Effects of supplementing a *Saccharomyces cerevisiae* fermentation product during the periparturient period on the immune response of dairy cows fed fresh diets differing in starch content

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ABSTRACT

The objective of this study was to evaluate the effects of supplementing a *Saccharomyces cerevisiae* fermentation product (SCFP; NutriTek, Diamond V, Cedar Rapids, IA) during the periparturient period (d -28 ± 3 to 44 ± 3 relative to calving) on mRNA abundance of genes in the rumen epithelium, inflammation indicators, oxidative status, and adaptive immunity of dairy cows fed diets with different starch content after calving. From d 28 ± 3 ($\pm$ standard deviation) before the expected calving date to calving, Holstein cows ($n = 38$) received a common basal controlled-energy close-up diet (1.43 Mcal/kg, net energy for lactation; 13.8% starch) with (SCFP; $n = 19$) or without (CON; $n = 19$) SCFP, and cows within each treatment (CON or SCFP) were fed either a low- (LS; 22.1% starch) or high-starch (HS; 28.3% starch) diet from d 1 to 23 ± 3 after calving (fresh period). There were 4 treatment groups: LS + CON ($n = 9$), LS + SCFP ($n = 10$), HS + CON ($n = 10$), and HS + SCFP ($n = 9$). From d 24 ± 3 to 44 ± 3 after calving, all cows were fed the HS diets (post-fresh period). Animal assignment to treatments was balanced for parity, body condition score, and expected calving date. An interaction was observed between dietary starch content and SCFP on indices of oxidative stress; plasma concentrations of total antioxidant capacity tended to be reduced on d 21 after calving for SCFP compared with CON cows when a LS fresh diet was fed, but did not differ for cows fed HS fresh diets. Regardless of starch content, SCFP supplementation increased plasma concentrations of malondialdehyde at d 21 after calving compared with CON. Supplementing with SCFP reduced serum concentrations of haptoglobin on d 7 after calving, indicating reduced inflammation, and feeding LS fresh diets reduced mRNA abundance of IL receptor associated

kinase-1 in rumen tissue at d 21 after calving, suggesting reduced immune activation in rumen tissue. Other than the anti-inflammatory effects indicated by lower serum haptoglobin concentration, no other effects of treatment on adaptive immunity were detectable. These results indicate that supplementing SCFP through the transition period and feeding low-starch diets during the fresh period may reduce inflammation.

Key words: inflammation, acute phase protein, calving transition

INTRODUCTION

During the calving transition period, cows can experience depressed immune function, depressed immunity, and an inability to initiate a strong immune response (Mallard et al., 1998). An adequate immune response is necessary to defend against harmful pathogens that the animal faces after calving, such as bacterial infiltration into the uterus and mammary gland (LeBlanc, 2008; Mulligan and Doherty, 2008). Some inflammation is beneficial to help counter some of these early bouts of infections. However, continual inflammation has detrimental effects on cow health and production, and multiple studies have noted an association between chronic inflammation and a reduction in milk yield (Berton et al., 2008; Bionaz et al., 2007; Yuan et al., 2013). The change from a high-forage close-up diet to a high-concentrate fresh diet increases the risk of SARA (Zebeli et al., 2010). Subacute rumen acidosis is associated with an overactivation of the immune system, tight junction failure, and an increase in serum concentrations of acute phase proteins such as serum amyloid-A (SAA) and haptoglobin (Hp; Plaizier et al., 2008). Oxidative stress, the imbalance between oxidants and antioxidants, is common during the transition period and can have detrimental effects on cow health (Bernabucci et al., 2005; Bouwstra et al., 2008). During the calving transition period, cows can face not only a depressed innate immune system, but also a decreased activity of the adaptive immune system (Herr et al., 2011).

