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# Performance of dairy cows administered probiotic in water troughs

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## ABSTRACT

The purpose of this trial was to determine if a yeast product (YP: ProDairy, Donaghys, Christchurch, New Zealand) given in water troughs increased milk production or altered rumen pH and blood parameters. Multiparous cows (930) in a commercial herd were randomly assigned to 1 of 4 pens as they reached 30 DIM. Milk yield, fat, and protein were measured every other week for 11 wk. Two of the 4 pens received YP at the rate of 9 mL/cow per day. All 4 pens were fed the same diet (525 q/kq of DM. 186 g/kg of CP, 220 g/kg of ADF, 329 g/kg of NDF, 43.8 g/kg of fat, 188 g/kgof starch, 41.3 g/kg of lignin, and 81.9 g/kg of ash). Statistics were performed using PROC MIXED with random effects pens nested within treatment and the fixed effects of DIM, week, and parity. Average daily milk yield (43.1 and 44.8 kg, P = 0.042 for control and supplemented pens, respectively, were greater in YP pens. But milk fat (1.47 and 1.45 kg, P = 0.13) and milk protein (1.24 and 1.23 kg, P = 0.045) for control and supplemented pens, respectively, were lower in YP pens. Overall rumen pH(7.7 and 7.4, P = 0.044) and blood

ketone bodies (0.73 and 0.64 mEq/L, P = 0.011) were also reduced in supplemented pens. Therefore, YP did increase milk yield and affect rumen pH and blood ketone bodies, but other conditions on the commercial dairy may have influenced the milk response to YP. Depending upon the ability of the dairy to manage a consistent water supply, the delivery of YP via water should be considered by nutritionists and managers. More research is needed to determine the influence of other factors on milk response to YP supplemented in the water supply.

**Key words:** probiotic, rumen pH, dairy cow performance

#### INTRODUCTION

Improvements in milk production, feed intake, rumen pH, and metabolic function with supplementation of yeast culture products have been inconclusive. Past studies have shown modest increases in milk yield, milk fat yield, DMI, rumen pH, and OM digestibility (Desnoyers et al., 2009; Poppy et al., 2012). However, these results may not be significant due to insufficient sample sizes and management effects, such as concentrate level of the diet, number of feedings or push-ups per day, length of time of TMR mixing, how cows are grouped,

and so on (Piva et al., 1993; Desnoyers et al., 2009). Cows that are close to calving and in early stages of lactation have a greater response to yeast supplementation than mid- to latelactation cows (Erdman and Sharma, 1989; Wohlt et al., 1998; Erasmus et al., 2005; Nocek et al., 2011). Inclusion of a yeast product into a TMR diet that is lower in NDF concentration showed a greater increase in milk vield (Desnovers et al., 2009) and may alleviate milk fat depression (Erdman and Sharma, 1989) and decrease rumen pH and fiber digestion associated with subacute ruminal acidosis (Wallace 1994; Krause and Oetzel, 2006; Marden et al., 2008; Calsamiglia et al., 2012). Effects of feeding strategies, such as frequency of feeding and time since last feeding, on rumen pH are also moderated by feeding yeast products (Bach et al., 2007).

In addition to feeding and management effects on milk production and rumen function, it is also unknown how other feed ingredients may influence performance of the yeast product. As most yeast products are incorporated into a mix pellet and then added to a TMR, the acidity, moisture level, and oxidizing potential of other TMR ingredients may be altering the efficacy of yeast products (**YP**). Growth factors, pro-vitamins,

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or micronutrients and availability of these factors through different processing methods may influence their performance in the rumen (Nocek et al., 2011). Inclusion into TMR that is commonly 50% DM, fairly acidic (if based on corn silage), or processed into a pellet may change the functionality of the YP. Studies that focus on direct inclusion of a yeast product to the rumen (Harrison et al., 1988; Chung et al., 2011), in vitro continuous culture (Miller-Webster et al., 2002), or more stable feeding environment (water) may better represent results of yeast supplementation. Therefore, the purpose of the current study was to evaluate supplementation of a YP (ProDairy, Donaghys, Christchurch, New Zealand) containing a spectrum of yeast and bacterial extracts administered in water troughs on milk production, milk components, blood parameters, rumen pH, BCS, and fecal scores (**FS**).

