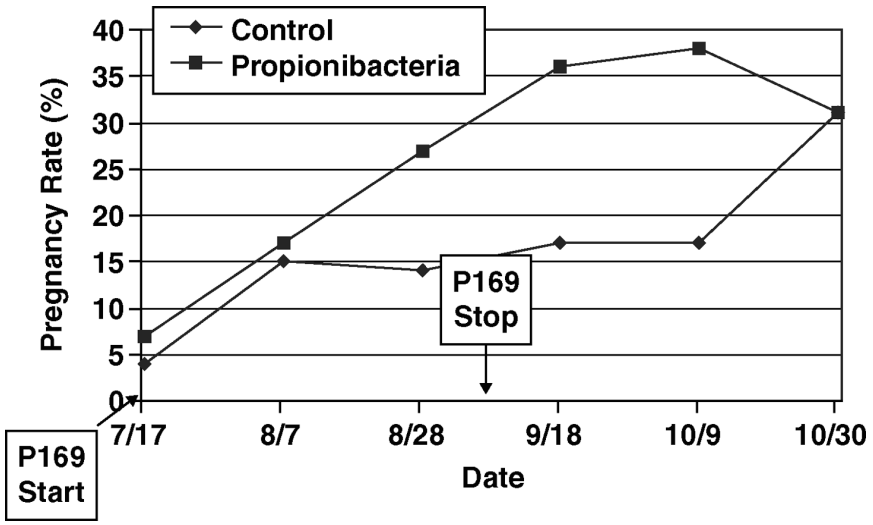


Figure 1. The percentage of cows who were eligible to become pregnant and became pregnant (pregnancy rate, %) by 21-d period for 18 wk following trial start.

did not impact utilization of nitrogen by the cow. Somatic cell count was not affected by treatment (Table 4).

Reproduction

Pregnancy rate (%) for six 21-d periods during 18 wk from the start of the trial is shown in Figure 1. Pregnancy rate was numerically greater ($P = 0.12$) with propionibacteria supplementation (15 vs. 23% for control vs. treatment). A larger multi-site study would be helpful to better understand the impact of propionibacteria supplementation on reproduction.

IMPLICATIONS

It appears from the milk and milk component responses in this study that supplemental P169 may have increased absorbed propionate from the rumen and enhanced gluconeogenesis. It would be beneficial to prove this mode of action with more basic research. Any positive effects of P169 supplementation on rumen pH were not evident from this work, but future studies with continuous measurement of rumen pH should provide more insight. This study also suggests possible reproductive benefits from propionibacteria supplementation possibly due to increased net energy absorption by way of increased propionate yield in the rumen. Future studies with more cows and multiple sites are needed to prove this.

LITERATURE CITED

AOAC. 1990. Official methods of analysis. Vol. 1. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2000. Official methods of analysis. Vol. 1. 17th ed. Assoc. of Official Analytical Chemists, Arlington, VA.

Chagunda, M. G. G., N. C. Friggens, M. D. Rasmussen, and T. Larsen. 2006. A model for detection of individual cow mastitis based on an indicator measured in milk. J. Dairy Sci. 89:2980.

CPM-Dairy, 1998. Version 3.07a., Cornell University, Ithaca, NY, University of Pennsylvania, Kennett Square, PA, W. H. Miner Agricultural Research Institute, Chazy, NY.

Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.

Ghorbani, G. R., D. P. Morgavi, K. A. Beauchemin, and J. A. Z. Leedle. 2002. Effects of bacterial direct-fed microbials on rumen fermentation, blood variables, and the microbial populations of feedlot cattle. J. Anim. Sci. 80:1977.

Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

FASS. 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1st rev. ed. Fed. Anim. Sci. Soc., Savoy, IL.

Francisco, C.C., C.S. Chamberlain, D.N. Waldner, R.P. Wettemann, and L.J. Spicer. 2002. Propionibacteria fed to dairy cows: effects on energy balance, plasma metabolites and hormones, and reproduction. J. Dairy Sci. 85:1738.

Holm, J., I. Bjorck, A. Drews, and N.G. Asp. 1986. A rapid method for the analysis of starch. Starch 7:224.

Jones, L. R., S. L. Spahr, and H. B. Puckett. 1994. Variability and reliability of real-time milk conductivity data. J. Dairy Sci. 77:80.

Kim, S. W., D. G. Standorf, H. Roman-Rosario, M. T. Yokoyama, and S. R. Rust. 2000. Potential use of *Propionibacterium acidipropionici* strain DH42, as a direct-fed microbial for cattle. J. Anim. Sci. 78(Suppl.1):292. (Abstr.).

Krehbiel, C.R., S.R. Rust, G. Zhang, and S.E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. J. Anim. Sci. 81(E. Suppl.2):E120.

Krishnamoorthy, U., T. V. Muscato, C. J. Sniffen, and P. J. Van Soest. 1982. Nitrogen fractions of selected feedstuffs. J. Dairy Sci. 65:217.

Kung, L., Jr., A. Hession, R. S. Tung, and K. Maciorowski. 1991. Effect of *Propionibacterium shermanni* on ruminal fermentations. p. 33 in Proc. 21st Biennial Conf. on Rumen Function. Chicago, IL. (Abstr.).

Lehloenya, K. V., D. R. Stein, D. T. Allen, G. E. Selk, D. A. Jones, M. M. Aleman, T. G. Rehberger, K. J. Mertz, and L. J. Spicer. 2008. Effects of feeding yeast and propionibacteria to dairy cows on milk yield and components and reproduction. J. Anim. Physiol. Anim. Nutr. 92:190.

NFTA. 2002. 2.2.2.5. Dry matter by oven drying for 3 hr at 105°C. http://www.foragetesting.org/lab_procedure/sectionB/2.2/part2.2.2.5.htm Accessed Mar. 17, 2008. NFTA, Omaha, NE.

Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1998. Acidosis in cattle: A review. J. Anim. Sci. 76:275.

Raeth-Knight, M. L., J. G. Linn, and H. G. Jung. 2007. Effect of direct-fed microbials on performance, diet digestibility, and rumen characteristics of Holstein dairy cows. J. Dairy Sci. 90:1802.

Stein, D. R., D. T. Allen, E. B. Perry, J. C. Bruner, K. W. Gates, T. G. Rehberger, K. Mertz, D. Jones, and L. J. Spicer. 2006. Effects of feeding propionibacteria to dairy cows on milk yield, milk components, and reproduction. J. Dairy Sci. 89:111.