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The *Saccharomyces cerevisiae* fermentation product (SCFP) used in this study is a dried product that contains multiple vitamins and antioxidants, including polyphenols (Jensen et al., 2008). In addition to the antioxidants and vitamins found in SCFP, other bioactive compounds including fermentation end products, β -glucans, and other components of the yeast cell have been shown to modulate the immune response in humans as well as animals by priming the innate and adaptive immune response through activation of immune cells (Li et al., 2005; Volman et al., 2008). *Saccharomyces cerevisiae* fermentation products reduce variability of rumen pH and increase VFA production without decreasing pH, potentially reducing the risk of developing SARA (Harrison et al., 1988; Erasmus et al., 2005; Li et al., 2016). In vitro, SCFP inclusion in growth medium increased activation of innate and adaptive immune responses, as well as increased antioxidant protection of cells (Jensen et al., 2008). We hypothesized that supplementation of SCFP during the calving transition period may reduce excessive immune system activation after calving.

Feeding a low-starch fresh diet reduces the effect of the dietary change after calving, and may reduce tight junction failure, immune system activation, and an acute phase response (Plaizier et al., 2008; Zebeli et al., 2010). Tight junctions act as a fence between cells, and the breakdown of tight junction proteins may be caused by periods of low pH (Steele et al., 2011). The breakdown of tight junctions in the rumen may enable the translocation of endotoxin, a potent immune activator, from the rumen into circulation and activate immune responses including an acute phase response (Plaizier et al., 2008). Consistent with this, a low-starch diet fed after calving reduced serum Hp concentrations (McCarthy et al., 2015) although the effects of reducing dietary starch content on the adaptive immune response are not known. As such, we hypothesized that reducing dietary starch content after calving may reduce excessive immune activation.

The objective of this study was to evaluate the effects of SCFP supplementation during the periparturient period on expression of immune-related genes in rumen papillae, the acute phase response, oxidative status, and adaptive immunity in dairy cows fed diets with different starch content during the fresh period.

MATERIALS AND METHODS

All experimental procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP#1915) and conducted according to the guidelines of the Canadian Council on Animal Care (2009).

Experimental Design, Animal Description, and Treatments

Thirty-eight Holstein dairy cows were enrolled into the study at $d 28 \pm 3$ before the expected calving date and were assigned to 1 of 2 treatments (**CON**: no SCFP supplementation; **SCFP**: SCFP supplementation). These cows were a subset selected from 117 cows used in the study of Shi et al. (2019). Animal assignment to treatments was balanced for parity, BCS, and BW (Table 1). The basal close-up diet contained 13.8% starch and 1.43 Mcal/kg of DM (Table 2; Shi et al., 2019), and SCFP was supplemented at 0.173 and 0.095% of dietary DM in prepartum and postpartum diets, respectively, to provide 19 g/cow per d (as-fed basis) based on expected DMI of the cows (prepartum, 11 kg/cow per d; postpartum, 20 kg/cow per d on a DM basis). After calving, cows within CON or SCFP treatment were assigned to either the low-starch (**LS**) or high-starch (**HS**) treatment for the first 23 ± 3 d after calving (fresh period) in a 2×2 factorial arrangement of treatments. Barley grain was replaced with beat pulp to reduce the starch content for LS diets, and additional diet modifications were made to keep all other nutrients similar. All cows were fed the HS diets from $d 24 \pm 3$ to 44 ± 3 after calving (post-fresh period); diet changed on a certain day of week (i.e., Friday), which allowed up to 3 d of deviations from the target day relative to calving. A timeline of experimental design was presented in the companion paper (Shi et al., 2019). Animals were housed in individual tiestalls bedded with wood shavings and fed a TMR once daily at 0730 h, accounting for 5 to 10% refusals. No feed was pushed up throughout the study as cows were fed using mangers allowing them to have full access to feed throughout the day. Cows were milked twice daily at 0330 and 1500 h. Animals were allowed to exercise outside 3 times a week for 3 h a day. Blood and ruminal papilla samples were collected on a certain day of week (i.e., Tuesday), except for d 1 samples, and this protocol allowed deviations of up to 3 d from the target sampling day relative to calving (± 3).