### MATERIALS AND METHODS

All procedures involving animals were approved by the Animal Care and Use Committee of the University of California, Davis.

# Animals and Experimental Design

Multiparous cows (930) in a commercial herd were randomly assigned to 1 of 2 treatments (2 pens/ treatment) as they reached 30 DIM. Two of the 4 pens received YP in 4 water troughs per pen at the rate of 9 mL/d per cow beginning August 1, 2011, through October 13, 2011 (11 wk). The other 2 pens received the dairy TMR without YP (control). The commercial dairy moved cows into and out of the pens according to their herd protocol during the study. That is, cows were moved from the pen after being confirmed pregnant (average of 132 + 66 DIM). Once cows were moved, they did not return to the pen. Cows were housed in a freestall barn that contained 2 pens on each side with 220 headlocks per pen. The same number and location

of water troughs were available in each pen and pens were identical in layout. Control cows were not able to access water in treated cow pens and feed and water space accessibility was equal in all pens. Water meters were installed in all water troughs to estimate water intake by pen.

Dispenser nozzles from Donaghys were used with 2-L bottles that were tethered to rebar cages around the water trough floats to prevent cow interference. Dispensers were refilled and replaced every other day and any residual was emptied into the water trough. All 4 pens were fed the same TMR, with a control pen and a treated pen delivered from the same mixer wagon loads. Cows were fed 3 times in a 24-h period. Diets were formulated by the dairy herd nutritionist using CPM Dairy software (Cornell-Penn-Miner, version 3.0.1, published by Cornell University, Ithaca, NY; University of Pennsylvania, Philadelphia, PA; Miner Institute, Chazy, NY; and University of Maryland, College Park, cooperating).

#### Measurements

Ration samples were collected from each pen once a week for nutrient analyses. Three empty feed tubs were placed in feed bunks just before the mixer wagon dropping a load. Tubs (approximately 8 to10 kg of TMR, as fed, per tub) were then collected and its contents were mixed on a large, clean cement floor. The TMR pile was then quartered and opposite quarters were mixed an llected into a quart Ziploc[AU1: Either add manufacture name and location for Ziploc or change the sentence to read \_resealable bags\_.] bag for nutrient

analyses by Analab (Agriking, Fulton, IL). Ration samples were analyzed for DM, ADF, NDF, CP, fat, ash, and lignin using wet chemistry analyses (American Association for Analytical Chemists reference methods[AU2: Add these methods to the Refs list.] 29, 973.18, 2002.04, 990.03, 920.39, 942.05, 973.18, respectively), starch using near-infrared spectom-

etry based on predictive equations

developed at Analab, and mineral analyses (Ca, P, Mg, K, S, Na, Cl, Fe, Cu, Mn, and Zn) using an inductively coupled plasma-mass spectrophotometry (American Association for Analytical Chemists reference methods 985.01 for Ca, P, Mg, K, Na, Fe, Cu, Mn and Zn, 923.01 for S, and 915.01 for Cl).

The DMI was estimated from daily group feed delivery weights from the mixer wagon and recorded using the FeedWatch feed management software (Valley Agricultural Software, Tulare, CA) for each pen. Dry matter intakes were corrected for residual feed, which was collected and weighed every other day and recorded using FeedWatch. Then, total corrected DMI was divided by numbers of cows in the pen that day to estimate individual cow DMI.

Water intake was measured using water meters installed at each water trough within each pen. Meters were read once a week on 2 consecutive days to obtain an estimate of water intake over 7 d and 24 h, respectively. On 2 occasions the water troughs did leak, but the troughs were repaired as soon as the leaks were identified. On those 2 occasions, data were corrected by comparing 24-h and 7-d intakes and eliminating values that were out of the range of possibility (approximately 95–170 L/d per cow; Murphy et al., 1983).

