AN ABSTRACT OF THE THESIS OF

<u>Erica M. Ramsing</u> for the degree of <u>Master of Science</u> in <u>Animal Science</u> presented on <u>April 22</u>, 2011.

Title: <u>Yeast Culture Improves Lactation Performance and Metabolic Status in Transition Dairy</u> Cows

Abstract approved:

Gerd Bobe

Yeast culture supplementation has been used to improve the health and performance of dairy cows for the last 25 years. To evaluate the effect of a *Saccharomyces cerevisiae* fermentation product (Diamond V Original XPTM) on the health and production of peripartal Holstein cows, two research studies were conducted.

The first experiment took place on the Oregon State University research dairy where feed intake behavior was continuously monitored. Treatments (administered 3 wks before anticipated calving date through 3 wks postpartum) included 0, 57, and 228 g/d XP. Yeast culture was reported to affect primi- and multiparous cows similarly by improving milk yield and prepartum intake, with little effect on metabolic parameters.

To test the effect on a larger scale, a second experiment was conducted using 96 multiparous cows on a commercial dairy. A method for individually feeding cows in a freestall barn was created and implemented to preserve the cow as the experimental unit. Cows were given treatments (0, 56, or 112 g/d XP) daily from 4 wks prior to expected calving date through 4 wks postpartum. Yeast culture supplementation significantly improved lactation performance in second parity cows and improved metabolic status of all cows, especially older animals.

Yeast culture supplementation was beneficial for improving lactation performance and health of dairy cows through the transition period. Further research is required to elucidate the mode of action and determine the optimum dosage.

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Yeast Culture Improves Lactation Performance and Metabolic Status in Transition Dairy Cows

by Erica M. Ramsing

A THESIS

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Master of Science thesis of Erica M. Ramsing presented on April 22, 2011.
APPROVED:
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Head of the Department of Animal Sciences
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.
Erica M. Ramsing, Author

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CONTRIBUTION OF AUTHORS

Chapter 2:

Dr. Davidson – performed statistical analysis and provided oversight of data entry

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Dr. Males – provided departmental support for project and assistance with manuscript writing

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Dr. Bobe – obtained funding, oversaw project design and sample collection and analysis, performed statistical analysis, and manuscript writing assistance

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YEAST CULTURE IMPROVES LACTATION PERFORMANCE AND METABOLIC STATUS IN TRANSITION DAIRY COWS

CHAPTER 1.INTRODUCTION

INTRODUCTION

Transition Cow Health

Most of the health problems of dairy cows occur during the early lactation period and can be linked to depressed peripartal intake (Ingvartsen and Andersen, 2000). This intake depression, combined with the increased energy demands of the onset of lactation, create a negative energy balance. Several metabolic parameters can be easily measured to evaluate the metabolic health and wellbeing of dairy cattle.

Serum glucose concentrations provide insight into the availability of readily oxidizeable substrate. Typically, serum glucose concentrations decrease as the requirement for glucose for lactose synthesis and subsequent milk production increases, and non-esterified fatty acid (NEFA) concentrations increase to meet the body's demands for gluconeogenic precursors (Ingvartsen and Andersen, 2000). As milk production increases, oxidation of glucose by peripheral tissues decreases allowing glucose to be directed to the mammary glands for lactose synthesis (Overton and Waldron, 2004). The primary determinant of milk volume is lactose concentration (Kronfeld, 1982). Thus, as serum glucose concentrations dip immediately postpartum we can assume that available glucose is being diverted to the mammary glands and NEFA concentrations must increase accordingly to provide energy for maintenance functions. A parity effect has been observed, where primiparous cows exhibited higher glucose concentrations postpartum (Janovick et al., 2011), possibly due to the higher glucose demands of the higher-producing multiparous cows (Janovick and Drackley, 2010). While it is possible that the postpartum decrease in lactose could be associated in part with the decline in intake associated with the day of calving, "there is little evidence that glucose concentration or utilization rate has a significant role in dry matter intake (**DMI**) regulation of ruminants." (Baile and Della-Fera, 1981).

Closely related to glucose, serum NEFA concentrations are an indirect measure of the mobilization of adipose triacylglyceride stores (Mashek et al., 2001). NEFA concentrations usually spike immediately following calving, then drop off in subsequent weeks (Ingvartsen and Andersen, 2000) with circulating levels peaking 0-7days postpartum (Hayirli et al., 2011, Janovick et al., 2011). Skeletal muscle uses NEFA for fuel, especially as reliance on glucose decreases during early lactation (Overton and Waldron, 2004). NEFA and DMI are usually inversely correlated (Overton and Waldron, 2004), with the intake regulation effect, potentially related to fatty acid oxidation in the brain and liver (Ingvartsen and Andersen, 2000).

Finally, serum β-hydroxybutyrate (**BHBA**) and blood urea nitrogen (**BUN**) concentrations are correlated with negative energy balance. At day 0 (parturition), serum BHBA concentrations typically begin increasing, reaching a maximum concentration about 7 days postpartum (Hayirli et al., 2011; Janovick et al., 2011). BHBA concentrations serve as a marker for ketosis, with a cutoff for subclinical ketosis at 0.97 mmol/L (Enemark et al., 2009). BUN concentrations are not directly correlated with negative energy balance, but elevated concentrations suggest an increase in hepatic urea production associated with tissue mobilization and negative energy balance (Rastani et al., 2006).

Microbial Supplementation of Ruminants

Manipulation of the production animal GI microbial ecosystem in order to improve health and performance has increased considerably in the last 25 years (Chaucheyras-Durand and Durand, 2010). Rumen ecosystem plays a large role in the response of ruminants to their diet (Desnoyers et al., 2009). For ruminant animals, the rumen is the target compartment for microbial supplementation, as it contains a diverse range of bacteria, protozoa, archaea, and fungi which are responsible for fermentation and degradation of 70-75% of ingested organic matter (Chaucheyras-Durand and Durand, 2010). *Saccharomyces cerevisiae* is one such common supplement, used either as an isolated, active product (**live yeast**) or combined with its culture media as a fermentation product (**yeast culture**).

Effects of Live Yeast Supplementation

Studies of the effect of live yeast on health, production, and metabolism of dairy cows have been relatively inconclusive. However, a meta-analysis by Desnoyers et al. (2009) of 157 live yeast supplementation experiments including lactating and growing cattle, sheep, goats, and buffalos nicely summarizes the bulk of the literature on the topic: Live yeast supplementation increased rumen pH and volatile fatty acid (VFA) concentration. No effect was detected of live yeast supplementation on acetate to proprionate ratio, and dietary crude protein (CP) did not influence the effect of live yeast. As DMI increased, so did the positive effects on rumen pH, although this was reduced as the proportion of NDF in the diet increased. The positive effect of yeast on VFA concentration in the rumen was also increased in conjunction with DMI. Yeast supplementation also tended to decrease rumen lactic acid concentration. As yeast dose increased, so did organic matter digestibility. This effect was increased by increasing the proportion of neutral detergent fiber (NDF) in the diet, CP in the diet, and increasing DMI. Yeast culture increased DMI and milk yield in a dose-dependent manner, and tended to increase milk fat

content. The effect on milk yield increased in conjunction with an increase in DMI and an increased proportion of dietary concentrate, CP, and NDF.

Effects appear to be consistent during periods of heat stress, when live yeast supplementation has been shown to increase feed intake, milk yield, and feed efficiency, and decrease rumen ammonia of lactating dairy cows (Moallem et al., 2009). However, live yeast supplementation does not appear to have immediate effects. Mid-lactation cows supplemented with live yeast required an adaptation period of four weeks, without which there was no discernable difference in milk yield between control cows and those receiving the supplemental yeast (de Ondarza et al., 2010).

Effects of Yeast Culture Supplementation

While there is a large literature base of live yeast supplementation studies, yeast culture studies are more limited. Large variability exists between yeast culture products. Many experiments have resulted in slight changes associated with yeast culture supplementation, but dramatic results are few and far between, and often statistical significance is limited by sample size.

Yeast culture supplementation has been researched to varying extents with cows of different parities, stages of lactation, and under normal and stressful environmental conditions (Table 1). The interactions between parity and effect of supplementation have not been closely examined. Robinson and Garrett (1999) clearly separated primi- and multiparous transition cows for analysis. While a significant improvement in milk yield was observed in primiparous cows, a smaller improvement was reported in multiparous cows. However, other studies have either focused exclusively on either primi- (Wohlt et al., 1991; Putnam et al., 1997) or multiparous cows (Williams et al., 1991; Robinson, 1997; Wohlt et al., 1998; Bruno et al., 2009; Longuski et al., 2009; Hippen et al., 2010; Hristov et al., 2010; Fortina et al., 2011), or combined all cows regardless of parity (Dann et al., 2000; Wang et al., 2001; Lehloenya et al., 2008) for analysis. Different stages of lactation studied in previous research include the transition phase (Wohlt et al., 1991; Robinson, 1997; Wohlt et al., 1998; Dann et al., 2000; Wang et al., 2001; Schingoethe et al., 2004; Lehloenya et al., 2008), early lactation (Harrison et al., 1988; Putnam et al., 1997; Bruno et al., 2009; Hristov et al., 2010), and mid to late lactation (Arambel and Kent, 1990; Williams et al., 1991; Piva, 1993; Schingoethe et al., 2004; White et al., 2008; Longuski et al., 2009; Fortina et al., 2011). Within each stage, results are mixed, ranging from significant improvements in milk yield, to numerical but non-significant improvements, to no differences or

slight decreases in milk yield. Our focus is specifically on the transition cow, but research from other stages of lactation provides valuable information about the possible mode of action. A final focus of several previous studies has been the effect of yeast culture supplementation on cows during periods of stress. Most commonly, this has applied to heat stress (Schingoethe et al., 2004; Bruno et al., 2009). As with the categorization by lactation phase, results have been mixed. Additional studies have examined the interaction of yeast culture during periods of nutritional stress, such as a fermentable starch challenge (Longuski et al., 2009).

The effects of varying concentrations of yeast culture supplementation on specific parities have not been closely examined. The objectives of these studies were to 1) identify the optimum dose of yeast culture for maximum health and lactation performance of peripartal Holsteins, 2) examine the interaction between parity and yeast culture supplementation, and 3) study the effect of yeast culture on feed intake behavior. Our hypothesis was that yeast culture supplementation would enhance lactation performance and reduce the feed intake depression associated with calving, modulating the changes in metabolic parameters during the transition period.

Table1. Literature Review of the Effects of Yeast Culture Supplementation on Feed Intake and Milk Production in Early-**Lactation Cows**

Study	Parity	Cows (#)	Prepartum Feed In (kg/d)	ntake	Postpartum (kg/d)	Feed Intake	Milk Prod (kg/d)	uction
		. /	Control	Trt	Control	Trt	Control	Trt
Arambel& Kent (1990)*	Not Defined	20	Not measured (NM)	NM	21.9	21.8	37.9	36.5
Williams et al (1991) ¹ **	Multi	8	NM	NM	15.7	16.5	22.5	21.5
Williams et al (1991) ² **	Multi	8	NM	NM	18.1	18.8	23.4	23.3
Williams et al (1991) ³ **	Multi	8	NM	NM	17.8	18.7	23.3	23.5
Williams et al (1991) ⁴ **	Multi	8	NM	NM	17.3	19.6	23.3	27.4
Wohlt et al (1991)***	Primi	24	NM	NM	19.2	18.5	26.0	27.2
Swartz et al (1994)****	Not Defined	306	NM	NM	NM	NM	31.8	31.6
Putnam et al (1997) ⁵ **	Primi	4	NM	NM	18.1	19.2	31.0	32.1
Putnam et al (1997) ⁶ **	Primi	4	NM	NM	18.3	19.0	31.6	31.9
Robinson (1997)*****	Multi	20	10.97	10.79	17.38	17.62	34.09	34.65
Wohlt et al (1998)***	Multi	36	11.2	11.2	18.0	17.6	34.3	34.1
Robinson & Garrett (1999)*****	Primi	18	9.31	9.32	14.34	15.40	25.36	27.81 ‡
	Multi	26	12.89	13.19	19.45	20.76‡	38.60	40.35
Soder& Holden (1999)***	Primi	12	12.1	11.3	22.1	21.0	40.2	40.4
_	Multi	36	12.1	11.5	22.1	21.0	40.2	70.7
Dann et al (2000) ⁷ *****	Primi	14	7.7	9.8†	10.2	12.0‡	18.9	20.3
	Multi	25	7.7	7.01	10.2	12.04	10.7	20.5
Wang et al (2001) ⁸ *****	Primi	4			18.1	18.5	36.6	35.6
	Multi	20			10.1	10.5	30.0	33.0
Wang et al (2001) ⁹ *****	Primi	4	13.9	14.6	19.4	21.3	38.2	41.0
	Multi	20	13.9	14.0	17.4	21.3	36.2	41.0
Wang et al (2001) ¹⁰ *****	Primi	2			NM	18.7	NM	36.4
	Multi	10			INIVI	10.7	INIVI	30.4
Lehloenya et al (2008)*****	Not Defined	NM	NM	NM	NM	NM	37.6	37.6
White et al (2008)*****	Not Defined	260	NM	NM	NM	NM	41.9	42.0
Ramsing et al (2009)*****	Primi	26	12.45	13.97	16.60	17.26	31.3	24.24
	Multi	40	12.43	†	10.00	1 / .20	31.3	34.2†
Ramsing et al (2009)******	Primi	See above	See above	12.50 †	See above	16.97	See above	33.4†

† indicates a *P*-value for the difference between Trt and Control of 0.05 or less

tindicates a *P*-value for the difference between Trt and Control between 0.05 and 0.10

- 50:50 concentrate:forage, straw
- 50:50 concentrate:forage, hay
- 60:40 concentrate:forage, straw
- 60:40 concentrate:forage, hay
- Low CP Diet
- High CP Diet
- Jersey breed instead of Holsteins
- 17% Forage NDF Diet
- 9. 21% Forage NDF Diet 10. 25% Forage NDF Diet *Diamond V YC

- **Unspecified
- ***Biomate Yeast Plus, 10g/d ****West Yeast Cell-con

- *****Diamond V XP Yeast Culture, 56g/d
 *****Diamond V XP Yeast Culture, 227 g/d

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Study	Parity	Cows	Fat (%)		Fat (kg/d)		Protein (%)		Protein (kg/d)	
		(#)	Control	Trt	Control	Trt	Control	Trt	Control	Trt
Arambel& Kent (1990)*	Not Defined	20	3.33	3.37	NM	NM	2.97	2.94	NM	NM
Williams et al (1991)1**	Multi	8	3.78	3.81	NM	NM	NM	NM	0.702	0.692
Williams et al (1991)2**	Multi	8	3.44	3.35	NM	NM	NM	NM	0.835	0.783
Williams et al (1991)3**	Multi	8	3.19	3.66	NM	NM	NM	NM	0.771	0.806
Williams et al (1991)4**	Multi	8	3.45	3.26	NM	NM	NM	NM	0.790	0.969
Wohlt et al (1991)***	Primi	24	NM	NM	NM	NM	NM	NM	NM	NM
Swartz et al (1994)****	Not Defined	306	3.69	3.76	1.17	1.16	3.15	3.17	1.00	0.99
Putnam et al (1997)5**	Primi	4	3.04	3.24	0.939	1.03 5	2.82	2.86	0.875	0.916
Putnam et al (1997)6**	Primi	4	3.23	3.23	1.017	1.01 8	2.87	2.87	0.903	0.912
Robinson (1997)*****	Multi	20	4.17	4.33	1.38	1.46	3.26	3.19	1.07	1.09
Wohlt et al (1998)***	Multi	36	4.26	4.14	1.44	1.38	3.09	3.10	1.04	1.03
Robinson & Garrett	Primi	18	3.88	3.59	0.96	0.99	3.16	3.00	0.79	0.83
(1999)****	Multi	26	3.88	3.82	1.48	1.53	3.05	3.05	1.16	1.22
Soder& Holden	Primi	12	3.92	3.82	1.46	1.33	3.15	3.13	1.27	1.20
(1999)***	Multi	36								
Dann et al (2000)7****	Primi	14	4.27	4.44	NM	NM	3.64	3.78	NM	NM
	Multi	25								
Wang et al (2001)8*****	Primi	4	4.21	4.22	1.48	1.48	3.24	3.34	1.16	1.17
	Multi	20								
Wang et al (2001)9*****	Primi	4	4.07	4.04	1.54	1.61	3.35	3.38	1.26	1.36
	Multi	20								
Wang et al	Primi	2	NM	3.97	NM	1.44	NM	3.43	NM	1.22
(2001)10****	Multi	10								
Lehloenya et al (2008)*****	Not Defined	NM	4.57	4.19	2.13	1.70	2.90	2.95	1.12	1.15
White et al (2008)*****	Not Defined	260	3.61	3.69†	1.49	1.53 ‡	3.21	3.19 †	1.33	1.32
Ramsing et al (2009)*****	Primi	26	4.57	4.77	1.36	1.57	3.55	3.60	1.06	1.17
	Multi	40								
Ramsing et al (2009)*****	Primi	See above	See above	e 4.70	See above	1.48 ‡	See above	3.58	See above	1.14

† indicates a P-value for the difference between Trt and Control of 0.05 or less

indicates a P-value for the difference between Trt and Control between 0.05 and 0.10
50:50 concentrate:forage, straw
50:50 concentrate:forage, hay

^{60:40} concentrate:forage, straw 60:40 concentrate:forage, hay Low CP Diet High CP Diet

^{**}Unspecified
***Biomate Yeast Plus, 10g/d

^{****}West Yeast Cell-con

*****Diamond V XP Yeast Culture, 56g/d

******Diamond V XP Yeast Culture, 227 g/d/

CHAPTER 2.EFFECTS OF YEAST CULTURE ON PERIPARTUM INTAKE AND MILK PRODUCTION OF PRIMIPAROUS AND MULTIPAROUS HOLSTEIN COWS

Our first study was conducted to examine the dose effect of Diamond V XP Yeast Culture on feed intake behavior, lactation performance, and metabolism of primi- and multiparous Holstein cows through the transition period. To increase statistical power, a relatively large sample size was used. However, the duration of the on-farm data collection was 13 months, with a small number of cows enrolled in the study at a given time to ensure careful monitoring and detailed intake behavior data. Milk yield and composition were analyzed weekly to monitor lactation performance, and blood samples were taken weekly (or more frequently around calving) to evaluate metabolism.