Plasma and Serum Sampling and Analysis

Approximately 15 mL of blood was taken on $d -10$ relative to expected calving date, $d 1$ within 12 h after calving, and on $d 7$, 21 , and 42 relative to actual calving date. The samples were collected from the coccygeal vessels into tubes containing sodium heparin (Fisher Scientific Company, Nepean, ON, Canada) and a glass tube containing no anticoagulant (Fisher Scientific Company). Tubes containing sodium heparin were placed on ice immediately after collection, and

spun within 30 min of collection. Glass tubes were allowed to sit at room temperature for 2 h to coagulate. All samples were spun at $3,000 \times g$ at 4°C for 20 min. Plasma and serum samples were stored at -20°C until time of analyses. Plasma samples were analyzed using commercial kits for concentrations of reactive oxygen metabolites (**ROM**; d-ROMs Test, MO003, Diacron Labs, Grosseto, Italy) and malondialdehyde [**MDA**; TBARS (TCA Method) Assay Kit, Item No. 700870, Cayman Chemical, Ann Arbor, MI], and total antioxidant capacity (**TAOC**; Antioxidant Assay Kit, Item No. 709001, Cayman Chemical). Serum samples were analyzed using commercial kits for concentrations of SAA (Multispecies SAA ELISA kit, catalog no. TP 802, Tridelata Development Ltd., Maynooth, Ireland) and Hp (PHASE Haptoglobin Assay, catalog no. TP 801, Tridelata Development Ltd.).

Ovalbumin Challenge

An ovalbumin challenge was conducted on d 7 and 21 after calving. Cows were injected with 1 mL of an ovalbumin solution consisting of 1 mg of ovalbumin (catalog no. A5503, Sigma-Aldrich, Oakville, Ontario, Canada) and 0.25 mg of a Quil-A vaccine adjuvant (Brenntag, Frederikssund, Denmark) per 1 mL of PBS (catalog no. P3813, Sigma-Aldrich). Approximately 5 mL of blood was taken into glass tube on d 7 and 21 before the injection of the ovalbumin solution, and on d 28 and 35. Tubes were allowed to sit at room temperature for 2 h to coagulate and spun at $3,000 \times g$ at 4°C for 20 min. Serum samples were stored at -20°C until time of analysis. Serum samples were analyzed for IgG concentration using a commercial kit (Bethyl IgG ELISA Kit, catalog no. E11-100, Bethel Laboratories Inc., Montgomery, TX).

Rumen Papillae Sampling

A subset of 18 cows, selected from the 38 cows, were ruminally cannulated for previous studies, and rumen papillae samples ($n = 18$) were taken on d -10 relative to expected calving date, and on d 21 relative to actual calving date. Rumen was partially emptied through the rumen cannula into insulated trash cans to keep contents warm. The ventral sac was pulled through the cannula, and approximately 20 to 30 papillae were cut and immediately placed into ice-cold 0.01 M PBS and rinsed to dislodge rumen content and placed into tubes containing RNAlater solution (catalog no. AM7020, Thermo Fisher Scientific, Waltham, MA). The tubes were placed into -20°C until analysis. Extraction of RNA from the rumen papillae was conducted following the protocol developed by Chomczynski and Sacchi (1987).

mRNA Abundance

β -Actin was used as a housekeeping gene after testing for presence and minimal fluctuation in all samples using quantitative real-time (qRT)-PCR. Genes analyzed were toll-like receptor 4 (*TLR4*; Moyes et al., 2010), interleukin-1 receptor associated kinase 1 (*IRAK1*; Moyes et al., 2010), nuclear factor kappa- β 1 (*NFKB1*; Moyes et al., 2014), occludin (*OCLN*; Minuti et al., 2015), claudin 1 (*CLDN1*; Minuti et al., 2015), claudin 4 (*CLDN4*; Malmuthuge et al., 2013), and tight junction protein 1 (*TJP1*; Minuti et al., 2015). Expression of these genes was measured by qRT-PCR using gene-specific primers (Table 3). The qRT-PCR was performed using the StepOnePlus Real-Time PCR System (Applied Biosystems by Life Technologies, Foster City, CA) using

Plasma and Serum Sampling and Analysis

Table 1. Animal information at enrollment (mean $\pm$ SD, $n = 38$ cows) and calving date)

Item	Treatment ¹			
	CON + LS	SCFP + LS	CON + HS	SCFP + HS
38 cows				
Primiparous cows, no.	3	3	3	3
Multiparous cows, no.	6	7	7	6
Average parity	2.00	2.00	1.90	1.89
BCS	3.31	3.25	3.35	3.29
BW, kg	639	620	655	632
Subset ²				
Multiparous cows, no.	4	5	5	4
Average parity	2.25	2.20	2.40	2.25
BCS	3.21	3.13	3.35	3.42
BW, kg	674	650	724	711

¹CON = control; LS = low-starch fresh diet; HS = high-starch fresh diet; SCFP = *Saccharomyces cerevisiae* fermentation product.