Rumen pH was measured on 6 fistulated cows with indwelling pH meters (Kahn Animal Health, Auckland, NZ) in the rumen that recorded pH and rumen temperature every 10 min. Cows were allocated 3 to a control pen and 3 to a treated pen. Meters were retrieved once a week to download data and perform calibration for pH 4 and 7 at 40°C in a water bath. Data (pH) used in the statistical analyses did not include calibration data or any pH data recorded when temperatures were outside the range of 36 to 42°C.

Milk yield, fat, and protein, were measured every 2 wk (wk 0, 2, 4, 6, 8, and 10) using Tulare County Dairy Herd Improvement Association milk testers and milk samples

Table 1. Ingredient composition
of diet

Ingredient	DM basis, %
Alfalfa hay	1.94
Whole cottonseed	4.52
Almond hulls	11.6
Rolled corn	11.2
Green chop alfalfa	9.94
Corn silage	16.7
Whey	1.40
Dairy premix <sup>1</sup>	42.7

<sup>1</sup>Dairy premix: 10.0% canola meal, 11.7% corn distillers, 4.24% wheat middlings, 10.4% soybean meal, 1.96% ground limestone, 1.27% blood meal, 1.14% sodium sesquicarbonate, 1.58% Megalac (Arm and Hammer Nutrition Group. Princeton, NJ), 1.45% Soybest (SoyBest, West Point, NE), 0.518% salt, 0.324% urea, 0.101% Alimet (Novus International, St. Charles, MO), 0.156% magnesium oxide, 0.0216% Zinpro 100 (Zinpro, Eden Prairie, MN), 0.0144% Rumensin 90 (Elanco, a division of Eli Lilly, Indianapolis, IN), and 0.324% mineral-vitamin mix.

were analyzed by the Tulare County Dairy Herd Improvement Association (Bentley Instruments ChemSpec 150, Chaska, MN). Body condition scores (1–5 scale; Wildman et al., 1982) and FS (1–4 scale; Ireland-Perry and Stallings, 1993) were estimated by 2 observers every week for 11 wk. Cows were scored for both FS and BCS when locked up in head stanchions for pregnancy check.

Blood plasma samples were also collected on approximately 25 cows per pen during pregnancy check at morning feeding via coccygeal vein or artery puncture into an evacuated sterile tube containing sodium fluoride and potassium oxalate to prevent glycolysis (Becton Dickinson Vacutainer Systems **B**: Add manufacturer name and location.]). Tubes were placed on ice after collection and then centrifuged[AU = lease add a duration, speed times g, and temperature for centrifugation.] and stored at  $-20^{\circ}$ C until analysis. Nonesterified fatty acids (**NEFA**) were determined using a commercially available kit (NEFA C kit, Wako Chemicals USA Inc., Richmond, VA). Blood glucose and BHBA were determined using a Precision Xtra hand-held meter (Ab-



Figure 1. Water intake (L/cow per day) by week and pen. YP = yeast product; error bars represent SEM[ Figures 1-4 were submitted in color but were not indicated as online only for r images. There is no charge for this service, would you like to make these figures online only color?]. Color version available in the online PDF.

bott Diabetes Care Inc., Alameda, CA).

#### **Statistics**

Statistical analyses for DMI, water intake, milk production, BCS, FS, blood glucose, BHBA, NEFA, and nutrient analyses were analyzed using the MIXED Procedure of SAS (SAS Institute Inc., Cary, NC) with pen nested within treatment. For milk production, BCS, FS, blood glucose, BHBA, and NEFA, the model was

$$\begin{split} \mathbf{Y}_{ijklm} &= \mathbf{\mu} + \mathrm{trt}_i + \mathrm{pen}_j(\mathrm{trt}_i) + \mathbf{wk}_k \\ &+ \mathrm{parity}_i + \mathrm{DIM}_m + \mathbf{E}_{iiklm}, \end{split}$$