EFFECTS OF YEAST CULTURE ON PERIPARTUM INTAKE AND MILK PRODUCTION OF PRIMIPAROUS AND MULTIPAROUS HOLSTEIN COWS

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ABSTRACT

Multiparous (n = 40) and primiparous (n = 26) Holstein cows were fed a common TMR with one of the following top-dress treatments of yeast culture supplementation at 57 g/d (XP-2) or 227 g/d (XP-8), or no yeast culture (control) from approximately 21 dprepartum to 21 d postpartum. Dry matter intake, lactation performance, metabolism, and feeding behavior were monitored. Prepartum DMI of XP-2 cows was greater by more than 1.4 kg/d compared to XP-8 cows (P< 0.01). Postpartum DMI and pre- and postpartum BW were similar for all groups. Milk yield was greater (P < 0.01) and 3.5% FCM (P = 0.09), ECM (P = 0.10), and milk fat yield (P = 0.09)tended to be 10% greater for cows that were supplemented with yeast culture compared to nonsupplemented cows, and were not different for XP-2 compared with XP-8 cows. Milk protein yield, milk protein percent, milk fat percent, and linear somatic cell score were not influenced by treatment. There were no significant effects of yeast culture supplementation on plasma BHBA, glucose, or NEFA concentrations pre- or postpartum. Yeast culture supplementation tended to increase the average number of prepartum meals per cow per day (P = 0.07) and XP-8 increased average daily meal duration (P = 0.05) and feeding time (P < 0.01) postpartum compared to XP-2. Yeast culture supplementation improved prepartum DMI and postpartum performance and improved the ability of cows to transition during the periparturient period. Primiparous and multiparous cows responded similarly when supplemented with yeast culture.

Key words: yeast culture, dairy cow, milk, intake

INTRODUCTION

Periparturient supplementation with yeast culture has increased DMI pre- and postpartum by 2.1 kg/d (Dann et al., 2000), or increased peak milk yield by 0.8 kg/d (Wohlt et al., 1991). Yeast culture supplementation of lactating cows tends to increase DMI and milk yield (Williams et al., 1991 and Piva, 1993) and increases feed efficiency (kg of ECM per kg of DMI) during periods of heat stress (Schingoethe et al., 2004). However, in other experiments no significant effect of yeast culture supplementation on intake or milk yield during periparturient or early lactation has been observed (Swartz et al., 1994; Robinson, 1997; Wohlt et al., 1998; Robinson and Garrett, 1999; Soder and Holdren, 1999).

Variation in responses to yeast culture supplementation is not well understood. Williams et al. (1991) observed an increase in the initial rate of degradation of fibrous materials in the rumen when Frisian steers were supplemented with yeast culture, and demonstrated that lactating cows being fed diets with higher concentrate to forage ratios had greater milk yield responses to

yeast culture supplementation. Yeast culture supplementation increases ruminal digestion of alfalfa more than corn silage or other forages (Adams et al., 1995; Miranda et al., 1996). Parity may also affect the influence of yeast culture supplementation on milk yield. Robinson and Garrett (1999) observed a parity x treatment interaction. Primiparous cows had a 2.5 kg/d increase ($P \le 0.09$) in milk yield, while multiparous cows did not show a significant response.

Effects of yeast culture supplementation on periparturient metabolism and feed intake behavior have yet to be clearly defined. Therefore, the objectives of this experiment were to determine: 1) the impact of periparturient yeast culture supplementation on dry matter intake, lactation performance, metabolism, and feed intake behavior of primi- and multiparous peripartum Holstein cows and 2) the effects of increased concentration of yeast culture.

MATERIALS AND METHODS

Animals and Diets

All procedures involving animals were conducted in accordance with Oregon State University Institutional Animal Care and Use (IACUC #3243). Forty multiparous and 26 primiparous Holsteins from the Oregon State University Dairy Center were blocked by expected calving date and parity (primiparous or multiparous). Within each block cows were assigned at random to one of three dietary treatments (n = 13 to 14 multiparous and n = 8 to 9 primiparous per treatment). One cow died during the experiment and was removed from the study. Dietary treatments were unsupplemented (**control**), XPTM (fully fermented yeast culture of *S. cerevisiae*, Diamond V Mills, Cedar Rapids, IA) supplemented at 2 oz/d (57 g/d, XP-2), and XPTM supplemented at 8 oz/d (227 g/d, XP-8). Cows were group housed in a freestall barn and fed individually using Calan® gates (American Calan, Northwood, NH) beginning 4 wk prior to expected calving date.

Feed tubs were located on digital scales set to auto send data when stable after motion and were linked to a computer that collected tub weight, time, and date of feeding bouts using a software program (Collect 4.0, Labtronics, Guelph, Ontario, Canada). Meal criteria were calculated for each cow according to the process described by DeGroot (2005) and the methods of DeVries et al. (2003), using a natural logarithmic frequency. Prepartum and postpartum meal criteria were calculated separately. Weight event intervals less than 20 s were removed prior to analysis due to interference with statistical modeling (DeVries et al., 2003; DeGroot 2005). To be considered a feeding meal, more than 0.35 kg of feed (as fed) must have disappeared. Number of

visits per day, meal size, meal duration, and feeding time were calculated for each cow using macros in Microsoft® Excel with meal criteria from behavior data.

Cows were offered a TMR ad libitum twice daily with approximately 67 and 33% of daily feed allowance offered at 0700 and 1300 h, respectively. Feed offered and refused was recorded at each feeding. Treatments were applied on a common TMR by top dressing 227 g of premixes at a morning feeding (227 g common ground grain mixture, 57 g XP plus 170 g common ground grain mixture and 227 g XP, for control, XP-2 and XP-8, respectively). Data were collected beginning 21 d prepartum and ending 21 d postpartum.

Ingredient and nutrient composition of diets is shown in Table 1. Ingredients were sampled weekly, dried to constant weight at 55°C in a forced air oven, and ground through a 1-mm screen in a Thomas Wiley Mill (Thomas Scientific, Swedesboro, NJ). Weekly ingredient samples were composited monthly and analyzed for CP, NDF, Ca, and P. Prepartum and postpartum diets were formulated using the CPM Dairy (version 3.0) ration evaluator.

Body weights were measured weekly. Cows were milked twice daily and total milk production was recorded daily. Milk was analyzed for fat, protein and SCC on two consecutive milkings (am and pm) each wk by Willamette Valley DHIA (Salem, OR).

Plasma Sampling and Analysis

Blood samples were collected by venipuncture d -21, -14, -10, -7, -3, and -1 prepartum. After calving, blood samples were collected on d 1, 3, 7, 14, and 21. Sampling time (approximately 1200 h) corresponds to approximately 5 h after morning feeding. Blood samples were collected in vacutainer tubes (Becton Dickson, Franklin Lanes, NJ) containing K EDTA or Na heparin plus NaFl and placed on ice immediately after collection. Plasma was separated after centrifugation at 1600 x g for 15 min at 5°C, and frozen at -80°C until analysis.

One subsample of prepartum plasma (d -21, -14, -10, -7, -3, and -1) and one subsample of postpartum plasma (d 1, 3, 7, 14, 21) were used for analysis of BHBA, glucose, and nonesterified fatty acid (**NEFA**). Plasma collected from K EDTA-coated tubes were analyzed for BHBA (Procedure 2440, Stanbio Laboratory, Boerne, TX) and NEFA (NEFA-C, WAKO Pure Chemical Industries, Richmond, VA). Plasma from Na heparin plus NaFl tubes were analyzed for glucose (Procedure No. 1070, Stanbio Laboratory). All spectrophotometric measurements were conducted using a BIO-TEK (Winooski, VT) EL-800 microplateautoreader.

Statistical Analysis

Data was analyzed as repeated measures using the Proc Mixed procedure of SAS (SAS User's Guide, 2001). The experimental design was a randomized block of parity and expected calving date. Cow within parity by treatment was defined as the random effect. Autoregressive covariance structure was specified for repeated measures of equal spacing, such as milk yield, milk composition yield, DMI, and BW. Unstructured covariance structure was utilized for unequally spaced repeated measures (i.e. plasma samples) as identified with Akaike's information criteria to select the best covariance structure. Orthogonal contrasts were control vs. XP (XP-2 and XP-8) and XP-2 vs. XP-8. Prepartum and postpartum data were analyzed separately. Energy corrected milk yield (ECM) was calculated as 0.3246 x milk yield (kg) + 12.86 x fat yield (kg) + 7.04 x true protein yield (kg) (Smith et al., 2002), and fat corrected milk yield (3.5%) calculated as 0.432 x milk yield (kg) + 16.23 x fat yield (kg) (Tyrrell and Reid, 1965). Due to heterogeneity of variance, plasma NEFA concentrations were natural logarithmically transformed prior to statistical analyses.

Model used for all dependant variables was $Y_{ijkl} = \mu + P_i + T_j + C_{(ij)l} + D_k + TD_{jk} + e_{ijkl}$ where μ = overall mean, P_i = ith parity (heifer or cow), T_j = jth treatment (control, XP-2, and XP-8), $C_{(ij)l}$ = lth cow within the ith parity and jth treatment, D_k = day or week (repeated measure), and e = residual error. A parity by treatment interaction was included in the original model, but was removed after it was determined to be not significant for any of the dependant variables. Data was considered significant when $P \le 0.05$, with trends from P < 0.05 to $P \le 0.10$.

RESULTS AND DISCUSSION

Prepartum and postpartum body weights were similar across all treatment groups. Supplementation of yeast culture increased (P< 0.02) prepartum DMI (Table 2). Cows supplemented with XP-2 had an increased DMI of more than 1.4 kg/d compared to cows supplemented with XP-8. As parturition neared, DMI declined similarly for all groups, and after parturition intakes of all groups increased in a similar manner (Figure 1). Postpartum DMI was not affected by concentration of yeast culture. Primiparous cows had lower (P< 0.0001) DMI (11.9 and 15.5 kg/d for prepartum and postpartum, respectively) compared with multiparous cows (14.1 and 18.4 kg/d for prepartum and postpartum, respectively). The prepartum DMI increase supports the findings of Wohlt et al. (1991) and Dann et al. (2000), where primi- and multiparous dairy cows exhibited improved pre-partum DMI when supplemented with yeast cultures. In contrast to the current study, these studies also had greater DMI during postpartum after pre- and

postpartum yeast culture supplementation from at least 7 d prepartum through the first 6 wks of lactation (Wohlt et al., 1991; Dann et al., 2000).

Feeding supplemental yeast culture increased (P< 0.05) milk yield and tended to increase (P<0.10)3.5% FCM, ECM, and milk fat yield (kg/d), by approximately 10% compared to yields from cows that were not supplemented (XP-2 and XP-8 vs. control, Table 3). However, additional increases in milk, 3.5% FCM, ECM, and milk fat yield were not observed with the higher inclusion rate of yeast culture (XP-8). Milk protein yield, fat and protein percent, and linear somatic cell score were not different among treatment groups. Primiparous cows had lower (P< 0.0001) milk yield (28 kg/d) compared with multiparous cows (38 kg/d). The interaction of treatment by day was significant only for milk fat and protein percentages (Table 3). These improvements in lactation performance (Figure 2) agree with results of early- and mid-lactation studies where yeast culture supplementation improved milk yield and FCM (Williams et al., 1991; Piva et al., 1993; Kung et al., 1997; Wohlt et al., 1998; Wang et al., 2001; Lehloenya et al., 2008). Yeast culture supplementation also increased lactation efficiency (kg ECM per kg of DMI) during mid-lactation heat stress (Schingoethe et al., 2004). Wohlt et al. (1991) observed an earlier and higher peak milk yield when cows were supplemented with yeast culture pre-and postpartum. However, pre- and postpartum yeast culture supplementation generally failed to significantly improve milk yield or component yield prior to week 5 postpartum (Robinson, 1997; Wohlt et al., 1998; Soder and Holden, 1999; Lehloenya et al., 2008).

Overall plasma concentrations of glucose, NEFA, and BHBA were not different among treatment groups (Table 4). As expected, NEFA and BHBA concentrations were higher during the postpartum period as compared to the prepartum concentrations (Figure 3). During the prepartum period, NEFA concentrations were affected by treatments as parturition neared (treatment by day interaction, P = 0.01, Figure 3). However, the magnitudes of these differences were small and may not have a physiological significance. Postpartum NEFA concentrations of 0.36 mM were within the range of mild negative energy balance (Adewuyi et al., 2006) but not considered alarming. Circulating BHBA concentration means both pre- and postpartum remained below the sub-clinical ketosis cutoff of 10 mg/dL (Enemark et al., 2009) for all treatments. Plasma glucose was lower after parturition compared with the prepartum concentrations. Direct fed microbial supplements containing S. cerevisiae and bacterial strains have affected circulating metabolites by lowering BHBA concentrations prepartum and d 1 postpartum (Nocek and Kautz, 2006), increasing blood glucose (Nocek and Kautz, 2006) and insulin postpartum, and lowering

NEFA levels postpartum (Nocek et al., 2003). In contrast, plasma glucose and insulin concentrations were not affected by yeast culture supplementation in Angus x Hereford steers (Lehloenya et al., 2008a) or mid lactation dairy cows (Piva et al., 1993). Metabolism as indicated by plasma glucose, NEFA, and BHBA concentrations does not seem to be influenced by yeast culture supplementation alone.

Yeast culture supplementation affected feeding behavior variables (Table 5). Prepartum cows receiving XP-2 or XP-8 treatments had greater number of meals per day (P=0.02) than control cows, and number of meals were similar for both concentrations of yeast culture. Prepartum average daily meal size tended to be greater (P=0.07) when supplemented with XP-2 compared to XP8 (Table 5). Prepartum total daily feeding time tended to be increased (P=0.08) with yeast culture supplementation. Postpartum total daily feeding time was greater (P<0.01) for cows on the XP-8 treatment compared with XP-2 cows, and a treatment by day interaction (P=0.04) indicated that feeding time increased slightly more rapidly for the first 5 days postpartum for cows receiving yeast culture supplementation compared to cows receiving the control. Robinson and Garrett (1999) observed that multi- and primiparous Holsteins supplemented with yeast culture pre- and postpartum maintained diurnal feeding patterns closer to calving than non-supplemented controls and were able to resume regular diurnal patterns sooner postpartum. Thus, yeast culture supplementation may positively alter feeding behavior patterns during the critical periparturient period.

In the present study, additional amounts of supplementation (XP-8) did not result in further enhancement of intake, lactation performance, or metabolism compared to XP-2. Yeast culture supplementation has improved modulation of ruminal pH, and decreased ruminal lactate concentrations, and acetate to propionate ratios (Williams et al., 1991). A proposed mechanism credits yeast culture with stimulating the growth and activities of cellulose digesting and lactic acid utilizing bacteria by supplying nutrients (Wiedmeier et al., 1987; Harrison et al., 1988). Both pre- and postpartum diets contained alfalfa hay, the forage upon which yeast cultures have the greatest digestibility effect (Adams et al., 1995; Miranda et al., 1996).

If the improvement in digestibility associated with greater yeast culture supplementation (XP-8) may have also increased satiety responses, highly digestible diets would have a decreased DMI due to increased propionate concentrations which signal an excess of metabolic fuels and increase satiety (Allen, 2000; Forbes, 2006). While supplementation of 57 g/d yeast culture improved intake, 227 g/d did not. This may be due to an increase of digestibility promoting

satiety signals, thus no additional gains in DMI in our study. Additional studies are needed to increase the understanding of amount of yeast culture supplementation on digestibility for various forages and resulting effect on intake signal.

IMPLICATIONS

When supplemented to periparturient diets, yeast culture can increase DMI prior to calving and increase milk yield postpartum. Yeast culture supplementation enhances productivity through the transition period with no additional benefits from higher supplementation level. Supplementation of yeast culture at a higher level (above 56 g/d) may diminish returns on cow performance although levels between 56 and 227 g/d warrant further study. Further study is also necessary to determine the role of diet composition in the effect of yeast culture supplementation and speculation on the mechanisms of satiety and hunger controls.

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2.1 Table 2. Ingredient and nutrient composition of pre- and postpartum diets.

	% of Diet Dry	Matter
Ingredient	Prepartum	Postpartum
Corn silage	34.0	18.3
Grass silage	17.0	14.8
Alfalfa hay	13.6	18.3
Ground corn	23.8	
Soybean meal	8.5	
Mineral/Vitamin ¹	3.1	
Mixed Grain ²		48.6

	Chemical Com	position, % DM
	Prepartum	Postpartum
CP	12.9	16.5
NDF	33.4	32.3
ADF	18.7	18.6
Crude Fat	3.2	5.1
Ash	8.9	9.4
Ca	1.06	0.78
P	0.42	0.41

¹Contains (DM basis) 23% Ca, 4.5% P, 0.73% Mg, 0.11% K, 5.0% S, 1.98% Na, 15.6% Cl, 6 mg Co/kg, 700 mg Cu/kg, 300 mg Mn/kg, 9 mg Se/kg, 989 mg Zn/kg, 188 KIU A/kg, 55 KIU D/kg, and 2.8 KIU E/kg.

²Contains (as-fed basis) 26% rolled corn, 26% rolled barley, 16% whole cottonseed, 13% soybean meal, 13% dried corn distillers grain, 3.2% Na bicarbonate, 1.3% limestone, and 1.5% other mineral/vitamin sources. Contains (DM basis) 19.7% CP, 22% NDF, 0.82% Ca, 0.65% P, 0.36% Mg, 0.82% K, 0.25% S, 1.2% Na, 0.29% Cl, 1.4 mg Co/kg, 18 mg Cu/kg, 31 mg Mn/kg, 0.7 mg Se/kg, 59 mg Zn/kg, 7.7 KIU A/kg, 2.4 KIU D/kg, and 79 IU E/kg.

Table 2. Effect of yeast culture on dry matter intake and body weight in periparturient cows. 2.7

'		Treatments ²		P-va	P -value 3	P-value of contrasts ⁴	ıtrasts ⁴
Independent Variables	Control	XP-2	XP-8	Н	TxD	Control vs. XP-2 and XP-8	XP-2 vs. XP-8
	22	22	22				
Prepartum DMI, kg/d	12.45 ± 0.34	13.97 ± 0.33	12.50 ± 0.33	0.02	0.51	90.0	0.002
Postpartum DMI, kg/d	16.60 ± 0.40	17.26 ± 0.40	16.97 ± 0.40	0.49	0.97	0.29	0.61
Prepartum BWT, kg	712 ± 16	741 <u>+</u> 16	720 ± 16	0.42	0.19	0.34	0.36
Postpartum BWT, kg	639 ± 14	663 ± 14	642 ± 14	0.39	0.74	0.43	0.26

Least square means and standard error of mean.

Treatments were Control = 0 g/d, XP-2 = 57 g/d, and XP-8 = 227 g/d of supplemental yeast culture.

The structure of Significance of T = main effect of treatment and T x D = interaction of treatment by day or week.

Probability values of significance for orthogonal contrasts.

No values by parity are as follows: for primiparous cows, Control = 8, XP-2 = 9, XP-8 = 9; for multiparous cows, Control = 14, XP-2 = 13, XP-8 = 13.