where pen is the experimental unit;  $Y_{iiklm}$  = the dependent variable (observed production variable) in treatment *i*, in pen *j*, for week *k*, parity *l*, and DIM m;  $\mu$  = overall mean; trt = treatment i (i = 1 to 2); pen(trt) = random effect of the *i*th pen nested within the *i*th treatment (j = 1 to 2); wk = fixed effect of week  $k \ (k = 1 \text{ to})$ 11); parity = fixed effect of parity l(l)= 1 to 9); DIM = fixed effect of DIM  $m (m = 19 \text{ to } 513); \text{ and } \mathbf{E}_{iikm} = \text{ran-}$ dom residuals. No significant interaction among treatment and week was observed; therefore, it was removed from the model. Rumen pH was analyzed with a similar model, but cow was nested within treatment and parity was not included because all cows were the same parity. Rumen pH was measured in a total of 6 cows, with 3 cows in a control pen and 3 cows in a treatment pen. The model was

$$\begin{split} \mathbf{Y}_{ijklm} &= \mathbf{\mu} + \mathrm{trt}_i + \mathrm{cow}_j(\mathrm{trt}_i) + \mathbf{wk}_k \\ &+ \mathrm{trt}_i \times \mathbf{wk}_k + \mathrm{temp}_l + \mathrm{DIM}_m + \mathbf{E}_{ijklm} \end{split}$$

where  $Y_{ijklm}$  = the dependent variable (rumen pH) in treatment *i*, for cow *j*, week *k*, rumen temperature *l*, and DIM *m*;  $\mu$  = overall mean; trt = treatment *i* (*i* = 1 to 2); cow(trt) = random effect of the *j*th cow nested within the *i*th treatment (*j* = 1 to 3); wk = fixed effect of week *k* (*k* = 1 to 11); trt<sub>i</sub> × wk<sub>k</sub> = the *i*th treatment in the *k*th week; temp = effect of rumen temperature *l*; DIM = effect of

Nutrient, % (unless otherwise noted)	Control	YP	SEM	<i>P</i> -value
	Control			1 -Vulue
DMI, kg/cow per day	26.1	26.7	0.63	0.19
Water intake, L/cow per day	115	117	5.2	0.64
DM	50.6	50.6	2.5	1.0
CP	18.5	18.5	0.58	1.0
Starch	19.6	19.6	1.5	1.0
ADF	21.5	21.5	1.5	1.0
NDF	32.4	32.4	1.4	1.0
Lignin	4.07	4.07	0.41	1.0
Fat	4.40	4.40	0.50	1.0
Ash	7.79	7.79	0.32	1.0
Са	0.937	0.937	0.058	1.0
Р	0.441	0.441	0.024	1.0
Mg	0.268	0.268	0.013	1.0
к	1.69	1.69	0.11	1.0
S	0.272	0.272	0.010	1.0
Na	0.387	0.387	0.058	1.0
CI	0.525	0.525	0.061	1.0
Fe. ppm	213	213	264	1.0
Cu. ppm	15.5	15.5	1.1	1.0
Zn ppm	94 7	94 7	6.2	1.0
Mn ppm	73.5	73.5	7.0	1.0

Table 2. Dry matter intake, water intake, and chemical composition of diet by treatment (control and yeast product; YP) and pen

DIM m (m = 64 to 156); and  $E_{ijkm} =$  random residual. Statistical analyses for DMI, water intake, and nutrient analyses was simplified to

 $Y_{ijk} = \mu + trt_i + pen_j(trt_i)$ 

 $+ wk_{k} + E_{iik},$ 

where  $Y_{ijk}$  = the dependent variable (observed variable) in treatment *i*, in pen *j*, for week *k*;  $\mu$  = overall mean; trt = treatment *i* (*i* = 1 to 2); pen(trt) = random effect of the *j*th pen nested within the *i*th treatment (*j* = 1 to 2); wk = effect of week *k* (*k* = 1 to 11); and  $E_{ijkm}$  = random residual.



Figure 2. Milk yield (kg) by week and pen. YP = yeast product; error bars represent SEM. Color version available in the online PDF.

Cows were continually moving into and out of the pens during the trial as is typical in a commercial dairy herd; therefore, only cows that were exposed to treatments for at least 2 wk before data collection (milk test) were included in the data analyses and wk 0 is considered baseline for all 4 pens (i.e., YP was administered beginning at wk 0).

## **RESULTS AND DISCUSSION**

#### Intakes and Ration Composition

All pens were fed the same diet (Table 1) and DMI and nutrient composition were not different between treatments (Table 2). Differences in supplied feed?] (SEM) are and within range of deviations observed due to daily variations in feeding (loading, mixing and unloading) observed by Weiss et al. (2012) for TMR starch and Rossow and Alv (2013) for all TMR nutrients; thus it is unlikely that this level of TMR variation would have an effect on milk production and components. Water intake, however, was different among pens for YP (P <0.0001), but not control treatments. The YP pen that had the least water consumption also was greatest in milk yield, and the YP pen that had the highest water consumption also had the least milk yield (Figure 1). All water intakes are within normal ranges for water consumption (Castle and Thomas, 1975; Murphy et al., 1983). Main factors influencing water intake are DMI, DM of the diet, milk production, environmental conditions (temperature), and sodium and protein content of the diet (Murphy et al., 1983). Cows producing more milk may drink more water, but an increase in water intake does not increase or decrease milk production (Murphy et al., 1983). None of these factors were different among pens except milk production. It is unlikely that YP dilution in the lowestproducing pen was responsible for lower milk production and high water intake, as dosing was based on cow

Table 3. Effect of yeast product	(YP) on milk production, BCS, fecal
score (FS), blood glucose, I🧮	U11: Spell out KB.], and nonesterified
fatty acids (NEFA)	

Control	YP	SEM	P-value 📒
448	482		<b>~</b>
43.1	44.8	1.3	0.042
1.24	1.23	0.016	0.045
2.84	2.80	0.043	0.13
1.47	1.45	0.013	0.13
3.39	3.35	0.059	0.72
3.1	3.0	0.019	0.70
2.6	2.8	0.088	0.26
59	59	2.1	0.30
0.69	0.59	0.031	0.011
0.20	0.20	0.033	0.52
	Control 448 43.1 1.24 2.84 1.47 3.39 3.1 2.6 59 0.69 0.20	ControlYP44848243.144.81.241.232.842.801.471.453.393.353.13.02.62.859590.690.590.200.20	ControlYPSEM44848243.144.81.31.241.230.0162.842.800.0431.471.450.0133.393.350.0593.13.00.0192.62.80.08859592.10.690.590.0310.200.200.033

<sup>1</sup>Differences between treatments are at the *P* < 0.05 level**[AU12: Add footnote 1 to the table or delete it.]**.

numbers per pen per day and not concentration of YP in the water trough.

#### **Production Performance**

The YP increased milk and decreased milk protein yield (P < 0.042 and P < 0.045, respectively). A tendency was also noted for milk protein percentage and milk fat yield to be improved in control pens (P < 0.13 and P < 0.13, respectively). No difference was observed between control and YP milk fat percentage (P < 0.72). Parity, DIM, and week were also different for milk yield and milk protein yield (P < 0.0001 for all), but no interaction between treatment and week was seen. Increases in milk yield of 1 to 2 kg of milk/d (Piva et al., 1993; Bruno et al., 2009; Nocek et al., 2011; Poppy et al., 2012) are within average results from other studies. Several factors have been postulated to affect response of cows to yeast supplementation, such as stage of lactation, age, DMI, feed composition (Piva, 1993[AU6:



**Figure 3.** Fat yield (kg) by week and pen. YP = yeast product; error bars represent SEM. Color version available in the online PDF.

# Please correct the citation, add the reference to the list, or delete

the citation.]; Desnoyers et al., 2009; Nocek et al., 2011). Early lactation cows on high-concentrate diets with increased DMI or under conditions in which rumen pH was lower are more likely to respond to yeast supplementation. However, in the current study, diets and intakes were similar among pens and cows were within the same stage of lactation and similar in size and facilities. In addition, the number of cows on treatment was fairly consistent among pens and across weeks of the study (Table 3). How YP is administered can change its effectiveness; however, the advantage of giving YP in the water trough is the ability to have a consistent environment that is unlikely to be affected by weather or other feed ingredients. Variation in milk, milk fat, and milk protein yield (Figures 2, 3 and 4) was observed from week to week, but this change was not due to YP administration. Over 10 wk, milk vield varied by approximately 5 kg, milk fat by 0.4 kg, and milk protein by 0.15 kg. For both milk yield and milk protein yield, 1 YP pen had a different pattern from the other 3 pens, but the increase in milk yield was greater with YP.