P-value of contrasts⁴

Table 3. Effect of yeast culture on milk yield and composition yield in periparturient cows.¹ Treatments² Treatments² 2.3

Independent Variables	Control	XP-2	XP-8	Н	TxD	Control vs. XP-2 and XP-8	XP-2 vs. XP-
N^5	22	22	22				
Milk yield, kg/d	31.1 ± 0.8	34.2 ± 0.8	33.4 ± 0.8	0.02	96.0	0.005	0.45
Milk fat yield, kg/d	1.36 ± 0.08	1.57 ± 0.08	1.48 ± 0.08	0.17	0.91	60.0	0.42
Milk fat percent, %	4.57 ± 0.17	4.77 ± 0.16	4.70 ± 0.17	89.0	0.10	0.42	0.75
Milk protein yield, kg/d	1.06 ± 0.06	1.17 ± 0.05	1.14 ± 0.06	0.33	0.53	0.16	99.0
Milk protein percent, %	3.55 ± 0.09	3.60 ± 0.09	3.58 ± 0.09	0.94	60.0	0.74	06.0
ECM, kg/d	34.6 ± 1.9	39.4 ± 1.8	37.3 ± 1.8	0.18	0.81	0.10	0.41
3.5% FCM, kg/d	35.0 ± 1.9	40.0 ± 1.8	37.7 ± 1.9	0.16	0.88	60.0	0.37
Linear somatic cell score	3.48 ± 0.44	3.43 ± 0.43	3.49 ± 0.44	66.0	0.53	96.0	0.92
Efficiency, kg/d ECM per kg/d DMI	2.10 + 0.10	2.29 + 0.10	2.26 + 0.10	0.37	0.93	0.16	0.87
Efficiency, kg/d FCM per kg/d DMI	2.12 + 0.11	2.52 + 0.10	2.29 + 0.10	0.36	0.97	0.16	0.84

¹ Least square means and standard error of mean.

² Treatments were Control = 0 g/d, XP-2 = 57 g/d, and XP-8 = 227 g/d of supplemental yeast culture.

³ Probability values of significance of T= main effect of treatment and T x D= interaction of treatment by day or week.

⁴ Probability values of significance for orthogonal contrasts.

⁵N values by parity are as follows: for primiparous cows, Control = 8, XP-2 = 9, XP-8 = 9; for multiparous cows, Control = 14, XP-2 = 13, XP-8 = 13

Table 4. Effect of yeast culture on plasma concentrations of glucose, natural logarithmically transformed non-esterified fatty acids (In NEFA), and beta-hydroxy butyrate (BHBA) in periparturient cows.¹

rasts ⁴	XP-2 vs. XP-8		0.40	99.0	0.59	0.61	0.71	0.77
P-value of contrasts ⁴	Control vs. XP-2 and XP-8		0.64	0.20	0.21	0.92	0.50	0.48
lue³	TxD		99.0	0.52	0.01	0.89	0.67	0.12
P-value ³	H		0.63	0.40	0.39	0.88	0.74	0.74
	XP-8	22	82 ± 3.1	79 <u>+</u> 2.7	5.2 ± 0.07	5.9 ± 0.08	6.7 ± 0.27	7.9 ± 0.73
Treatments ²	XP-2	22	86 ± 3.2	77 ± 2.5	5.2 ± 0.07	5.8 ± 0.08	6.8 ± 0.27	7.5 ± 0.73
	Control	22	82 ± 3.1	74 <u>+</u> 2.7	5.3 ± 0.07	5.8 ± 0.08	6.6 ± 0.27	8.3 ± 0.73
	Independent Variables	$ m N^{5}$	Prepartum glucose, mg/dL	Postpartum glucose, mg/dL	Prepartum In NEFA, In(μeq/L)	Postpartum ln NEFA, ln(μeq/L)	Prepartum BHBA, mg/dL	Postpartum BHBA, mg/dL

Least square means and standard error of mean.

Treatments were Control = 0 g/d, XP-2 = 57 g/d, and XP-8 = 227 g/d of supplemental yeast culture.

The specificance of T = main effect of treatment and T x D = interaction of treatment by day or week.

Probability values of significance for orthogonal contrasts.

Note: A probability values of significance for orthogonal contrasts.

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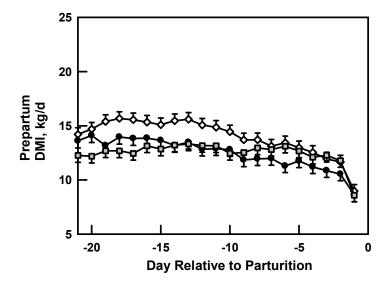
Note: A probability values of significance for orthogonal contrasts.

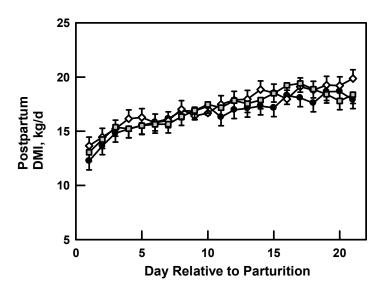
Table 5. Effect of yeast culture on dry matter intake, number of meals, intake per meal, meal duration, and total feeding time in periparturient cows.1

		Treatments ²		P-Vi	P -value 3	P-value of contrasts ⁴	rasts ⁴
Independent Variables	Control	XP-2	XP-8	\vdash	TxD	Control vs. XP-2 and XP-8	XP-2 vs. XP- 8
$ m N^{5}$	18	16	18				
Prepartum							
Visits, meals/d	11.4 ± 0.3	12.3 ± 0.3	12.3 ± 0.3	0.07	0.46	0.02	0.87
DMI, kg per meal	1.10 ± 0.04	1.13 ± 0.04	1.04 ± 0.04	0.18	0.37	0.73	0.07
Meal Duration, min/meal	13.4 ± 0.5	12.8 ± 0.5	13.5 ± 0.5	0.51	0.10	0.67	0.28
Feeding Time, min/d	143.2 ± 4.9	147.7 ± 4.8	158.1 ± 4.7	0.08	0.32	0.10	0.12
Postpartum							
Visits, meals/d	12.9 + 0.4	12.6 + 0.4	13.0 + 0.4	0.74	0.43	0.88	0.44
DMI, kg per meal	1.22 ± 0.04	1.30 ± 0.04	1.24 ± 0.04	0.38	0.65	0.34	0.30
Meal Duration, min/meal	12.7 ± 0.4	12.9 ± 0.4	14.1 ± 0.4	0.05	0.93	0.15	0.05
Feeding Time, min/d 154.9 ± 4 . Least solution means and standard error of mean	154.9 ± 4.3 lerror of mean	153.1 ± 4.4	171.3 ± 4.2	0.005	0.04	0.17	0.003

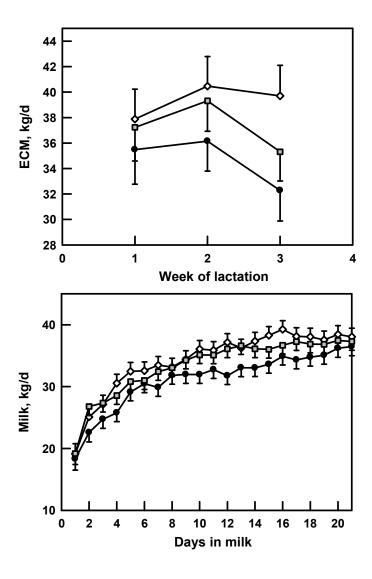
Least square means and standard error of mean. ² Treatments were Control = 0 g/d, XP-2 = 57 g/d, and XP-8 = 227 g/d of supplemental yeast culture. ³ Probabilities of T= main effect of treatment and T x D= interaction of treatment by day or week.

Probabilities of orthogonal contrasts 4 Probabilities by parity are as follows: for primiparous cows, Control = 8, XP-2 = 9, XP-8 = 9; for multiparous cows, Control = 10, XP-2 = 7, XP-8 = 9

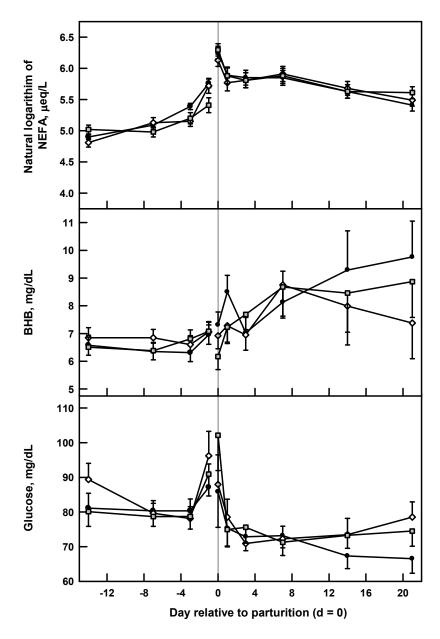




2.1 Figure 1. Top panel: Prepartum DMI for control (\bullet), XP-2 (\Diamond), and XP-8 (\flat treatment groups relative to parturition. Bottom panel: Postpartum DMI for treatment groups relative to parturition. Treatment by day interaction tended to be significant for prepartum DMI (P=0.20), but not for postpartum DMI (P>0.05).



2.2 Figure 2. Top panel: Energy-corrected milk (ECM) yield for control (\bullet), XP-2 (\Diamond), and XP-8 (\square) treatment groups during lactation. Bottom panel: Milk yield for treatment groups relative to parturition. Treatment by day interaction was not significant for ECM or milk yield (P>0.05).



2.3 Figure 3. Top panel: Natural logarithmic transformed plasma concentrations of non-esterified fatty acids (NEFA) for control (\bullet), XP-2 (\Diamond), and XP-8 (\square) treatment groups relative to parturition. Middle panel: Plasma concentrations of beta-hydroxy butyrate (BHB) for treatment groups relative to parturition. Bottom panel: Plasma glucose concentrations for treatment groups relative to parturition. Treatment by day interaction was significant for the NEFA concentrations during the prepartum period (P=0.01). No effect was detected for postpartum concentrations for any of the metabolites or prepartum BHB and glucose concentrations.

CHAPTER 3.A NEW METHOD FOR INDIVIDUALLY FEEDING A SUPPLEMENT TO DAIRY COWS IN A FREE STALL

After the first study, we realized the need to conduct a study with an even larger sample size to increase statistical power. To reduce duration of the study and increase sample size, a Calan gate setup would not be practical. Instead, a top-dressing method was devised and proposed. Originally, the goal was to have a two by three factorial with three doses (0, 56, and 112 g/d in hopes that the dose curve is parabolic with a peak between 56 and 228 g/d) and two administration methods: top-dressing and bolusing. However, dairy owners were not willing to have animals bolused daily for a 56-day period (28 d prepartum through 28 d postpartum) so only one administration method was used.

TECHNICAL NOTE: A NEW METHOD FOR INDIVIDUALLY FEEDING A SUPPLEMENT TO DAIRY COWS IN A FREE STALL

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ABSTRACT

Previously, nutrition research on commercial farms was limited to treatments applied across entire pens or utilization of forced-intake techniques such as bolusing. Our hypothesis was to develop a reproducible, non-invasive procedure for individually feeding supplements to dairy cows on commercial dairy farms. One hundred and fifty-five multiparous Holstein cows, housed in free-stall barns, received Saccharomyces cerevisiae fermentation product (Diamond V Original XPTM) as a top dressing during the morning feeding lock-up period. The supplement consisted of 0, 56, or 112 g of Original XP mixed with 84 g of molasses and 168, 112, or 56 g of corn meal, respectively. After creating an indentation (25 to 30 cm deep and 10 cm diameter) in front of each cowin the newly delivered TMR, the supplement was placed in the indentation so adjacent cows could not consume it. Intake of the supplement and other feed was monitored and given a score on a 5-point scale (0 = no supplement consumption, 1 to 4 = partial consumption, 5 = complete consumption). To prevent supplement consumption by other cows, leftovers of the supplements were removed after 15 min from the feed bunk. Cows accepted the new feeding method within three d. The greatest differences in feed consumption between cows were observed at calving (no supplement consumption: 45 cows; partial consumption: 12 cows; complete consumption: 98 cows). Using PROC GLM, complete supplement consumption on the day of calving was associated with lower serum β -hydroxybutyrate concentrations the following day (P = 0.02). The impact of the differing supplements on intake behavior is reported in companion manuscripts. In conclusion, the newly developed method is non-invasive to cows, requires minimal investment and no modification to existing facilities, and enables three technicians to feed and monitor up to 50 cows during a 30-minute lockup period.

Commercial nutrition studies are valuable for determining the efficacy of a feed supplement. However, current methods are impractical to individually feed animals in a large-scale production setting. When applying treatments to pens, statistical power is compromised to accommodate animal and facility availability and reduce costs (St-Pierre and Jones, 1999). Individual feeding systems such as use of tie-stalls or Calan® gates (American Calan, Northwood, NH) are expensive to install and require maintenance and careful monitoring of animals and equipment (Cole, 1995). Bolusing cows is invasive, labor-intensive, and can result in injury of handler and cows (Andersen and Barrett, 1983). We propose a feeding method that is non-invasive and does not interrupt regular management practices or require any additional facilities. Our method provides investigators with a means to individually feed cattle on a commercial free-stall dairy. We hypothesize top-dressing a semi-formed supplement in wells dug in a total-mixed ration during a daily lock-up period will be an effective individual feeding method for dairy cattle.

The feeding procedure was tested on a 1000-head dairy farm in Oregon's central Willamette Valley. Lactating cows were milked two, three, or six times daily in a 50-stall rotary parlor. Treatments were randomly assigned to 155 cows blocked by parity, with expected calving dates and previous 305ME evenly represented amongst treatments. Supplementation began 28 days prior to each cow's expected calving date and continued through 28 days postpartum. Cows that calved less than 14 days after beginning supplementation were removed from the study.

Until three weeks prior to calving, dry cows were housed in a freestall barn with locking stanchions at the feed bunk. The morning feed was delivered at 0800 and head gates were locked from the time of feed delivery until 0900. During the final three weeks prepartum cows were housed in a large straw-bedded maternity pen, also equipped with locking stanchions at the feed bunk. This pen was fed at 0730 and head gates were locked until 0830. After calving, cows were initially moved to the hospital pen for 48 hours, then to the fresh cow pen. Both of these freestall pens were equipped with locking head gates. The hospital pen was fed at 0700 and stanchions remained locked until 0830 or treatments were complete, and feed was delivered to the fresh pen during the morning milking at 0900. All cows returned from the parlor by 0930 and were locked in headgates until 1000. In all pens, there was sufficient bunk space to lock up all cows.

Composed of 84 g molasses, 168, 112, or 56 g ground corn, and 0, 56, or 112 g/d *Saccharomyces cerevisiae* fermentation product (Diamond V Original XPTM, Diamond V Mills, Cedar Rapids, IA), the treatments were mixed by daily batch in a commercial grade mixer and

weighed into individual 252g allotments. The molasses held the supplement together in large, loose "cookies".

Treatments were administered during the morning lock-up period for each pen. The supplement was top-dressed on the fresh TMR, inside a 10 cm diameter well dug into the feed approximately 45-60 cm in front of the head gates and 25-30 cm down into the feed pile, out of reach of neighboring cows. Cows were given 15 minutes to consume the entire supplement, during which time they were visually monitored. Behaviors such as eating around the supplement, burying the supplement, complete feed refusal, or attempting to consume a neighbor's supplement were recorded. Intake scores of 0-5 were awarded, where 0 = no supplement consumption, 1 to 4 = partial consumption, 5 = complete consumption. If a cow failed to consume her entire treatment, leftovers were removed prior to the release of the cows from the stanchions. Using this method, three technicians were able to feed and monitor up to 50 cows during a 30 minute lock-up period. The greatest difference in feed intake score was observed on day 0 (Figure 1.) when 98 cows consumed their entire supplement (score = 5), 12 cows consumed part of their supplement (score = 1-4), and 45 cows did not consume any of their supplement (score = 0).

On d-28 (at first supplement feeding; baseline sample), -21 (-26 to -18), -14 (-17 to -11), -7 (-10 to -5), -3 (-4 or -3), and -1 (-2 or -1) prepartum and on d 0, 1, 3, 7, 14, 21, and 28 postpartum, one additional technician collected blood samples from the coccygeal vein or artery. Sampling time (during morning lockup period) corresponds to the time of the morning feeding, +/- 10 minutes. Blood samples were collected in serum vacutainer tubes (BD Vacutainer® Plus Plastic Serum Tubes, BD Diagnostics, Franklin Lakes, NJ) and placed on ice immediately after collection. Serum was separated after centrifugation at 1600 x g for 20 min, and frozen at -80°C until analysis. Samples were analyzed for BHBA (β-hydroxybutyrate; Liquicolor, Stanbio Laboratories, Boerne, TX) according to manufacturer's instructions. Spectrophotometric measurements were conducted using a FLUOstar Omega (BMG LabtechInc, San Fransisco, CA) microplateautoreader. To compare serum BHBA values and intake scores on specific days relative to calving, a Pearson correlation of coefficients in the PROC GLM procedure of SAS (SAS User's Guide, 2001) was used. Day 0 feed intake was negatively correlated with serum BHBA concentrations the following day (P = 0.02). Effects of different treatments on intake and BHBA concentrations are discussed in detail in a companion article (Ramsing et al., 2011).

For nutrition studies in a commercial setting, our method provides the most cost effective, least invasive form of supplement administration to large numbers of cows without sacrificing statistical power or erroneously identifying individual cows as the experimental unit when treatments are administered by pen.

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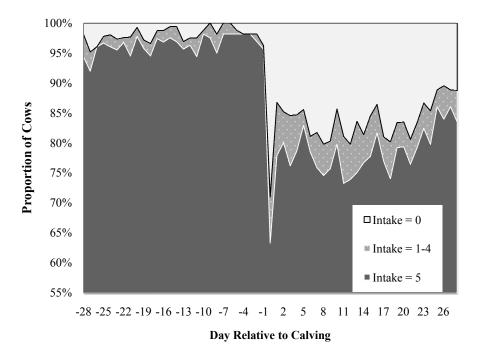
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3.1 Figure 1. Distribution of feed intake scores (0, 1-4, or 5) relative to parturition (d = 0) for cows supplemented individually using the new feeding method

CHAPTER 4.EFFECT OF VARIOUS DOSAGES OF SACCHAROMYCES CEREVISIAE FERMENTATION PRODUCT ON MILK PRODUCTION OF MULTIPAROUS DAIRY COWS

As was previously mentioned, we wanted to compare the effect of varying doses of yeast culture (0, 56, and 112 g/d) on milk production and blood metabolites in a commercial setting. To reduce variation in management, only multiparous cows were included in the study. The study took place during the summer months (May-September), and cows experienced the additional stress of frequent milking (6 times/day). This resulted in a large-scale study of the effects of the two doses of yeast culture on stressed multiparous peripartal Holstein cows.

EFFECT OF VARIOUS DOSAGES OF *SACCHAROMYCES CEREVISIAE*FERMENTATION PRODUCT ON MILK PRODUCTION OF MULTIPAROUS DAIRY COWS

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ABSTRACT

Feeding 56 g/d of Saccharomyces cerevisiae fermentation product (Diamond V Original XPTM) to transition dairy cows increased milk production in most studies. Doubling feeding rates of Original XP was suggested during times of increased stress such as around parturition, which is an especially challenging time period for older cows. The objective of the current study was to evaluate whether greater dosages of Original XP than 56 g/d are beneficial during the transition period. Multiparous Holstein cows housed in the same pen were given a supplement containing either 0 (control; n = 32), 56 (n = 33); or 112 g (n = 31) of Original XP daily during morning lock-up as a top dressing to their TMR. The supplement consisted of 0, 56, or 112 g of Original XP mixed with 84 g of molasses and 168, 112, or 56 g of corn meal, respectively. Supplement feeding started 28 d before predicted calving date (at least 14 d prepartum) and ended 28 d postpartum. The study was conducted on a commercial dairy. Milk weights and samples were collected twice weekly from the afternoon milking on non-consecutive d and analyzed for milk fat, protein, lactose, and somatic cell counts. Overall, supplementation with Original XP did not significantly increase milk production, however, in second lactation Holstein cows (n = 25; 8 or 9 cows per group), Original XP supplementation, regardless of dosage, increased milk production by 5.5 kg/d (P = 0.05). Doubling feeding rates of Original XP (112 g/d) additionally benefitted milk production in the last supplementation week in fourth or higher lactation Holstein cows (n = 27; 8 to 10 cows per group; +10.6 kg/d versus control, P = 0.08, and +9.8 kg/d versus 56 g Original XP; P = 0.10). Although there were several potential confounding factors that could not be controlled on the commercial dairy, our results support the original hypothesis that greater dosages of Original XP than 56 g/d may be required to support increased nutritional demands and milk production during time periods of increased stress.

Key Words: dairy, milk, parity, yeast culture.

INTRODUCTION

Yeast culture supplements have been used in production to enhance lactation performance. During the last twenty years, numerous studies have been conducted to elucidate the response of early lactation dairy cattle to yeast culture supplementation, with inconclusive results. Multiple studies of primi- and multiparous dairy cattle revealed increases in milk yield in conjunction with yeast culture supplementation during the transition phase (Williams et al., 1991; Wohlt et al., 1991; Putnam et al., 1997; Dann et al., 2000; Wang et al., 2001; Fortina et al., 2011), but results from only two studies (Robinson and Garrett, 1999; Ramsing et al., 2009) were statistically significant. Other studies revealed no difference or even a numerical negative effect of yeast culture supplementation on milk yield (Arambel and Kent, 1990; Williams et al., 1991; Swartz et al., 1994; Wohlt et al., 1998; Wang et al., 2001; Lehloenya et al., 2008). Similarly, slight numerical increases were seen in milk fat of yeast culture supplemented cows compared to control cows in some studies (Arambel and Kent, 1990; Williams et al., 1991; Swartz et al., 1994; Putnam et al., 1997; Robinson, 1997; Dann et al., 2000, Ramsing et al., 2009) and increases were seen in milk protein of yeast culture supplemented cows compared to control cows (Swartz et al., 1994; Putnam et al., 1997; Dann et al., 2000; Wang et al., 2001; Lehloenya et al., 2008). A significant increase in milk fat was reported by White et al. (2008) in conjunction with a decrease in milk protein.

In early testing, Harrison et al. (1988) observed more stable ruminal fermentation of cows supplemented with yeast culture. This has been supported by recent studies, where yeast culture supplementation increased milk production in mid-lactation multiparous Holsteins during a fermentable starch challenge (Longuski et al., 2009) and tended to decrease rumen ammonia and increase microbial protein synthesis in the rumen when fed a 50:50 forage: concentrate diet (Hristov et al., 2010), and increased NDF digestibility of early lactation Holsteins (White et al., 2008). In a systematic review of previous studies, Robinson and Erasmus (2009) observed a negative correlation between increased NDF of the diet and an increase in milk yield, with an even stronger negative correlation between ADF and milk yield. They also found the increase in milk production to be absolute across the 22 studies they examined, rather than proportional to current production, possibly due to the dosage of yeast culture being constant and not proportional to DMI (Robinson and Erasmus, 2009). However, dose-response studies of yeast culture are limited. Ramsing et al. (2009) compared three treatment levels of yeast culture (0, 57,

and 227 g/d fermented yeast product) but results were inconclusive, suggesting the possibility of an optimum dose between 56 and 227 g/d.

The objective of this experiment was to evaluate the effects of yeast culture supplementation at varying doses on the milk yield and milk composition of periparturient dairy cattle in a commercial setting. We hypothesized that yeast culture supplementation at double the recommended dose of 56 g/d would aid cows in overcoming the metabolic challenges associated with parturition and onset of lactation, resulting in an increase in milk yield.

MATERIALS AND METHODS

Animals and Diets

All procedures involving animals were conducted in accordance with Oregon State University Institutional Animal Care and Use (IACUC #3991). One hundred and eight Holsteins from the VanBeek Dairy in Monroe, OR were blocked by expected calving date, parity (second, third, or fourth and higher), and previous adjusted milk production (305ME). Within each block cows were assigned at random to one of three dietary treatments. Dietary treatments contained a supplement (Table 1.) including either 0 (control; n = 36), 56 (n = 36); or 112 g (n = 36) of Original XP TM (fully fermented yeast culture of *S. cerevisiae*, Diamond V Mills, Cedar Rapids, IA).

Throughout the course of the study, cows were lost to causes unrelated to supplementation. Nine cows did not start the study due to abortions, culling, or conformation concerns (the supporting ligaments of one cow's udder were severely degenerated, and another cow was unstable due to extensive muscle atrophy in her hindquarters, making blood sampling unsafe). An additional three cows were removed from the study for calving too early (less than 14 days of supplementation) or late (outside the collection period). Ninety six cows remained in the study through parturition (control = 32 cows, 56 g/d = 33 cows, 112 g/d = 31 cows). Prior to milk sample collection, an additional five cows were lost due to death or culling. Specifically, two control cows were lost (one culled with a neurologic disorder, one died from caesarian section surgery complications), two 56 g/d cows died (one from toxic mastitis and one from stepping on and tearing off her prolapsed uterus), and one 112 g/d cow was lost due to weakness. Finally, six cows were unable to complete the study. Two cows (control and 112 g/d) were lost to toxic mastitis, two were lost to weakness (56 g/d), one died with fatty liver (control), and one from injuries from tetany (56 g/d). Eighty-five cows completed the study (control = 28 cows, 56 g/d = 28 cows, 112 g/d = 29 cows).

Cows were group housed in a freestall barn and supplemented individually at the time of the morning feeding using a top dressing method as described in Ramsing et al., 2011 (pending acceptance). Data were collected beginning 28 d prepartum and ending 28 d postpartum. Ingredient and nutrient composition of diets as fed on farm is shown in Table 2. Each cow was body condition scored once weekly during the supplementation period by the same three independent technicians, as described by Bewley and Schutz (2008).

Fresh cows were milked four or six times daily except in the hospital pen, where they were milked twice daily. Milk was collected twice weekly during the mid-afternoon milking by Willamette Valley DHIA (Salem, OR), at d 7, 10, 14, 17, 21, 25, and 28 postpartum. Samples were analyzed for fat, protein, and lactose.

Serum Sampling and Analysis

Blood samples were collected from the coccygeal vein or artery during morning lockup. Prepartum blood samples were collected weekly starting at 28 d before predicted calving and, close to calving, every other day. After calving, blood samples were collected on d 0, 1, 3, 7, 14, 21, and 28. Sampling time (during morning lockup period) corresponds to approximately 5-10 minutes after morning feeding. Blood samples were collected in serum vacutainer tubes (BD Vacutainer® Plus Plastic Serum Tubes, BD Diagnostics, Franklin Lakes, NJ) and placed on ice immediately after collection. Serum was separated after centrifugation at 1600 x g for 20 min, and frozen at -80°C until analysis.

Samples were analyzed for glucose (Glucose Enzymatic, Stanbio Laboratories, Boerne, TX), BUN (Urea Nitrogen Liqui-UV, Stanbio Laboratories, Boerne, TX), BHBA (β-hydroxybutyrate Liquicolor, Stanbio Laboratories, Boerne, TX), and NEFA (NEFA-HR, WAKO Pure Chemical Industries, Richmond, VA). All spectrophotometric measurements were conducted using a FLUOstar Omega (BMG Labtech Inc, San Fransisco, CA) microplate autoreader.

Statistical Analysis

Data were analyzed as repeated measures randomized block design using the PROC MIXED procedure of SAS (SAS User's Guide, 2001). Cows were randomly assigned to treatments within parity (second, third, or fourth and higher). Calving dates and previous 305ME values were evenly represented across treatments. A completely unrestricted variance-convariance structure was specified for repeated measures in time within time for milk yield and composition. Orthogonal contrasts were control vs. XP (56 and 112 g/d) and 56vs. 112 g/d. Fat

corrected milk yield (4%) was calculated as: 4% FCM = [0.4 x milk (kg/d)] + [15 x fat (kg/d)] (NRC, 2001). Due to heterogeneity of variance, plasma NEFA concentrations were log10 transformed prior to statistical analysis.

Model used for all dependant variables was $Y_{ijkl} = \mu + P_i + T_j + D_k + H_l + F_m + TD_{jk} + C_m + e_{ijklmn}$ where μ = overall mean, P_i = the fixed effect of the ith parity (second,third, fourth or higher parity, T_j = the fixed effect of the jth treatment (control, 56, and 112g/d XP), D_k = the fixed effect of the kth day or week (repeated measure), H_l = lth weather(temperature heat index on sampling day, calculated according to NOAA (1976)), F_m = milking frequency (2, 4, or 6, included in model only for milk yield and components), TD_{jk} = the fixed effect of jth treatment by kth day or week, and the random effects of cow (C_m , using a completely unrestricted variance covariance structure for measurement across time within the same cow) and residual error (e_{ijklm}). To evaluate the effect of special treatment on lactation performance, data were also stratified according to the following protocol for "health" instead of by parity for additional analysis: cows following the typical management scenario of two days or fewer in the hospital pen following parturition (d 0 and 1) postpartum were considered "healthy", while cows who remained in the hospital pen beyond d 1 postpartum or were moved back to the hospital pen from the fresh cow pen were considered "sick".

To evaluate the effect of treatment on supplement consumption, the PROC GLM procedure of SAS (SAS User's Guide, 2001) was used to evaluate the model $Y_{ijkl} = \mu + T_i + D_j + TD_{ij} + cow_k + e_{ijkl}$ where T_i = the ith treatment and D_j = the jth day. The same contrasts were applied as for the PROC MIXED analysis. Data was considered significant when $P \le 0.05$, with trends from P < 0.05 to $P \le 0.10$.

RESULTS

Lactation performance of second parity cows was significantly affected by yeast culture supplementation. Compared to control cows, XP-supplemented cows produced +5.4 kg/d (P = 0.05) for the first 4 wk postpartum. The greatest benefit was observed during wk 2 postpartum (Table 3). Overall, 4% FCM also tended to increase by at least 4.3 kg/d for cows receiving 56 or 112 g/d XP compared with control cows (P = 0.06). Overall increases in milk protein (0.15 kg/d) and lactose (1.2 kg/d) were observed in supplemented cows (56 and 112 g/d XP) compared to control cows (P = 0.05 and 0.04 for protein and lactose, respectively). However, a slight but significant decrease in milk protein concentration of 0.25% was observed during week 1 (P = 0.04) at 112 g/d XP compared to 56 g/d XP. Lactose production consistently tended to increase

with XP supplementation at wks 2 and 4 (P = 0.07 and 0.09, respectively) and was accompanied by an increase in lactose concentration during wk 2 (P = 0.08). No significant interactions between treatment and milk production were observed for third parity cows (Table 4), but fourth-plus lactation cows (Table 5.) supplemented with 112 g/d XP vs. 56 g/d XP tended to produce more milk during wk 4 (P = 0.10), more milk fat and a higher protein concentration during wk 3 (P = 0.07 and P = 0.09, respectively), and more protein during wk 4 (P = 0.08). No significant differences were noted between production of fourth-plus parity cows supplemented with XP and controls.

Limited effects were noted between healthy and sick cows. XP supplementation of healthy cows (56 g/d and 112 g/d) tended to increase milk fat concentration in wk 1 postpartum (Table 5) by 0.28 kg/d (P = 0.10). Conversely, when compared with control cows XP supplementation of sick cows tended to decrease milk fat concentration (Table 6) in wk 3 (P = 0.06). When all data were evaluated without stratification by parity or health (Table 7), XP supplementation tended to decrease milk fat concentration in week 3 (P = 0.08).

Serum metabolites were affected by treatment, especially in the first wk postpartum (Table 9.). Compared to controls, cows supplemented with XP had higher serum glucose concentrations (76.5 vs 86.0 and 84.5mmol/L, for 0, 56, and 112 g/d XP, respectively, P = 0.08). Serum BUN concentrations of yeast culture supplemented cows increased on d 0 (+5.9 mg/dL for 56 and +7.2 mg/dL for 112 g/d XP compared to control, P = 0.02) and d 1 postpartum (+2.3 mg/dL for 56 and +1.1 mg/dL for 112 g/d XP compared to control, P = 0.03). BHBA concentrations for yeast culture supplemented cows at parturition were -1.7 and -0.9 mmol/L relative to control for 56 and 112 g/d XP treatments, respectively (P = 0.04). On d 3 postpartum, NEFA values were decreased by for 56 and 112 g/d XP (P = 0.10).

Like lactation performance, metabolic responses to treatment varied by parity. Second lactation cows (Table 10.) exhibited significant differences in day 1 glucose concentrations (80.7 mg/dL vs. 62.0 mg/dL for 56 and 112 g/d, P = 0.01), but no significant difference between control and supplemented cows. Yeast culture supplementation tended to increase BUN concentration one wk prior to calving (P = 0.10) with a significant difference between treatments (11.4 mg/dL vs. 17.0 mg/dL for 56 and 112 g/d, P = 0.01). Postpartum, XP supplementation tended to increase BUN concentration on d 0 (P = 0.09), 21 (P = 0.10), and 28 postpartum (P = 0.08), with an overall tendency to increase BUN concentration in a dose-dependent manner. Increased XP supplementation inversely affected NEFA concentrations on d 3 (P = 0.03), and

lower NEFA concentrations were observed in conjunction with higher XP doses (56 vs. 112 g/d) on d 7 (6.83 vs 6.29 $\ln(\mu Eq/L)$, P = 0.04), d 14 (6.60 vs 5.89 $\ln(\mu Eq/L)$, P = 0.008), and d 21 $(6.48 \text{ vs } 5.90 \ln(\mu \text{Eg/L}), P = 0.03)$. Additional XP supplementation tended to increase BHBA concentrations on d 3 postpartum (56 vs. 112 g/d XP, P = 0.07), but supplementation was beneficial on d 14 (control vs. XP, P = 0.008). Glucose concentrations of third lactation cows (Table 11) were not significantly affected by yeast culture supplementation on d 1 parturition, but an increase was observed in conjunction with supplementation at parturition (control vs. XP, P =0.008). BUN concentrations were affected by dose on d 1 postpartum (P = 0.002) with less XP supplementation resulting in a greater increase in BUN (56 vs. 112 g/d XP). On d 1, supplementation increased BUN concentrations compared to no supplementation (P = 0.02), with a tendency for treatment at 56 g/d to have a greater effect than 112 g/d XP (P = 0.08). NEFA concentration of third parity cows was not affected by yeast culture supplementation, although control cows tended to have higher baseline values (day -21, P = 0.09). Unlike second lactation cows, where yeast culture supplementation increased BHBA concentrations on d 3 and had the opposite effect on d 14, third lactation cows supplemented with XP were observed to have increased BHBA concentrations on d 7 postpartum (P = 0.03). Fourth-plus lactation cows (Table 12) tended to have increased glucose concentrations at parturition in conjunction with XP supplementation (P = 0.07). However, on d -1 (prepartum), glucose concentrations were lower in cows supplemented with 112 g/d XP compared to 56 g/d (P = 0.06). BUN concentrations were increased by XP supplementation on d 0 (P = 0.04) and 7 (P = 0.02), but supplementation tended to have the opposite effect on d 28 postpartum (P = 0.10). Yeast culture supplementation had no effect on NEFA concentrations of fourth-plus lactation cows, but significantly decreased BHBA concentrations on d 21 (P = 0.008) and d 28 postpartum (P = 0.03).

Supplement consumption differences between treatments were observed when cows of all parities were grouped together (Figure 1). An overall treatment by day effect was observed on d 0 (p = 0.02), d 2 (P = 0.0003), d 17 (P = 0.03), and d 19 (P = 0.02). When control cows were compared to those receiving XP supplementation, a treatment by day effect on both d 2 (P = 0.03) and d 3 postpartum (P = 0.03) was observed where control cows appeared to increase intake scores more rapidly following parturition than supplemented cows. Similarly, on d 17 (P = 0.05) and d 19 postpartum (P = 0.05) control cows were still observed to have a higher average intake score than supplemented cows. When supplementation of 56 and 112 g/d XP are compared, cows receiving 112 g/d had significantly higher feed intake scores on day 0 (P =

0.02), day 2(P = 0.001), and day 19(P = 0.040). Body condition score was not affected by treatment (Table 13).

DISCUSSION

Effects of yeast culture on milk yield of early lactation dairy cattle vary, from a non-significant slight negative impact or no improvement (Arambel and Kent, 1990; Swartz et al., 1994; Wohlt et al., 1991) to the significant increase of more than 5.4 kg/d observed in 2nd lactation cows in this study. In most transition studies a slight numerical benefit of less than 2 kg/d was observed (Putnam et al., 1997; Robinson, 1997; Robinson and Garrett, 1999; Soder and Holden, 1999; Dann et al., 2000; White et al., 2008; Fortina et al., 2011). While larger numerical increases in milk yield of greater than 2 kg/d were attributable to yeast culture in some studies (Williams et al., 1991; Wang et al., 2001), the only other statistically significant differences observed were a 2.9 kg/d increase in production of primi- and multiparous cows (Ramsing et al., 2009) and a 2.4 kg/d increase in production of primiparous cows (Robinson and Garrett, 1999).

Parity appears to affect the responses of cows to yeast culture supplementation during the transition period. While Ramsing et al. (2009) did not observe any significant parity by treatment interactions, Robinson and Garrett (1999) observed an increased DMI by primiparous cows while multiparous cows had a greater DMI and a higher CP intake due to selection of a more energydense diet. Putnam et al. (1997) also observed a numerical increase in DMI of 0.9 kg/d when primiparous cows were supplemented with yeast culture, resulting in an increase of milk fat production and 4% FCM, and a tendency for increased milk and milk protein yields. We observed similar improvements in 4% FCM and overall milk protein and lactose yield in second lactation cows. However, these improvements were not observed in older cows or when all cows were pooled for analysis. The effects observed by Putnam et al. (1997) were stronger when cows were fed a low CP diet (16.1 vs 18.8% for a 40% forage diet), supporting Robinson and Garrett's findings that selection for a higher CP diet did not necessarily increase the effect of yeast culture on milk production. Although CP intake was not measured during this study, significant increases in serum BUN concentrations of yeast culture supplemented cows were observed in third and fourth-plus lactation cows (Tables 11 and 12). These values could be attributable to a short-term increase in protein intake, possibly due to preferential selection for higher CP diets by multiparous cows, as was observed by Robinson and Garrett (1999), or possibly resulting from deamination of body proteins for use as an energy source. Regardless, more variability and higher overall serum BUN concentrations were observed for 2nd lactation cows across the entire supplementation period (Table 10).