#### Health Parameters

Average glucose, BHBA, and NEFA values were within normal ranges [60– 80 mg/dL, <1.2 mmol/L (Geishauser et al., 1998), >0.4 mEq/L (Adewuyi et al., 2006 [/ Please add the reference to the list or delete the citation.]), respectively]. No differences were noted in BCS, FS, or NEFA, but BHBA was different by treatment and pen and plasma glucose was different by pen (Table 3). Cows supplemented on YP had lower BHBA (P= 0.011), but no differences in NEFA and glucose were seen. In other YP supplementation studies, no effect on plasma glucose, BHBA, or NEFA was noted (Piva et al., 1993; Bruno et al., 2009).

Rumen pH was higher (P = 0.044)with YP overall and from wk 1 to 9 (P < 0.10; Table 4). Week 0 is when



Figure 4. Protein yield (kg) by week and pen. YP = yeast product; error bars represent SEM. Color version available in the online PDF.

the previously fed yeast product was removed from the ration and is actually 2 wk before wk 1. One week after the product was removed, YP was added to the water; therefore, wk 1 represents the completion of 1 wk of supplementation with YP. Thus, at wk 0, no difference was observed in rumen pH between control and YP, but rumen pH was consistently higher (0.03–0.28 pH units) after supplementation began until wk 9 to 11. Consistently higher rumen pH values could affect milk yield substrates, such as glucogenic precursors (i.e., plasma glucose). In a review of yeast supplementation studies, supplementation of live yeast products consistently lead to increases in rumen pH of 0.03 units, and the difference between supplemented and not supplemented cows seems to increase with level of

Table 4. Rumen pH by treatment (control and yeast product; YP) and week

Week	Treatment			<i>P</i> -value	
	Control	YP	SEM	Treatment	
Overall	6.32	6.40	0.028	0.044	
0	5.95	5.98	0.10	0.14	
1	5.84	6.07	0.099	0.011	
2	6.11	6.18	0.098	0.086	
3	6.13	6.26	0.098	0.048	
4	6.30	6.50	0.098	0.016	
5	6.30	6.47	0.098	0.028	
6	6.27	6.35	0.098	0.083	
7	6.42	6.70	0.098	0.0053	
8	6.52	6.61	0.098	0.071	
9	6.61	6.64	0.099	0.13	
10	6.60	6.51	0.10	0.38	
11	6.73	6.47	0.10	1.0	

concentrate in the diet and DMI level (Desnovers et al., 2009). Similar to the current study, Bach et al. (2007)found a response to yeast supplementation after 1 wk, where rumen pH increased with supplementation. However, in our study, the increase in rumen pH with YP started to decline at wk 8. Week 8 is also when milk and protein yield began to decline. Peaks in milk and protein yield correspond with greater increases in rumen pH in wk 3 to 5 and 7 (Figures 2 and 3). Bruno et al. (2009) reasoned that milk yields were increased with yeast supplementation because yeast increased DM digestibility and therefore increased microbial protein yield and glucogenic substrates for milk production increasing milk yield, as observed in the current study. Palmonari et al. (2010) also supported these conclusions by measuring rumen pH in 8 ruminally cannulated Holstein cows with indwelling pH electrodes to study the effect of changes in rumen pH on bacterial community compositions. He emphasized that rumen pH was an important factor in bacterial adherence and, therefore, fiber digestion. Therefore, YP increased rumen pH and, coupled with potential variability in nutrient content of the TMR and management factors that affected water intake on commercial dairy farms (from treatment and pen interactions), increased milk yield.

#### IMPLICATIONS

Administration of YP in the water trough has some management advantages in that water is a more consistent temperature and anaerobic environment with little chance of other feeds affecting its effectiveness. Supplementation of YP in the water did correspond with an increase milk yield, rumen pH in wk 1 to 8 of the study, and decrease in BHBA. Depending upon the economic importance of the declines in milk components and the ability of the dairy to manage a consistent water supply, the delivery of YP via water should be considered by nutritionists and managers.

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