The interaction between diet, particularly forage properties, and effect of yeast culture has been consistently observed. Yeast culture supplementation has been reported to have a stronger effect when used in conjunction with feeding a high-concentrate diet. Previously, Williams et al. (1991) observed a 4.1 kg/d increase in milk yield when cows receiving a 60% concentrate: 40% hay-based forage diet were supplemented with yeast culture and a slight (+2 kg/d) increase in milk yield when cows were fed the same concentrate: forage ratio with a strawbased forage and supplemented with yeast culture. When cows were fed a 50% concentrate, 50% forage diet, yeast culture had a slight negative effect, regardless of forage source (Williams et al. 1991). Yeast culture has been suggested to provide factors stimulatory to proteolytic bacteria, resulting in an increase of the percent of cellulolytic bacteria in the rumen and increasing total tract digestibility of crude protein and hemicellulose (Wiedmeier et al., 1987). In midlactation dairy cows, Piva et al. (1993) observed tendencies for yeast culture supplementation to improve plasma glucose concentrations and reduce ruminal pH and ruminal ammonia, suggesting yeast culture may help modulate metabolic variation within the rumen. Overall, cows in our study supplemented with XP tended to have higher serum glucose concentrations on day 0 compared to control cows. Interestingly, the strongest effects were observed in third and fourth-plus parity groups with no effect observed in the second parity cows. Increased glucose concentrations can be indicative a more positive net energy balance, possibly due to enhanced digestibility and subsequent improvements in feed efficiency. Lehloenya et al. (2008) observed slight decreases in intake of NDF, ADF, OM, and N coupled with increases of total tract digestibility of the same components in steers supplemented with yeast culture. Similarly, when mid-lactation cattle were examined in heat-stress and normal environmental conditions, yeast culture supplementation improved feed efficiency as measured by kg ECM/kg DMI and kg 4% FCM/kg DMI (Schingoethe et al., 2004, Fortina et al., 2011).

Yeast culture supplementation appeared to reduce the negative energy balance immediately postpartum. NEFA concentrations, which are an indirect measure of triacylglyceride mobilization from adipose tissue (Mashek et al., 2001), usually spike immediately following calving, then drop off in subsequent weeks (Ingvartsen and Andersen, 2000) with circulating levels peaking 0 to 7d postpartum (Hayirli et al., 2011, Janovick et al., 2011). Overall, d 3 NEFA concentrations tended to decrease in a dose-dependent manner with yeast culture supplementation

(P = 0.10). A stronger effect was observed in second lactation cows (Table 10.) where yeast culture supplementation decreased circulating NEFA concentration in a dose-dependent manner on day 3 (P = 0.04), but on d 7 to 21 cows supplemented with 112 g/d XP had the lowest NEFA concentrations, followed by control cows, with greatest concentrations found in cows supplemented with 56 g/d XP. Typically, serum glucose concentrations decrease as the requirement for glucose for lactose synthesis and subsequent milk production increases, and NEFA concentrations increase to meet the body's demands for gluconeogenic precursors (Ingvartsen and Andersen, 2000). This appears to be exactly what happened in wk 3 with the second lactation cows: as milk production and lactose concentrations increased, by the numerically highest amount with 56 g/d cows, NEFA concentrations would have been required to increase to provide energy to support maintenance functions within the body. The mechanism behind the dosage effect is as of yet unknown. However, serum NEFA concentrations did not directly correlate with ketone synthesis. BHBA concentrations followed the expected curve, with parturition marking the first d of the postpartum increase in serum BHBA and a maximum concentration around 7 d postpartum (Hayirli et al., 2011, Janovick et al., 2011). Control cows were consistently in a state of subclinical ketosis, defined as a BHBA concentration above 0.97 mmol/L (Enemark et al., 2009), from d 3 through d 28 postpartum with yeast culture supplemented cows becoming ketotic only on d 7 (56 g/d XP) and d 3 and 7 (112 g/d XP). Again, trends varied by lactation group during the first four wks postpartum (Tables 10-12) with all second lactation cows maintaining serum BHBA concentrations above the subclinical ketosis diagnosis cutoff through the entire postpartum measurement period, regardless of treatment. Cows in their third or lactation responded to supplementation with increased BHBA concentrations at day 7 compared to control cows (P = 0.03), but XP-supplemented fourth-plus lactation cows tended to have lower d 3 BHBA concentrations (P = 0.09) and exhibited significantly lower BHBA concentrations than control cows on d 21 (P < 0.001) and 28 postpartum (P = 0.03).

Dose of yeast culture supplementation may play an interesting role in the observed effect on milk production and metabolic status. While second lactation cows experienced a significant reduction in NEFA and BHBA concentrations postpartum when supplemented with 112 rather than 56 g/d XP, numerical differences in milk production (over FCM, fat %, and protein %) would support supplementation at the latter dose. In third and fourth-plus parity cows, effects of

yeast culture on milk production are still unclear. Further research is necessary before sound dosage recommendations can be made.

CONCLUSION

Yeast culture is beneficial for improving lactation performance through the transition period. It may also assist with reduction of negative energy balance through the first four weeks of lactation and protect against metabolic diseases under some conditions. In fourth-plus lactation cows, a dose of 112 g/d XP through the transition period appears to be beneficial for enhanced lactation performance compared to the recommended dose of 56 g/d. However, more detailed work is necessary to elucidate the mode of action of yeast culture and interactions between parity diet composition.

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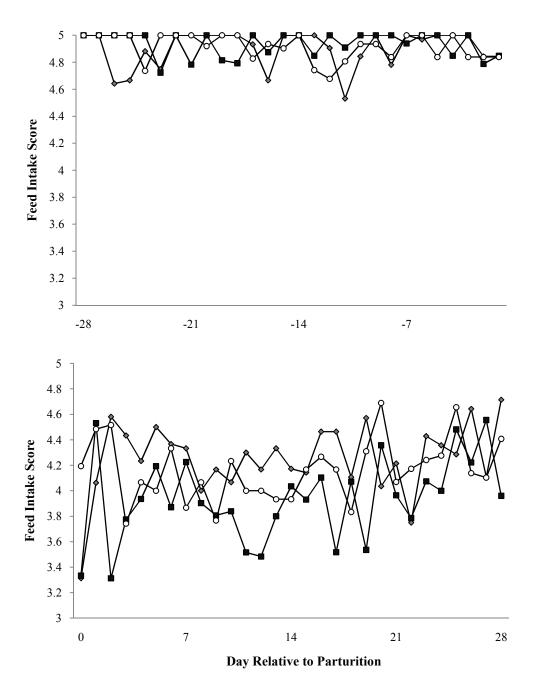
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4.1 Figure 1. Supplement intake scores by day for cows supplemented with $0 \ (\lozenge)$, $56 \ (\blacksquare)$, or $112 \ g/d \ XP \ (\circ)$. Top: prepartum intake, bottom: postpartum intake.

4.1 Table 1. Composition of dietary supplements

	Control	XP	2XP
$XP^{1}(g/d)$	0	56	112
$Corn^2$ (g/d)	168	112	56
Molasses ³ (g/d)	84	84	84

¹. Min. 12.0% CP, Min. 3.0% Crude Fat, Max. 6.5% Crude Fiber, Diamond V Mills, Cedar Rapids, IA

4.2 Table 2. Ingredient and nutrient composition of pre- and postpartum diets

	Percent of Diet I	Ory Matter
Ingredient	Prepartum	Postpartum
Grass Silage		2.13
Alfalfa Hay (20%CP)	13.42	19.26
Corn Silage	27.77	20.93
Triticale Hay (9%CP)	13.69	
Beet Pulp	3.41	
Mineral Premix ¹	4.95	
Molasses Mineral ²		2.96
MagOx ³	0.18	
Corn (Green Screens)	18.15	
Corn (High Moisture Ear Corn)		20.00
Corn Distillers Grain	8.06	12.33
Canola Meal	6.69	6.40
Wheat Distillers Grain		5.97
Bakery By-Product		6.39
EnerGII Regular ⁴	1.82	1.74
Limestone (ground)	1.85	0.94
Sodium Bicarbonate		0.94

	Chemical Composition, % DM				
Nutrient	Prepartum	Postpartum			
NE _L (MCal/kg, DM basis)	1.63	1.70			
Forage	75.91	42.32			
CP	12.72	17.33			
RUP (%CP)	29.04	36.85			
RDP (%CP)	70.96	63.15			
ADF	30.71	18.27			
NDF	46.77	29.99			
Sugar	6.45	6.57			
Starch	13.28	23.59			
EE	3.47	6.18			
Ash	9.16	8.67			

Tontains (DM Basis) 15.4% CP, 15.3% Non Protein Nitrogen, <0.01% Crude Fiber, 69.0% Ash, 13.6% Ca, 2.8% P, 16.2% Cl, 6.8% Mg, 0.003% K, 2.0%S, 3.4 ppm Co, 307 ppm Cu, 20.5 ppm I, 25.8 mg/kg Ethylenediamine dihydroidide, 155 ppm Mn, 6.20 ppm Se, 605 ppm Zn, 2182.6 KIU/kg Vitamin A, 93.0 KIU/kg Vitamin D, 3375.3 IU/kg Vitamin E, 23948.1 mg/kg Choline, 20247.4 mg/kg Niacin, 0.002 % Lysine, 0.001% Methionine, 542.3 mg/kg Monensin² Contains (DM Basis) 21.2% CP, 8.5% Non Protein Nitrogen, 0.14% Crude Fat, 0.02% Crude Fiber, 18.4% Ash, 42.3% Total Sugar, 0.83% Ca, 0.49% P, 0.91% Na, 5.33% Cl, 0.43% Mg, 4.0% K, 0.92%S, 3.9 ppm Co, 420 ppm Cu, 275 ppm Fe, 60.2 ppm I, 806 ppm Mn, 8.25 ppm Se, 1987 ppm Zn, 167.6 KIU/kg Vitamin A, 41.7 KIU/kg Vitamin D, 826.7 IU/kg Vitamin E, 0.09% Lysine, 0.02% Methionine³Guaranteed to contain no less than 56% Mg

². CHS Nutrition, Sioux Falls, SD

³. Min. 5.0% CP, Min. 33.0% total sugars inv., Max. 35% moisture, CHS Nutrition, Sioux Falls, SD

Table 3. Effect of yeast culture on milk yield and composition in second lactation periparturient cows¹

4.3 Table 3.				second lactation peri	parturient cows ¹
		ginal XP (LS Mean		P-value	of Contrasts ²
Ind. Variable	0 g/d XP	56 g/d XP	112 g/d XP		
No.	N = 9	N = 8	N = 8	Control vs. XP	56 vs. 112 g/d XP
Yield (kg/d)					
Overall	31.9 <u>+</u> 3.4	37.3 <u>+</u> 3.5	37.5 <u>+</u> 3.5	0.05	0.96
Week 1	25.8 <u>+</u> 4.1	28.9 <u>+</u> 4.9	31.7 <u>+</u> 4.3	0.28	0.59
Week 2	30.7 <u>+</u> 4.0	36.2 <u>+</u> 3.7	39.0 <u>+</u> 4.0	0.05	0.49
Week 3	36.6 <u>+</u> 4.1	43.2 <u>+</u> 4.5	38.6 <u>+</u> 4.1	0.24	0.28
Week 4	34.3 <u>+</u> 4.1	40.8 <u>+</u> 4.1	40.5 <u>+</u> 4.4	0.13	0.95
4% FCM (kg/d)					
Overall	39.9 <u>+</u> 3.3	46.1 <u>+</u> 3.4	44.2 <u>+</u> 3.5	0.06	0.57
Week 1	39.0 ± 4.5	50.7 ± 5.5	44.7 ± 4.7	0.09	0.34
Week 2	42.8 ± 3.7	45.4 ± 3.4	48.1 ± 3.8	0.19	0.46
Week 3	42.7 ± 4.2	49.1 ± 4.7	40.5 ± 4.3	0.62	0.10
Week 4	35.0 ± 4.0	39.0 ± 4.0	43.5 ± 4.2	0.13	0.34
Fat (kg/d)				****	
Overall	1.61 <u>+</u> 0.14	1.83 ± 0.15	1.71 <u>+</u> 0.15	0.19	0.44
Week 1	1.75 ± 0.19	2.25 ± 0.25	1.89 ± 0.20	0.14	0.21
Week 2	1.79 ± 0.16	1.83 ± 0.15	1.90 ± 0.17	0.59	0.68
Week 3	1.62 + 0.19	1.90 ± 0.21	1.46 ± 0.19	0.76	0.07
Week 4	1.29 ± 0.17	1.34 ± 0.18	1.60 ± 0.19 1.60 ± 0.18	0.27	0.18
Protein (kg/d)	1.27 <u>+</u> 0.17	1.54 1 0.16	1.00 <u>-</u> 0.10	0.27	0.10
Overall	0.91 <u>+</u> 0.10	1.09 <u>+</u> 0.11	1.06 + 0.11	0.05	0.74
Week 1	0.90 ± 0.10	1.08 ± 0.11 1.08 ± 0.16	1.00 ± 0.11 1.04 ± 0.13	0.23	0.74
Week 2	0.89 ± 0.12			0.23	0.76
Week 2 Week 3	0.89 ± 0.12 0.97 ± 0.12	1.07 ± 0.12 $1.13 + 0.13$	1.11 ± 0.13	0.30	0.76
	0.97 ± 0.12 0.88 + 0.12		1.02 ± 0.12		0.88
Week 4	0.88 ± 0.12	1.09 ± 0.12	1.07 <u>+</u> 0.13	0.12	0.88
Lactose (kg/d)	1 24 + 0 15	1.50 + 0.15	1 (2 + 0.15	0.02	0.74
Overall	1.34 ± 0.15	1.59 ± 0.15	1.63 ± 0.15	0.02	0.74
Week 1	0.96 ± 0.19	1.08 ± 0.22	1.30 ± 0.21	0.24	0.37
Week 2	1.25 ± 0.20	1.54 ± 0.19	1.68 ± 0.21	0.06	0.54
Week 3	1.64 ± 0.19	1.84 ± 0.21	1.73 ± 0.20	0.44	0.65
Week 4	1.53 <u>+</u> 0.19	1.90 <u>+</u> 0.20	1.82 ± 0.20	0.09	0.73
Fat (%)					
Overall	5.23 ± 0.30	5.15 ± 0.33	5.03 ± 0.32	0.65	0.76
Week 1	5.95 <u>+</u> 0.41	6.70 ± 0.54	6.60 <u>+</u> 0.44	0.19	0.88
Week 2	5.72 ± 0.37	5.18 ± 0.36	5.27 <u>+</u> 0.39	0.23	0.86
Week 3	4.96 <u>+</u> 0.37	4.81 <u>+</u> 0.40	4.07 <u>+</u> 0.39	0.22	0.15
Week 4	4.31 ± 0.32	3.90 ± 0.33	4.18 ± 0.33	0.40	0.47
Protein (%)					
Overall	3.12 ± 0.07	3.23 ± 0.07	3.16 ± 0.07	0.21	0.31
Week 1	3.68 <u>+</u> 0.09	3.87 ± 0.10^{a}	3.62 ± 0.10	0.53	0.04
Week 2	3.13 ± 0.10	3.21 <u>+</u> 0.09	3.12 ± 0.10	0.72	0.47
Week 3	2.88 ± 0.09	2.95 ± 0.10	2.94 ± 0.10	0.50	0.94
Week 4	2.79 ± 0.09	2.88 ± 0.10	2.94 <u>+</u> 0.07	0.20	0.57
Lactose (%)					
Overall	4.48 <u>+</u> 0.09	4.51 <u>+</u> 0.09	4.56 <u>+</u> 0.09	0.51	0.62
Week 1	4.19 <u>+</u> 0.12	4.16 <u>+</u> 0.13	4.32 ± 0.13	0.73	0.37
Week 2	4.39 ± 0.10	4.57 ± 0.10	4.60 ± 0.11	0.08	0.82
Week 3	4.68 ± 0.10	4.63 ± 0.10	4.67 ± 0.10	0.76	0.74
Week 4	4.65 ± 0.09	4.68 ± 0.10	4.66 ± 0.10	0.81	0.90
I east square mean	_		1.00 - 0.10	0.01	3.70

Week 4 4.65 ± 0.09 4.68 ± 0.09 Week 4 4.65 ± 0.09 4.68 ± 0.09 Week 4 4.65 ± 0.09 Week 4 4.65 ± 0.09 Heavy 1 Least square means and standard error of mean. 2 Probabilities of orthogonal contrasts

Table 4. Effect of yeast culture on milk yield and composition in third lactation periparturient cows¹

4.4 Table 4. Effect of yeast culture on milk yield and composition in third lactation periparturient cows ¹ Original XP (LS Means ± SEM) P-value of Contrasts ²						
T 1 T7 ' 11				P-value	1 -value of Contrasts	
Ind. Variable	0 g/d XP	56 g/d XP	112 g/d XP		56 110 /1TD	
No.	N = 12	N = 13	N = 14	Control vs. XP	56 vs. 112 g/d XP	
Yield (kg/d)						
Overall	41.5 <u>+</u> 3.2	40.1 <u>+</u> 3.0	38.2 ± 3.0	0.53	0.66	
Week 1	32.5 ± 3.0	31.7 <u>+</u> 3.1	33.1 <u>+</u> 2.9	0.98	0.74	
Week 2	39.0 <u>+</u> 3.5	38.9 <u>+</u> 3.2	37.0 <u>+</u> 3.1	0.80	0.67	
Week 3	45.6 <u>+</u> 3.8	46.7 <u>+</u> 3.6	40.7 <u>+</u> 3.5	0.68	0.23	
Week 4	49.1 <u>+</u> 5.2	43.0 <u>+</u> 4.9	42.3 <u>+</u> 4.9	0.29	0.91	
4% FCM (kg/d)						
Overall	47.3 <u>+</u> 3.4	43.6 <u>+</u> 3.2	42.9 <u>+</u> 3.2	0.32	0.87	
Week 1	44.0 <u>+</u> 3.9	44.3 <u>+</u> 4.1	46.0 <u>+</u> 3.7	0.81	0.76	
Week 2	47.1 <u>+</u> 4.1	43.4 <u>+</u> 3.8	42.4 <u>+</u> 3.7	0.39	0.85	
Week 3	49.6 <u>+</u> 4.6	47.1 <u>+</u> 4.3	40.7 <u>+</u> 4.2	0.30	0.29	
Week 4	48.5 ± 5.0	39.8 <u>+</u> 4.7	42.6 <u>+</u> 4.7	0.22	0.67	
Fat (kg/d)						
Overall	1.82 ± 0.14	1.61 ± 0.13	1.64 <u>+</u> 0.13	0.26	0.86	
Week 1	1.86 ± 0.17	1.81 ± 0.18	1.96 ± 0.17	0.89	0.54	
Week 2	1.87 ± 0.17	1.63 ± 0.16	1.65 ± 0.15	0.25	0.93	
Week 3	1.85 ± 0.21	1.66 ± 0.19	1.44 ± 0.19	0.22	0.42	
Week 4	1.69 ± 0.19	1.34 ± 0.18	1.52 ± 0.18	0.25	0.50	
Protein (kg/d)	1.09 _ 0.19	1.5 . <u> </u>	<u> </u>	0.20	0.00	
Overall	1.26 + 0.09	1.21 <u>+</u> 0.09	1.12 <u>+</u> 0.09	0.37	0.47	
Week 1	1.19 ± 0.10	1.17 ± 0.10	1.17 ± 0.09	0.90	0.99	
Week 2	1.22 ± 0.10	1.15 ± 0.09	1.08 ± 0.09	0.40	0.62	
Week 3	1.28 ± 0.10	1.32 ± 0.09	1.10 ± 0.10	0.60	0.13	
Week 4	1.36 ± 0.14	1.19 ± 0.14	1.10 ± 0.10 $1.11 + 0.14$	0.22	0.70	
Lactose (kg/d)	1.30 - 0.14	1.17 <u>-</u> 0.14	1.11 _ 0.14	0.22	0.70	
Overall	1.96 <u>+</u> 0.16	1.87 <u>+</u> 0.15	1.79 <u>+</u> 0.15	0.49	0.71	
Week 1	1.44 ± 0.15	1.42 <u>+</u> 0.15	1.47 <u>+</u> 0.13	0.96	0.79	
Week 2	1.85 ± 0.18	1.82 ± 0.15 1.82 ± 0.16	1.73 ± 0.14	0.74	0.79	
Week 2 Week 3	2.19 ± 0.19	2.20 ± 0.18	1.75 ± 0.10 1.95 ± 0.17	0.74	0.72	
Week 4	_	2.20 ± 0.18 2.04 + 0.25		0.01	0.91	
	2.37 ± 0.26	2.04 <u>+</u> 0.23	2.00 ± 0.25	0.27	0.91	
Fat (%)	4.51 + 0.22	4 19 + 0 21	4 22 + 0 21	0.25	0.61	
Overall	4.51 ± 0.23	4.18 ± 0.21	4.33 ± 0.21	0.35	0.61	
Week 1	5.60 ± 0.27	5.43 ± 0.29	5.71 ± 0.26	0.92	0.48	
Week 2	4.80 ± 0.24	4.30 ± 0.22	4.49 ± 0.22	0.17	0.54	
Week 3	4.14 ± 0.33	3.62 ± 0.30	3.50 ± 0.29	0.14	0.77	
Week 4	3.49 <u>+</u> 0.32	3.37 ± 0.30	3.63 <u>+</u> 0.29	0.98	0.52	
Protein (%)	2.00 + 0.07	2.02 + 0.06	2.02 + 0.06	0.44	0.00	
Overall	3.09 ± 0.07	3.03 ± 0.06	3.02 ± 0.06	0.44	0.90	
Week 1	3.67 ± 0.10	3.57 ± 0.11	3.61 ± 0.10	0.51	0.76	
Week 2	3.11 ± 0.09	2.94 ± 0.08	2.99 <u>+</u> 0.08	0.18	0.65	
Week 3	2.79 ± 0.08	2.87 ± 0.07	2.77 ± 0.07	0.72	0.31	
Week 4	2.77 ± 0.08	2.74 ± 0.07	2.70 ± 0.07	0.59	0.65	
Lactose (%)						
Overall	4.66 ± 0.09	4.58 ± 0.08	4.58 ± 0.08	0.48	0.97	
Week 1	4.40 <u>+</u> 1.12	4.31 ± 0.12	4.32 ± 0.11	0.51	0.94	
Week 2	4.70 ± 0.09	4.65 ± 0.09	4.63 <u>+</u> 0.08	0.62	0.87	
Week 3	4.77 ± 0.09	4.64 <u>+</u> 0.09	4.75 <u>+</u> 0.09	0.52	0.38	
Week 4	4.75 ± 0.09	4.72 ± 0.09	4.64 ± 0.09	0.50	0.50	

4.5 Table 5. Effect of yeast culture on milk yield and composition Original XP (LS Means ± SEM)				P-value of Contrasts ²	
Ind. Variable	0 g/d XP	56 g/d XP	112 g/d XP		
No.	N = 9	N = 10	N = 8	Control vs. XP	56 vs. 112 g/d XI
Yield (kg/d)					
Overall	39.0 <u>+</u> 3.6	40.6 ± 3.7	40.6 ± 3.7	0.91	0.62
Week 1	30.2 ± 3.4	29.4 ± 3.8	29.4 ± 3.8	0.78	0.42
Week 2	40.8 ± 4.3	37.1 ± 4.3	37.1 ± 4.3	0.97	0.22
Week 3	45.6 ± 5.1	35.7 ± 5.0	45.9 ± 5.1	0.42	0.14
Week 4	39.5 ± 4.5	40.3 ± 4.5	50.1 ± 4.5	0.27	0.10
4% FCM (kg/d)	_	_	_		
Overall	45.6 ± 5.1	45.4 <u>+</u> 5.1	46.9 <u>+</u> 4.9	0.90	0.73
Week 1	39.6 ± 5.4	43.5 + 5.6	43.8 ± 5.6	0.50	0.96
Week 2	47.2 ± 5.7	51.7 ± 5.5	44.4 ± 5.6	0.43	0.19
Week 3	51.0 ± 6.6	39.8 ± 6.6	50.4 ± 6.5	0.39	0.18
Week 4	44.8 <u>+</u> 5.7	46.5 ± 5.7	48.9 ± 5.7	0.53	0.67
Fat (kg/d)		<u>-</u>	<u>-</u>		
Overall	1.69 <u>+</u> 0.19	1.59 <u>+</u> 0.19	1.71 <u>+</u> 0.19	0.80	0.42
Week 1	1.55 ± 0.23	1.70 ± 0.23	1.88 ± 0.24	0.30	0.51
Week 2	1.80 ± 0.25	1.91 ± 0.24	1.64 ± 0.24	0.92	0.31
Week 3	1.90 ± 0.25	1.30 ± 0.25	1.81 ± 0.25	0.16	0.07
Week 4	1.49 ± 0.26	1.44 ± 0.27	1.52 ± 0.27	0.95	0.78
Protein (kg/d)	1	1 <u>-</u> 0.27	1.02 _ 0.27	0.50	0.70
Overall	1.30 ± 0.11	1.26 <u>+</u> 0.11	1.34 ± 0.12	0.98	0.58
Week 1	1.21 ± 0.13	1.34 ± 0.13	1.15 ± 0.12	0.81	0.29
Week 2	1.36 + 0.14	1.35 ± 0.13	1.24 ± 0.14	0.65	0.50
Week 3	1.42 ± 0.15	1.12 ± 0.15	1.42 ± 0.16	0.37	0.13
Week 4	1.21 + 0.13	1.23 ± 0.14	1.53 + 0.14	0.21	0.08
Lactose (kg/d)	1.21 _ 0.13	1.23 _ 0.11	1.55 <u>-</u> 0.11	0.21	0.00
Overall	1.85 <u>+</u> 0.19	1.77 <u>+</u> 0.19	1.90 <u>+</u> 0.19	0.96	0.59
Week 1	1.33 ± 0.17	1.42 ± 0.17	1.26 ± 0.19	0.94	0.47
Week 2	1.94 ± 0.22	2.03 ± 0.21	1.72 ± 0.13	0.80	0.29
Week 3	2.21 ± 0.27	1.67 ± 0.26	2.21 ± 0.28	0.41	0.14
Week 4	1.91 ± 0.22	1.97 ± 0.22	2.40 ± 0.23	0.26	0.14
Fat (%)	1.71 _ 0.22	1.57 _ 0.22	<u>_</u>	0.20	V.1.
Overall	4.84 ± 0.30	4.73 ± 0.29	4.89 <u>+</u> 0.29	0.90	0.54
Week 1	5.77 ± 0.36	5.99 ± 0.36	6.50 ± 0.27	0.20	0.24
Week 2	4.98 ± 0.38	5.09 ± 0.37	5.22 ± 0.38	0.63	0.76
Week 3	4.47 ± 0.40	3.90 ± 0.38	4.33 ± 0.40	0.35	0.33
Week 4	4.12 + 0.40	3.92 ± 0.41	3.50 ± 0.42	0.30	0.39
Protein (%)		3.52 <u> </u>	5.50 <u> </u>	0.50	0.57
Overall	2.89 ± 0.28	2.77 + 0.29	2.87 ± 0.28	0.55	0.47
Week 1	3.79 ± 0.29	3.59 ± 0.32	3.51 ± 0.29	0.30	0.75
Week 2	2.79 ± 0.29	2.67 ± 0.32	2.82 ± 0.31	0.79	0.40
Week 3	2.79 ± 0.30 2.53 ± 0.30	2.41 ± 0.29	2.64 ± 0.31 2.64 ± 0.30	0.94	0.09
Week 4	2.43 + 0.29	2.42 + 0.29	2.49 ± 0.29	0.79	0.55
Lactose (%)	2.13 - 0.27	2. 12 <u>·</u> 0.2)	2. 17 <u>-</u> 0.27	0.17	3.55
Overall	4.66 ± 0.13	4.60 <u>+</u> 0.14	4.60 ± 0.15	0.49	0.98
Week 1	4.30 ± 0.15	4.23 ± 0.16	4.13 ± 0.15	0.43	0.57
Week 2	4.69 ± 0.15	4.56 ± 0.15	4.60 ± 0.15	0.35	0.76
Week 3	4.82 ± 0.14	4.75 ± 0.14	4.88 ± 0.14	0.97	0.24
Week 4	4.83 ± 0.13	4.86 ± 0.13	4.77 + 0.13	0.84	0.19

Week 4 4.83 ± 0.13 4.86 ± 0 .

Least square means and standard error of mean.

Probabilities of orthogonal contrasts

4.6 Table 6.		fect of yeast culture on milk yield and composition in Original XP (LS Means ± SEM)		P-value of Contrasts ²	
Ind. Variable	0 g/d XP			1 value	0. 001111111111111111111111111111111111
No.	N = 15	N = 12	N = 12	Control vs. XP	56 vs. 112 g/d XF
Yield (kg/d)	11 13	11 12	11 12	Control vs. Al	30 V3. 112 g/u XI
Overall	40.1 <u>+</u> 2.7	40.1 <u>+</u> 2.9	40.7 <u>+</u> 2.9	0.91	0.85
Week 1	31.1 <u>+</u> 2.5	$\frac{40.1 \pm 2.9}{31.8 + 2.8}$	32.1 ± 2.8	0.73	0.92
Week 2	40.4 ± 2.5	38.8 <u>+</u> 2.4	39.6 ± 2.7	0.73	0.77
Week 2 Week 3	40.4 ± 2.3 43.7 ± 3.8	42.8 ± 4.1	41.9 <u>+</u> 4.1	0.76	0.86
Week 4	45.7 ± 3.8 45.2 ± 3.6	47.0 ± 4.1 47.0 ± 3.9	41.9 ± 4.1 49.2 ± 4.0	0.47	0.65
	43.2 <u>+</u> 3.0	47.0 <u>+</u> 3.9	49.2 <u>+</u> 4.0	0.47	0.03
4% FCM (kg/d)	45 4 + 2 2	441 + 24	110 + 21	0.70	0.92
Overall	45.4 <u>+</u> 3.2	44.1 <u>+</u> 3.4	44.8 <u>+</u> 3.4	0.70	0.83
Week 1	41.6 ± 3.2	44.3 <u>+</u> 3.7	43.7 ± 3.5	0.44	0.86
Week 2	48.2 ± 3.2	44.4 ± 3.0	46.6 ± 3.3	0.25	0.46
Week 3	46.8 <u>+</u> 4.3	43.2 <u>+</u> 4.7	41.8 <u>+</u> 4.6	0.34	0.80
Week 4	45.1 <u>+</u> 3.9	44.4 <u>+</u> 4.2	47.0 <u>+</u> 4.2	0.87	0.58
Fat (kg/d)	4.70 . 0.44	4 64 . 64 5	4 45 . 0 45	0.74	
Overall	1.72 ± 0.14	1.64 <u>+</u> 0.15	1.67 ± 0.15	0.56	0.87
Week 1	1.74 ± 0.14	1.89 ± 0.16	1.83 ± 0.15	0.35	0.71
Week 2	1.89 <u>+</u> 0.15	1.69 <u>+</u> 0.14	1.80 ± 0.15	0.21	0.46
Week 3	1.71 <u>+</u> 0.20	1.51 <u>+</u> 0.21	1.47 ± 0.20	0.28	0.87
Week 4	1.56 <u>+</u> 0.17	1.48 <u>+</u> 0.19	1.57 <u>+</u> 0.19	0.83	0.67
Protein (kg/d)					
Overall	1.20 <u>+</u> 0.08	1.18 <u>+</u> 0.08	1.17 <u>+</u> 0.08	0.76	0.94
Week 1	1.13 <u>+</u> 0.08	1.16 <u>+</u> 0.09	1.13 <u>+</u> 0.09	0.86	0.75
Week 2	1.23 ± 0.07	1.15 ± 0.07	1.15 <u>+</u> 0.08	0.17	0.98
Week 3	1.21 <u>+</u> 0.11	1.14 ± 0.12	1.13 <u>+</u> 0.11	0.53	0.95
Week 4	1.23 ± 0.10	1.28 ± 0.11	1.29 <u>+</u> 0.11	0.66	0.94
Lactose (kg/d)					
Overall	1.91 <u>+</u> 0.14	1.87 <u>+</u> 0.14	1.95 <u>+</u> 0.14	0.99	0.63
Week 1	1.39 ± 0.13	1.37 ± 0.14	1.44 ± 0.14	0.90	0.63
Week 2	1.91 ± 0.13	1.81 ± 0.12	1.91 ± 0.14	0.63	0.47
Week 3	2.13 ± 0.19	2.04 ± 0.20	2.05 ± 0.20	0.69	0.99
Week 4	2.20 ± 0.18	2.26 ± 0.20	2.39 ± 0.20	0.54	0.61
Fat (%)	_	_	_		
Overall	4.76 ± 0.24	4.65 ± 0.26	4.66 ± 0.25	0.64	0.99
Week 1	5.58 ± 0.22	6.05 ± 0.26	5.86 ± 0.24	0.10	0.51
Week 2	5.06 ± 0.25	4.78 ± 0.25	4.89 ± 0.27	0.34	0.72
Week 3	4.40 ± 0.31	4.12 ± 0.34	4.09 + 0.34	0.38	0.94
Week 4	4.00 ± 0.29	3.67 ± 0.32	3.79 ± 0.32	0.39	0.76
Protein (%)	0.2>	5.07 <u>-</u> 0.52	0.77 _ 0.52	0.57	0.70
Overall	3.18 ± 0.09	3.10 ± 0.09	3.10 ± 0.09	0.28	0.50
Week 1	3.77 ± 0.11	3.77 ± 0.12	3.66 ± 0.12	0.64	0.47
Week 2	3.20 ± 0.10	3.09 + 0.09	3.10 ± 0.12	0.15	0.89
Week 3	2.88 ± 0.09	2.86 ± 0.09	2.86 ± 0.09	0.73	0.96
Week 4	2.88 + 0.09	2.87 ± 0.09	2.78 ± 0.09	0.26	0.14
Lactose (%)	2.88 <u>+</u> 0.07	2.87 <u>-</u> 0.07	2.78 <u>+</u> 0.07	0.20	0.14
Overall	4.52 ± 0.07	4.44 <u>+</u> 0.07	4.53 <u>+</u> 0.07	0.61	0.21
Week 1	4.32 ± 0.07 4.29 ± 0.09	4.44 ± 0.07 4.56 ± 0.08	4.35 ± 0.07 4.26 ± 0.10	0.30	0.21
		_			
Week 2	4.52 ± 0.07	4.61 ± 0.08	4.60 ± 0.08	0.65	0.15
Week 3	4.63 ± 0.08	4.56 ± 0.08	4.70 <u>+</u> 0.08 4.59 <u>+</u> 0.08	0.89	0.11 0.80
Week 4	4.63 ± 0.07 ns and standard err	4.61 <u>+</u> 0.08	4.33 <u>+</u> 0.08	0.62	0.80

4.7 Table 7.	Effect of yeast cu	ılture on milk yiel	in sick periparturient cows ¹		
	Orig	inal XP (LS Mear		P-value of Contrasts ²	
Ind. Variable	0 g/d XP	56 g/d XP	112 g/d XP		
No.	N = 15	N = 19	N = 18	Control vs. XP	56 vs. 112 g/d XP
Yield (kg/d)					
Overall	35.5 ± 2.6	35.7 ± 2.4	35.8 <u>+</u> 2.3	0.94	0.97
Week 1	27.8 ± 2.6	28.7 ± 2.6	31.1 ± 2.5	0.50	0.50
Week 2	32.5 ± 3.3	36.5 ± 3.0	32.8 ± 2.9	0.58	0.36
Week 3	39.4 <u>+</u> 3.4	40.8 ± 3.1	38.8 <u>+</u> 2.9	0.92	0.64
Week 4	42.3 ± 4.1	36.7 ± 3.7	40.6 ± 3.7	0.45	0.45
4% FCM (kg/d)	_	_	_		
Overall	42.0 ± 3.0	40.1 ± 2.8	41.2 ± 2.6	0.70	0.76
Week 1	$40.0\overline{3.7}$	38.6 ± 3.6	44.0 ± 3.5	0.76	0.27
Week 2	40.1 <u>+</u> 3.7	42.4 ± 3.4	38.7 ± 3.2	0.92	0.43
Week 3	44.1 ± 4.3	42.9 ± 3.9	39.9 ± 3.7	0.60	0.58
Week 4	43.7 ± 4.3	36.4 ± 3.9	42.1 ± 3.9	0.37	0.29
Fat (kg/d)					
Overall	1.65 ± 0.12	1.53 ± 0.12	1.59 <u>+</u> 0.11	0.53	0.70
Week 1	1.73 ± 0.17	1.63 ± 0.16	1.90 ± 0.16	0.86	0.22
Week 2	1.61 ± 0.15	1.65 ± 0.14	1.52 ± 0.13	0.88	0.46
Week 3	0.17 ± 0.19	1.56 ± 0.17	1.44 ± 0.16	0.42	0.60
Week 4	1.58 ± 0.17	1.28 ± 0.15	1.51 + 0.15	0.33	0.27
Protein (kg/d)	_	—	_		
Overall	1.07 <u>+</u> 0.08	1.08 ± 0.07	1.09 <u>+</u> 0.07	0.85	0.95
Week 1	1.04 ± 0.10	1.07 ± 0.09	1.12 ± 0.09	0.57	0.68
Week 2	1.00 ± 0.10	1.10 ± 0.09	0.99 ± 0.09	0.72	0.37
Week 3	1.12 + 0.10	1.16 + 0.09	1.11 ± 0.09	0.87	0.73
Week 4	1.13 ± 0.12	1.00 ± 0.11	1.12 + 0.11	0.67	0.38
Lactose (kg/d)	_	—	_		
Overall	1.67 <u>+</u> 0.13	1.67 ± 0.12	1.67 <u>+</u> 1.11	0.99	0.99
Week 1	1.20 ± 0.13	1.25 ± 0.13	1.36 ± 0.12	0.48	0.52
Week 2	1.52 ± 0.16	1.69 ± 0.15	1.86 ± 0.14	0.65	0.40
Week 3	1.89 ± 0.17	1.94 ± 0.18	1.86 ± 0.14	0.98	0.69
Week 4	2.05 ± 0.20	1.78 ± 0.18	1.94 ± 0.18	0.42	0.53
Fat (%)	_	—	_		
Overall	4.83 ± 0.19	4.53 ± 0.18	4.58 ± 0.17	0.22	0.82
Week 1	5.91 ± 0.28	5.73 ± 0.27	6.08 ± 0.26	0.99	0.35
Week 2	5.16 ± 0.25	4.79 + 0.23	4.77 ± 0.22	0.21	0.94
Week 3	4.48 ± 0.28	3.95 ± 0.25	3.73 ± 0.24	0.06	0.54
Week 4	3.79 ± 0.25	3.65 ± 0.22	3.76 ± 0.22	0.74	0.71
Protein (%)	_	_	_		
Overall	3.12 ± 0.07	3.15 ± 0.07	3.12 <u>+</u> 0.06	0.83	0.71
Week 1	3.84 ± 0.11	3.86 + 0.11	3.65 ± 0.11	0.55	0.16
Week 2	3.08 ± 0.10	3.07 ± 0.09	3.07 ± 0.09	0.95	0.96
Week 3	2.82 ± 0.08	2.91 ± 0.07	2.90 ± 0.07	0.37	0.91
Week 4	2.75 ± 0.08	2.78 ± 0.07	2.86 ± 0.07	0.43	0.41
Lactose (%)	_	_	_		
Overall	4.63 ± 0.06	4.62 ± 0.06	4.58 ± 0.06	0.66	0.63
Week 1	4.27 ± 0.10	4.34 ± 0.10	4.23 ± 0.09	0.88	0.42
Week 2	4.62 ± 0.09	4.60 ± 0.08	4.53 ± 0.08	0.57	0.53
Week 3	4.78 + 0.08	4.71 + 0.07	4.78 ± 0.06	0.71	0.47
Week 4	4.84 ± 0.07	4.81 ± 0.06	4.76 ± 0.06	0.47	0.62

Least square means and standard error of mean.

2Probabilities of orthogonal contrasts

4.8 **Table 8.** Effect of yeast culture on milk yield and composition in all periparturient cows¹

4.8 Table 8.				in all periparturient c	
		ginal XP (LS Mean		P-value	of Contrasts ²
Ind. Variable	0 g/d XP	56 g/d XP	112 g/d XP		
No.	N = 30	N = 31	N = 30	Control vs. XP	56 vs. 112 g/d XP
Yield (kg/d)					
Overall	36.7 <u>+</u> 2.2	36.3 <u>+</u> 2.2	36.3 <u>+</u> 2.2	0.86	0.98
Week 1	28.4 <u>+</u> 2.1	29.1 <u>+</u> 2.1	30.1 <u>+</u> 2.1	0.57	0.67
Week 2	35.7 <u>+</u> 2.5	36.5 ± 2.3	34.4 <u>+</u> 2.4	0.94	0.44
Week 3	40.8 ± 2.7	40.0 ± 2.7	38.2 <u>+</u> 2.7	0.56	0.58
Week 4	41.9 ± 3.0	39.7 ± 3.0	42.4 ± 3.0	0.81	0.47
4% FCM (kg/d)					
Overall	42.4 <u>+</u> 2.7	40.5 ± 2.6	41.4 <u>+</u> 2.7	0.51	0.71
Week 1	39.5 ± 2.8	40.0 ± 2.9	42.4 ± 2.9	0.53	0.45
Week 2	43.3 ± 3.0	42.5 ± 2.7	40.9 ± 2.9	0.54	0.57
Week 3	44.5 ± 3.5	41.4 ± 3.5	40.0 ± 3.4	0.28	0.73
Week 4	42.2 ± 3.3	37.9 ± 3.3	42.3 ± 3.3	0.50	0.23
Fat (kg/d)					
Overall	1.64 ± 0.11	1.53 <u>+</u> 0.11	1.59 <u>+</u> 0.11	0.39	0.58
Week 1	1.69 ± 0.12	1.71 ± 0.13	1.82 ± 0.13	0.52	0.40
Week 2	1.73 ± 0.12	1.65 ± 0.12	1.61 ± 0.12	0.38	0.71
Week 3	1.65 ± 0.12	1.48 + 0.15	1.45 + 0.15	0.23	0.85
Week 4	1.49 + 0.14	1.3 ± 0.13	1.48 <u>+</u> 0.14	0.39	0.18
Protein (kg/d)	1. 4 2 <u>+</u> 0.14	1.5 - 0.14	1.40 _ 0.14	0.57	0.16
Overall	1.10 <u>+</u> 0.07	1.09 <u>+</u> 0.07	1.08 <u>+</u> 0.07	0.78	0.86
Week 1	1.06 ± 0.07	1.09 ± 0.07 1.09 + 0.07	1.08 ± 0.07 1.08 ± 0.07	0.71	0.95
Week 2	1.00 ± 0.07 1.09 ± 0.08		1.03 ± 0.07 1.03 ± 0.08	0.62	0.43
		1.09 ± 0.07		0.60	0.65
Week 3	1.13 ± 0.08	1.11 ± 0.08	1.07 ± 0.08		
Week 4	1.13 <u>+</u> 0.09	1.08 <u>+</u> 0.09	1.14 <u>+</u> 0.09	0.86	0.56
Lactose (kg/d)	1.74 + 0.11	1.70 + 0.11	1.72 + 0.11	0.76	0.97
Overall	1.74 ± 0.11	1.70 ± 0.11	1.72 ± 0.11	0.76	0.87
Week 1	1.25 ± 0.10	1.26 ± 0.10	1.33 ± 0.10	0.63	0.51
Week 2	1.68 ± 0.12	1.71 ± 0.12	1.63 ± 0.12	0.91	0.60
Week 3	1.99 ± 0.14	1.91 ± 0.14	1.86 ± 0.13	0.47	0.74
Week 4	2.05 ± 0.15	1.92 <u>+</u> 0.15	2.05 ± 0.15	0.71	0.49
Fat (%)	4.00	4.64 . 0.40	1.60 . 0.10	0.00	0 = 4
Overall	4.82 <u>+</u> 0.19	4.64 ± 0.19	4.69 <u>+</u> 0.19	0.33	0.76
Week 1	5.80 ± 0.20	5.89 ± 0.22	6.04 <u>+</u> 0.22	0.40	0.52
Week 2	5.12 ± 0.22	4.85 ± 0.20	4.90 <u>+</u> 0.21	0.21	0.83
Week 3	4.42 <u>+</u> 0.24	4.07 ± 0.24	3.95 ± 0.24	0.08	0.64
Week 4	3.92 ± 0.23	3.73 ± 0.23	3.87 ± 0.23	0.56	0.55
Protein (%)					
Overall	3.21 ± 0.06	3.21 ± 0.06	3.19 ± 0.07	0.78	0.79
Week 1	3.85 ± 0.08	3.87 ± 0.09	3.73 ± 0.09	0.51	0.17
Week 2	3.21 ± 0.08	3.13 ± 0.07	3.17 ± 0.08	0.38	0.64
Week 3	2.92 ± 0.07	2.95 ± 0.07	2.97 ± 0.07	0.44	0.77
Week 4	2.87 ± 0.07	2.88 ± 0.07	2.91 ± 0.07	0.68	0.65
Lactose (%)					
Overall	4.59 <u>+</u> 0.06	4.53 ± 0.06	4.56 ± 0.06	0.44	0.68
Week 1	4.29 ± 0.07	4.24 ± 0.08	4.25 <u>+</u> 0.08	0.50	0.91
Week 2	4.59 ± 0.07	4.56 ± 0.07	4.57 ± 0.07	0.75	0.79
Week 3	4.72 ± 0.06	4.64 ± 0.06	4.73 ± 0.06	0.51	0.14
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¹Least square means and standard error of mean. ²Probabilities of orthogonal contrasts

4.9 Table 9 Effect of yeast culture on metabolic parameters of periparturient cows¹

4.9 Table 9.	e 9. Effect of yeast culture on metabolic parameters of periparturier Original XP (LS Means ± SEM)			e of Contrasts ²	
Ind. Variable			112 g/d XP	***************************************	
No.	N = 30	N = 31	N = 30	Control vs. XP	56 vs. 112 g/d XP
Glucose (mg/dL)					<u> </u>
-21	69.2 + 1.8	66.7 + 1.8	67.1 + 1.8	0.28	0.89
-14	69.0 + 1.3	67.3 + 1.3	68.5 + 1.3	0.43	0.52
-7	65.7 + 1.5	66.6 + 1.5	66.2 + 1.7	0.70	0.84
-3	67.1 + 1.9	65.0 + 1.8	64.7 + 1.9	0.34	0.92
-3 -1	79.3 + 3.4	73.9 + 3.5	74.6 + 3.7	0.24	0.89
0				0.24	0.89
	76.5 + 4.0 $69.1 + 3.2$	86.0 + 4.0	84.5 + 4.0		0.80
1		76.3 + 3.3	71.8 + 3.2	0.21	
3	60.4 + 3.5	63.7 + 3.5	60.9 + 3.5	0.66	0.56
7	61.6 + 3.4	60.7 + 3.4	61.7 + 3.5	0.91	0.84
14	57.2 + 2.3	63.5 + 2.3	57.5 + 2.2	0.24	0.07
21	58.1 + 1.7	59.0 + 1.8	58.4 + 1.7	0.79	0.81
28	62.5 + 1.8	64.5 +1.8	58.8 + 1.8	0.70	0.03
Overall	66.3 + 1.1	67.8 + 1.1	66.2 + 1.1	0.59	0.29
BUN (mg/dL)					
-21	10.3 + 0.7	11.2 + 0.7	11.1 + 0.7	0.36	0.95
-14	9.92 + 0.6	10.8 + 0.6	10.6 + 0.6	0.25	0.77
-7	10.8 + 0.7	11.5 + 0.7	12.2 + 0.7	0.20	0.42
-3	11.1 + 0.7	11.5 + 0.6	11.5 + 0.6	0.57	0.96
-1	11.6 + 0.6	12.0 + 0.6	12.4 + 0.6	0.43	0.70
0	13.2 + 0.7	16.1 + 0.7	14.4 + 0.7	0.02	0.10
1	12.5 + 0.7	14.8 + 0.7	13.6 + 0.7	0.03	0.18
3	13.3 + 0.7	12.2 + 0.7	13.4 + 0.7	0.57	0.24
7	10.2 + 0.6	11.3 + 0.6	12.0 + 0.6	0.06	0.40
14	12.2 + 0.7	11.6 + 0.7	11.4 + 0.6	0.04	0.77
21	11.5 + 0.7	12.0 + 0.7	12.7 + 0.7	0.31	0.50
28	11.2 + 0.6	11.9 + 0.7	12.9 + 0.6	0.14	0.28
Overall	11.5 + 0.4	12.2 +0.4	12.3 + 0.0 $12.3 + 0.4$	0.08	0.85
LNEFA ln(µEq/L)?	11.3 ± 0.4	12.2 ±0.4	12.3 ± 0.4	0.08	0.83
	5.26 + 0.14	5 22 + 0 14	5 27 + 0 12	0.66	0.42
-21	5.36 + 0.14	5.22 + 0.14	5.37 + 0.13	0.66	0.43
-14	5.57 + 0.12	5.54 + 0.12	5.63 + 0.12	0.92	0.59
-7	5.77 + 0.11	5.79 + 0.10	5.81 + 0.11	0.81	0.85
-3	5.86 + 0.12	6.10 + 0.12	6.05 + 0.12	0.14	0.76
-1	6.36 + 0.12	6.25 + 0.12	6.37 + 0.13	0.74	0.48
0	6.22 + 0.09	6.19 + 0.09	6.18 + 0.09	0.75	0.92
1	6.48 + 0.10	6.47 + 0.10	6.55 + 0.10	0.75	0.56
3	6.79 + 0.09	6.63 + 0.09	6.58 + 0.10	0.10	0.67
7	6.63 + 0.10	6.60 + 0.10	6.58 + 0.10	0.71	0.90
14	6.27 + 0.11	6.22 + 0.12	6.18 + 0.11	0.61	0.82
21	6.29 + 0.11	6.31 + 0.11	6.02 + 0.11	0.38	0.07
28	6.19 + 0.10	6.03 + 0.10	6.13 + 0.10	0.38	0.49
Overall	6.15 + 0.06	6.11 + 0.06	6.12 + 0.06	0.64	0.90
BHBA (mmol/L)					
-21	0.49 + 0.03	0.51 + 0.04	0.49 + 0.03	0.60	0.74
-14	0.74 + 0.03	0.47 + 0.03	0.47 + 0.03	0.93	0.94
-7	1.04 + 0.08	0.50 + 0.03	0.48 + 0.03	0.34	0.55
-3	0.48 + 0.04	0.54 + 0.04	0.48 ± 0.04	0.25	0.25
-3 -1	0.59 + 0.05	0.59 + 0.05	0.48 + 0.04 0.66 + 0.05	0.54	0.30
0	0.84 + 0.05	0.67 + 0.05	0.75 + 0.05	0.04	0.30
			0.79 + 0.05		0.99
1	0.75 + 0.05	0.79 + 0.05		0.54	
3	0.99 + 0.08	0.89 + 0.08	1.10 + 0.08	0.93	0.05
7	1.01 + 0.11	1.15 + 0.11	1.23 + 0.11	0.18	0.59
14	1.02 + 0.14	0.90 + 0.14	0.74 + 0.13	0.21	0.40
21	1.08 + 0.12	0.79 + 0.14	0.96 + 0.12	0.16	0.32
28	1.04 + 0.11	0.81 + 0.12	0.90 + 0.11	0.19	0.57
Overall	0.78 + 0.03	0.72 + 0.03	0.75 + 0.03	0.29	0.45

 $[\]frac{\text{Overall}}{\text{Overall}} = \frac{0.78 + 0.03}{0.72 + 0.03} = \frac{0.72}{0.72}$ $\frac{1}{\text{Least square means and standard error of mean.}}$ $\frac{2}{\text{Probabilities of orthogonal contrasts}}$

4.10 Table 10. Effect of yeast culture on metabolic parameters of second lactation periparturient cows ¹						
		iginal XP (LS Means		P-value	e of Contrasts ²	
Ind. Variable	0 g/d XP	56 g/d XP	112 g/d XP			
No.	N = 9	N = 8	N = 8	Control vs. XP	56 vs. 112 g/d XP	
Glucose (mg/dL)	65.0 + 7.4	(2.1 + 7.4	(2.0 + 7.4	0.66	0.00	
-21	65.8 <u>+</u> 7.4	62.1 <u>+</u> 7.4	62.0 ± 7.4	0.66	0.99	
-14	64.2 ± 5.6	63.1 ± 5.9	61.9 ± 5.6	0.78	0.87	
-7	60.3 ± 5.9	62.2 ± 5.6	59.8 <u>+</u> 6.7	0.91	0.76	
-3	61.7 ± 6.7	58.0 ± 7.3	56.6 ± 6.8	0.57	0.88	
-1	70.9 ± 6.7	68.9 ± 6.2	82.8 ± 8.2	0.53	0.16	
0	72.0 ± 5.2	74.0 ± 5.6	72.0 ± 5.6	0.87	0.77	
1 3	63.2 <u>+</u> 5.4 58.9 + 5.4	80.7 ± 5.6	62.0 ± 5.6 58.4 + 5.6	0.17	0.01 0.16	
7	_	61.1 ± 5.5	_	0.64		
14	64.0 ± 5.3	49.9 <u>+</u> 5.6	58.4 ± 5.6	0.10 0.61	0.23 0.46	
21	52.4 <u>+</u> 5.2 58.5 <u>+</u> 5.2	58.1 ± 5.4	52.8 ± 5.5	0.57	0.40	
28	60.1 ± 4.8	53.6 ± 5.6 60.3 ± 5.0	56.6 ± 5.5 57.2 ± 5.1	0.82	0.66	
Overall	62.7 <u>+</u> 2.9	62.7 ± 3.0	61.1 ± 3.1	0.74	0.58	
BUN (mg/dL)	02.7 1 2.9	02.7 <u>+</u> 3.0	01.1 <u>+</u> 3.1	0.74	0.56	
-21	10.9 + 2.0	11.8 <u>+</u> 2.0	12.5 <u>+</u> 2.0	0.60	0.79	
-21 -14	9.7 + 1.5	$\frac{11.8 \pm 2.0}{11.1 + 1.6}$	$\frac{12.3 \pm 2.0}{11.0 + 1.5}$	0.42	0.79	
-7	11.1 + 1.6	11.4 + 1.5	17.0 ± 1.3 17.0 ± 1.8	0.10	0.01	
-3	11.9 + 1.8	12.8 + 1.9	17.0 <u>+</u> 1.8 12.7 <u>+</u> 1.8	0.70	0.95	
-1	12.5 ± 1.8 12.5 ± 1.8	12.7 ± 1.7	14.5 ± 2.1	0.60	0.50	
0	14.4 + 1.4	16.1 + 1.5	18.3 ± 1.5	0.09	0.28	
1	12.5 ± 1.5	14.2 ± 1.5	15.5 ± 1.5	0.15	0.50	
3	15.2 + 1.5	12.5 + 1.5	15.3 ± 1.5	0.42	0.17	
7	12.3 ± 1.5	12.6 ± 1.5	13.8 ± 1.5	0.60	0.54	
14	13.9 + 1.4	13.1 <u>+</u> 1.5	14.3 ± 1.5	0.91	0.56	
21	12.3 + 1.4	14.0 ± 1.5	16.2 ± 1.5	0.10	0.27	
28	11.4 <u>+</u> 1.3	13.7 ± 1.4	15.1 <u>+</u> 1.4	0.08	0.48	
Overall	12.4 + 0.8	13.0 + 0.9	14.7 + 0.9	0.06	0.08	
LNEFA ln(µEq/L)				****	****	
-21	5.40 + 0.26	5.21 + 0.26	5.61 + 0.26	0.97	0.25	
-14	5.65 ± 0.20	5.43 ± 0.21	5.81 ± 0.20	0.89	0.17	
-7	5.69 + 0.21	5.62 ± 0.19	5.97 ± 0.23	0.68	0.21	
-3	5.91 + 0.23	6.11 + 0.25	5.88 ± 0.24	0.75	0.47	
-1	6.32 + 0.23	6.18 ± 0.22	5.99 ± 0.27	0.39	0.57	
0	6.21 ± 0.19	6.11 ± 0.20	6.21 ± 0.20	0.81	0.70	
1	6.65 ± 0.19	6.52 ± 0.20	6.41 ± 0.20	0.40	0.67	
3	7.07 ± 0.19	6.90 ± 0.20	6.31 ± 0.20	0.04	0.03	
7	6.76 ± 0.19	6.83 ± 0.20	6.29 ± 0.20	0.36	0.04	
14	6.24 ± 0.19	6.60 ± 0.20	5.89 ± 0.20	0.99	0.008	
21	6.38 ± 0.19	6.48 ± 0.20	5.90 ± 0.20	0.39	0.03	
28	6.18 ± 0.18	6.18 ± 0.19	5.94 ± 0.19	0.58	0.36	
Overall	6.21 <u>+</u> 0.11	6.18 ± 0.12	6.01 ± 0.12	0.33	0.21	
BHBA (mmol/L)						
-21	0.92 ± 0.24	0.94 ± 0.24	0.93 ± 0.24	0.95	0.97	
-14	0.90 <u>+</u> 0.18	0.90 ± 0.19	0.91 <u>+</u> 0.18	0.97	0.94	
-7	0.97 <u>+</u> 0.19	0.91 ± 0.18	0.95 ± 0.21	0.86	0.90	
-3	1.02 ± 0.21	1.06 ± 0.23	0.93 ± 0.22	0.93	0.68	
-1	1.07 ± 0.21	1.04 ± 0.20	0.95 ± 0.26	0.77	0.77	
0	1.04 ± 0.17	1.14 ± 0.18	1.18 ± 0.18	0.54	0.88	
1	1.25 ± 0.17	1.17 ± 0.18	1.38 ± 0.18	0.88	0.36	
3	1.38 ± 0.17	1.14 ± 0.18	1.55 ± 0.18	0.83	0.07	
7	1.50 ± 0.17	1.44 ± 0.18	1.52 ± 0.18	0.95	0.73	
14	1.75 ± 0.17	1.27 ± 0.17	1.23 ± 0.18	0.008	0.86	
21	1.08 ± 0.17	1.36 ± 0.18	1.24 ± 0.18	0.25	0.58	
28	1.16 ± 0.15	0.98 ± 0.16	1.04 ± 0.16	0.44	0.80	
Overall	1.17 <u>+</u> 0.09	1.11 <u>+</u> 0.10	1.15 <u>+</u> 0.10	0.63	0.69	

Overall 1.17 ± 0.09 1.11

Least square means and standard error of mean.

Probabilities of orthogonal contrasts

4.11 Table 11		ginal XP (LS Means		ation periparturient cow P-value	e of Contrasts ²
Ind. Variable			112 g/d XP		
No.	N = 12	N = 13	N = 14	Control vs. XP	56 vs. 112 g/d XP
Glucose (mg/dL)					
-21	66.3 + 5.4	67.3 + 5.8	70.4 + 5.1	0.70	0.69
-14	69.0 + 4.4	67.6 + 4.2	69.6 + 4.3	0.94	0.74
-7	64.6 + 4.1	63.0 + 4.1	66.7 ± 4.5	0.96	0.54
-3	69.4 + 5.1	65.3 ± 5.0	65.0 + 4.7	0.49	0.97
-1	78.0 ± 5.0	67.2 ± 5.4	74.2 ± 5.4	0.25	0.35
0	73.2 + 4.3	88.1 <u>+</u> 4.1	85.5 ± 3.9	0.008	0.64
1	63.8 ± 4.1	70.9 ± 4.1	71.8 + 3.8	0.13	0.86
3	56.2 + 4.3	58.9 + 4.0	64.5 ± 3.8	0.28	0.31
7	58.4 + 4.1	60.1 ± 4.1	61.7 ± 3.8	0.61	0.78
14	55.9 ± 4.5	66.7 <u>+</u> 4.5	57.5 ± 3.8	0.25	0.12
21	57.3 ± 4.3 57.3 ± 4.3		54.9 ± 3.9	0.23	0.72
28		56.9 <u>+</u> 4.1		0.79	
	60.6 ± 4.3	66.6 ± 4.1	58.6 ± 3.9		0.17
Overall	64.4 <u>+</u> 1.5	66.5 <u>+</u> 1.5	66.7	0.20	0.93
BUN (mg/dL)	0.5 + 1.2	112 + 12	100 + 11	0.27	0.97
-21	9.5 ± 1.2	11.2 ± 1.2	10.9 ± 1.1	0.27	0.86
-14	9.9 ± 1.0	11.5 ± 0.9	10.9 ± 0.9	0.25	0.67
-7	11.1 ± 0.9	11.9 ± 0.9	10.6 ± 1.0	0.91	0.32
-3	11.3 <u>+</u> 1.1	11.1 ± 1.0	11.2 ± 1.0	0.91	0.97
-1	11.9 ± 1.0	12.1 ± 1.1	11.1 ± 1.1	0.85	0.52
0	13.6 ± 0.9	17.3 <u>+</u> 0.9	13.3 ± 0.9	0.13	0.002
1	11.9 <u>+</u> 0.9	15.6 ± 0.9	13.4 ± 0.9	0.02	0.08
3	12.3 ± 0.9	11.6 <u>+</u> 0.9	13.3 ± 0.9	0.87	0.16
7	9.6 <u>+</u> 0.9	10.9 ± 0.9	10.9 ± 0.9	0.23	0.99
14	12.1 <u>+</u> 1.0	12.1 ± 1.0	11.7 <u>+</u> 0.9	0.86	0.79
21	11.7 <u>+</u> 1.0	11.5 <u>+</u> 0.9	12.3 ± 0.9	0.84	0.50
28	11.2 <u>+</u> 1.0	12.1 <u>+</u> 0.9	13.9 <u>+</u> 0.9	0.13	0.15
Overall	11.3 ± 0.47	12.4 ± 0.5	12.0 ± 0.4	0.13	0.50
LNEFA					
ln(μEq/L)?					
-21	5.53 ± 0.23	5.04 ± 0.21	5.19 ± 0.19	0.09	0.60
-14	5.42 ± 0.17	5.56 ± 0.16	5.37 ± 0.16	0.80	0.42
-7	5.87 ± 0.16	5.89 ± 0.16	5.79 ± 0.17	0.88	0.67
-3	5.92 ± 0.19	6.07 ± 0.18	5.95 ± 0.18	0.67	0.65
-1	6.24 ± 0.18	6.16 ± 0.19	6.19 ± 0.19	0.79	0.91
0	6.21 ± 0.17	6.08 ± 0.16	6.02 ± 0.15	0.41	0.78
1	6.42 ± 0.16	6.52 ± 0.16	6.52 ± 0.15	0.61	0.98
3	6.55 + 0.17	6.46 ± 0.16	6.59 ± 0.15	0.92	0.55
7	6.44 + 0.16	6.42 + 0.16	6.70 + 0.15	0.54	0.22
14	6.23 ± 0.17	6.01 ± 0.17	6.33 ± 0.15	0.78	0.16
21	6.20 ± 0.17	5.88 ± 0.16	6.00 ± 0.15	0.41	0.82
28	6.14 ± 0.17	6.07 ± 0.08	6.20 ± 0.16	0.61	0.16
Overall	6.10 ± 0.08	6.01 ± 0.08	6.07 ± 0.08	0.57	0.59
BHBA (mmol/L)			**** = ****	***	***
-21	0.48 <u>+</u> 0.17	0.53 ± 0.18	0.45 ± 0.16	0.94	0.74
-14	0.45 ± 0.17	0.49 + 0.13	0.45 ± 0.10 $0.45 + 0.13$	0.94	0.82
-1 4 -7	0.56 + 0.113	0.59 ± 0.13	0.43 ± 0.13 0.43 + 0.14	0.73	0.39
-3	0.59 ± 0.16	0.55 ± 0.15	0.43 ± 0.14 0.50 ± 0.14	0.73	0.84
-1	0.64 + 0.15	0.62 ± 0.15 0.62 ± 0.16	0.50 ± 0.14 $0.55 + 0.16$	0.77	0.76
0	0.04 ± 0.13 0.90 ± 0.13	0.62 ± 0.10 0.67 + 0.13	0.76 ± 0.10 0.76 ± 0.12	0.77	0.70
1	0.90 ± 0.13 0.75 ± 0.13	0.07 ± 0.13 0.90 + 0.13	0.70 ± 0.12 0.73 ± 0.12	0.67	0.32
		_			
3	1.12 ± 0.13	1.02 ± 0.13	1.10 ± 0.12	0.71	0.62
7	1.02 ± 0.13	1.29 ± 0.13	1.42 ± 0.12	0.03	0.46
14	0.92 ± 0.14	0.90 ± 0.14	0.69 ± 0.12	0.45	0.24
21	1.07 ± 0.14	0.82 ± 0.13	1.00 ± 0.12	0.33	0.31
28	0.87 ± 0.14	0.70 ± 0.13	0.91 ± 0.12	0.68	0.26
Overall	0.78 <u>+</u> 0.05	0.76 ± 0.05	0.75 ± 0.05	0.66	0.91

Overall 0.78 ± 0.05 0.76

Least square means and standard error of mean.

Probabilities of orthogonal contrasts

	Effect of yeast culture on metabolic parameters of fourth-plu Original XP (LS Means ± SEM)			P-value of Contrasts ²	
Ind. Variable	0 g/d XP 56 g/d XP		112 g/d XP		
No.	N = 9	N = 10	N = 8	Control vs. XP	56 vs. 112 g/d XI
Glucose (mg/dL)					· ·
-21	75.2 + 6.4	67.5 <u>+</u> 6.4	61.6 <u>+</u> 6.4	0.17	0.51
-14	70.6 + 4.8	67.6 + 4.9	67.0 ± 5.4	0.58	0.93
-7	69.3 ± 4.8	71.4 ± 4.8	67.5 ± 4.9	0.98	0.56
-3	63.3 + 7.0	64.2 + 6.3	61.5 ± 9.8	0.97	0.82
-1	81.7 + 5.4	78.5 + 5.8	63.5 ± 5.4	0.11	0.06
0	80.4 + 4.6	89.7 + 4.6	91.3 + 4.7	0.07	0.82
ĺ	77.2 + 4.6	73.7 ± 4.7	76.1 ± 4.8	0.68	0.72
3	62.4 + 4.8	67.8 + 4.8	57.9 + 5.0	0.94	0.15
7	60.4 + 4.8	64.3 + 4.5	57.7 ± 5.4	0.92	0.35
14	59.3 + 4.8	62.9 + 4.7	58.1 ± 5.0	0.84	0.49
21	53.8 ± 5.0	64.0 ± 5.5	61.8 ± 5.0	0.14	0.77
28	64.8 + 5.0	66.3 + 5.4	60.0 ± 5.4	0.79	0.41
Overall	 -	69.8 + 1.8		0.79	0.08
	68.2 <u>+</u> 1.9	09.8 ± 1.8	65.3 <u>+</u> 1.9	0.78	0.06
BUN (mg/dL)	11 1 ± 1 2	11.0.1.1.2	115 ± 12	0.05	0.77
-21	11.1 <u>+</u> 1.3	11.0 ± 1.3	11.5 <u>+</u> 1.3	0.95	0.77
-14	11.0 ± 1.0	9.5 ± 1.0	10.9 ± 1.1	0.51	0.33
-7	10.6 ± 1.0	11.5 ± 1.0	11.2 ± 1.0	0.52	0.81
-3	10.7 <u>+</u> 1.3	12.0 <u>+</u> 1.2	11.9 <u>+</u> 1.7	0.44	0.97
-1	11.3 ± 1.1	11.8 <u>+</u> 1.1	12.5 ± 1.1	0.51	0.63
0	12.1 <u>+</u> 1.0	15.5 <u>+</u> 1.0	13.5 ± 1.0	0.04	0.14
1	13.5 ± 1.0	15.1 <u>+</u> 1.0	13.1 ± 1.0	0.63	0.17
3	13.2 <u>+</u> 1.0	13.3 <u>+</u> 1.0	12.5 ± 1.0	0.84	0.57
7	9.3 ± 1.0	10.8 <u>+</u> 1.0	13.5 <u>+</u> 1.1	0.02	0.06
14	10.6 <u>+</u> 1.0	10.5 <u>+</u> 1.0	8.9 <u>+</u> 1.1	0.44	0.28
21	11.3 <u>+</u> 1.0	11.1 <u>+</u> 1.1	10.1 <u>+</u> 1.1	0.60	0.48
28	11.3 <u>+</u> 1.1	9.4 <u>+</u> 1.1	8.7 <u>+</u> 1.1	0.10	0.69
Overall	11.3 ± 0.5	11.8 ± 0.5	11.5 ± 0.5	0.57	0.72
LNEFA					
ln(μEq/L)?					
-21	5.26 <u>+</u> 0.24	5.22 ± 0.24	5.51 ± 0.24	0.72	0.41
-14	5.68 ± 0.19	5.62 ± 0.19	5.81 ± 0.21	0.88	0.50
-7	5.72 + 0.19	5.83 + 0.18	5.91 ± 0.19	0.51	0.75
-3	5.91 ± 0.26	6.05 ± 0.23	6.62 ± 0.34	0.19	0.16
-1	6.56 + 0.21	6.45 + 0.21	6.63 ± 0.21	0.92	0.56
0	6.26 ± 0.18	6.44 ± 0.18	6.37 ± 0.19	0.50	0.80
1	6.42 + 0.18	6.44 + 0.18	6.73 + 0.19	0.46	0.27
3	6.84 + 0.19	6.62 + 0.18	6.79 + 0.20	0.57	0.52
7	6.74 + 0.19	6.65 + 0.18	6.67 + 0.21	0.72	0.92
14	6.43 ± 0.19	6.21 + 0.19	6.30 ± 0.20	0.45	0.75
21	6.36 + 0.20	6.50 + 0.21	6.30 ± 0.20	0.85	0.50
28	6.24 ± 0.20	6.04 + 0.21	6.15 + 0.21	0.58	0.72
Overall	6.20 ± 0.09	6.17 ± 0.09	6.32 ± 0.09	0.68	0.26
BHBA (mmol/L)	0.20 _ 0.07	0.17 - 0.07	0.52 1 0.07	0.00	0.20
-21	0.48 + 0.16	0.44 <u>+</u> 0.15	0.54 <u>+</u> 0.13	0.89	0.57
-14	_		0.54 ± 0.13 0.55 + 0.12		0.57
-14 -7	0.49 ± 0.12	0.43 ± 0.12	_	0.95	
	0.48 ± 0.12	0.46 ± 0.12	0.54 ± 0.23	0.88	0.59
-3	0.51 ± 0.17	0.46 ± 0.15	0.71 ± 0.13	0.95	0.80
-1	0.57 ± 0.13	0.54 ± 0.14	0.77 ± 0.12	0.73	0.38
0	0.90 ± 0.11	0.71 ± 0.11	0.77 ± 0.12	0.24	0.74
1	0.73 ± 0.11	0.72 ± 0.12	1.16 ± 0.12	0.92	0.77
3	0.92 ± 0.12	0.88 ± 0.12	0.91 ± 0.13	0.49	0.09
7	0.90 ± 0.12	1.05 ± 0.11	0.78 ± 0.12	0.57	0.41
14	0.72 ± 0.12	0.72 ± 0.12	0.95 ± 0.12	0.53	0.73
21	0.88 ± 0.12	0.61 ± 0.13	0.83 ± 0.13	0.0008	0.06
28	1.21 <u>+</u> 0.12	0.89 ± 0.13	0.71 ± 0.04	0.03	0.74
Overall	0.78 ± 0.05	0.66 ± 0.05	0.76 ± 0.05	0.22	0.16

4.13 **Table 13.** Effect of yeast culture body condition score in periparturient cows¹

4.13 Table 13. 1		inal XP (LS Mean	P-value of Contrasts ²		
Ind. Variable	0 g/d XP	56 g/d XP	112 g/d XP	Control vs. XP	56 vs. 112 g/d XP
Overall	n = 30	n = 31	n = 30		
Overall	3.31 ± 0.07	3.19 ± 0.07	3.23 ± 0.07	0.20	0.69
Week -3/4	3.73 ± 0.05	3.63 ± 0.05	3.69 ± 0.05	0.30	0.49
Week -1/2	3.66 ± 0.06	3.52 ± 0.06	3.59 ± 0.06	0.18	0.47
Week 1/2	3.20 ± 0.08	3.02 ± 0.08	3.09 ± 0.09	0.17	0.57
Week 3/4	2.67 ± 0.10	3.57 ± 0.09	2.54 ± 0.10	0.33	0.84
Second Lactation	n = 9	n = 8	n = 8		
Overall	3.34 ± 0.10	3.26 ± 0.10	3.23 ± 0.11	0.48	0.87
Week -3/4	3.74 ± 0.09	3.62 ± 0.09	3.63 ± 0.09	0.28	0.95
Week -1/2	3.56 ± 0.11	3.48 ± 0.11	3.51 ± 0.11	0.60	0.83
Week 1/2	3.29 ± 0.12	3.17 ± 0.12	3.14 ± 0.13	0.39	0.84
Week 3/4	2.75 ± 0.13	2.76 ± 0.14	2.65 ± 0.14	0.80	0.60
Third-Plus Lactation	n = 21	n = 23	n = 22		
Overall	3.30 ± 0.09	3.17 ± 0.08	3.23 ± 0.09	0.30	0.62
Week -3/4	3.73 ± 0.07	3.64 ± 0.07	3.71 ± 0.07	0.51	0.50
Week -1/2	3.70 + 0.08	3.55 + 0.07	3.62 + 0.08	0.22	0.51
Week 1/2	3.16 ± 0.11	2.97 ± 0.10	3.07 ± 0.11	0.30	0.48
Week 3/4	2.64 ± 0.12	2.51 ± 0.12	2.51 ± 0.12	0.36	0.99

Least square means and standard error of mean.

Probabilities of orthogonal contrasts

CHAPTER 5.CONCLUSION

Each of our studies provided key information or tools to continue piecing together the mode of action of yeast culture supplements.

In the first study, we observed a significant effect of yeast culture on milk yield of primiand multiparous Holstein cows during the transition period, validating its use in the dairy industry. A slight effect on metabolic parameters was observed, indicating that further testing with a larger sample size might clarify the influence of yeast culture on energy balance. Finally, a significant effect on prepartum intake demonstrates a need for further research on the interaction of yeast culture supplementation and intake regulators.

In the first component of our second study, we developed a low-cost, non-invasive method for individually feeding dairy cattle suitable for conducting research trials of nutritional supplements on a commercial dairy. Then, we implemented this method for a large-scale field study of the effects of yeast culture supplementation on milk production and metabolic parameters of transition-period multiparous Holstein cows. With the increased statistical power of a large sample size, we were able to clarify the positive effects of yeast culture supplementation on lactation performance and metabolism, specifically a tremendous increase in milk yield of second lactation cows, and a decrease in circulating BHBA concentrations three and four weeks postpartum in older cows.

The means by which yeast culture functions remains to be further elucidated. However, our results do not contradict the hypothesis that yeast culture increases fiber digestibility by providing stimulatory factors for cellulolytic ruminal bacteria, decreasing NEFA concentrations thus reducing their negative impact on intake, and decreasing rumen fill to cause a slight increase in meal frequency and increase in meal size prepartum. Additionally, it is possible that yeast culture increases protein digestibility, may enhance deamination of structural proteins for use as an energy source, or both. Regardless of the mechanism, it is unlikely that yeast culture will support enhanced milk yield beyond the maximum amount the cow is genetically gifted to produce. Such supplements may be useful to assist in reaching genetic potential, especially in younger cows or animals under stress (environmental or physical) but may be less helpful in cows already producing at or near their full capabilities.

Much research has already been completed in this area, but several specific areas would benefit from further exploration. Dose-response studies are limited, and when they have taken place no more than three treatments (including the control) have been tested within a single study.

Our results indicate a significant interaction between parity and dose response, so populations must be carefully considered. However, carefully defined criteria for yeast culture dosing would be a valuable tool for producers during challenging economic periods. Additionally the effect of yeast culture supplementation on intake regulators such as leptin, cholecystokinin, ghrelin, neuropeptide-Y, tumor necrosis factor- α , haptoglobin, and interleukin-6 should be explored. Tumor necrosis factor- α , haptoglobin, and interleukin-6 also provide key insight into the effect of yeast culture on systemic inflammation, which would help complete the knowledge base on this form of supplementation.

In conclusion, our research has contributed much information to the wealth of knowledge on the subject of yeast culture supplementation. While the mechanism by which yeast culture functions has not been determined, our results bring us closer to understanding its mode of action.

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