²A subset of 18 ruminally cannulated cows selected from the 38 cows.

ing SYBR green. The reaction consisted of 40 cycles at 95°C for 10 s followed by annealing/extension for 30 s at 60°C. Fluorescence was measured at 0.1°C intervals, and the point at which the signal crossed the threshold was the threshold cross value (Ct). Relative transcript quantity as compared with a control was expressed as fold change. One cow fed a high-starch fresh diet without SCFP supplementation was chosen as the control. The average Ct of each sample was compared with the corresponding average Ct of the housekeeping

gene by subtracting Ct of sample – Ct of housekeeping to calculate ΔCt . The ΔCt of the control was used as the reference value to calculate $\Delta\Delta\text{Ct}$ by subtracting ΔCt of a sample – ΔCt of the control. Fold change was calculated as $2^{-\Delta\Delta\text{Ct}}$ (Livak and Schmittgen, 2001).

Statistical Analysis

Data for mRNA abundance were analyzed separately for d –10 and 21 relative to calving using the Fit Model

Table 2. Ingredient and chemical composition of experimental diets (Shi et al., 2019)¹

Item	Prepartum		Postpartum			
	CON	SCFP	LS		HS	
			CON	SCFP	CON	SCFP
Ingredient, % of DM						
Barley silage	46.9	46.9	46.5	46.5	46.5	46.5
Alfalfa hay	—	—	2.8	2.8	2.8	2.8
Barley straw	29.1	29.1	—	—	—	—
Corn grain, ground, dry	—	—	12.5	12.5	12.5	12.5
Barley grain, rolled	7.5	7.5	8.9	8.9	18.1	18.1
Beet pulp	—	—	8.3	8.3	—	—
Canola meal	8.2	8.2	10.2	10.2	8.0	8.0
Soybean meal, 44% CP	—	—	3.0	3.0	3.0	3.0
Corn gluten meal	0.5	0.5	0.9	0.9	2.0	2.0
Amino Plus ²	1.8	1.8	2.0	2.0	2.0	2.0
Urea	0.5	0.5	—	—	—	—
F-100 Dairy Fat ³	—	—	1.1	1.1	1.1	1.1
Limestone	0.7	0.7	1.4	1.4	1.6	1.6
Potassium carbonate	—	—	—	—	0.1	0.1
Premix-CON ⁴	1.9	—	1.1	—	1.1	—
Premix-SCFP ⁵	—	1.9	—	1.1	—	1.1
Rumensin premix ⁶	0.8	0.8	0.6	0.6	0.6	0.6
Mineral and vitamin mix ⁷	2.1	2.1	0.8	0.8	0.8	0.8
Nutrient content						
% of DM	50.2	50.2	50.2	49.9	50.2	50.1
CP, % of DM	15.3	15.3	17.3	17.0	17.5	16.9
ADF, % of DM	31.6	31.5	20.9	20.3	19.3	19.2
NDF, % of DM	49.5	49.4	33.3	32.7	31.7	31.5
Forage NDF, % of DM	45.1	45.1	24.0	24.0	24.0	24.0
NFC, % of DM	25.6	25.6	39.3	41.3	41.1	42.8
Starch, % of DM	13.8	13.9	21.6	22.5	27.0	29.5
Ether extract, % of DM	2.5	2.5	3.6	3.6	3.7	3.7
NE _L , ⁸ Mcal/kg of DM	1.43	1.43	1.60	1.62	1.63	1.64
DCAD, mEq/kg	26.0	25.0	192	164	170	138
NE _L allowable milk, ⁸ kg/d	—	—	31.5	31.5	32.1	32.1
MP allowable milk, ⁸ kg/d	—	—	32.7	32.7	32.7	32.7

¹CON = control diet, no *Saccharomyces cerevisiae* fermentation product (SCFP) supplementation, premix-CON was used in the diet; SCFP = SCFP-supplemented diet, premix-SCFP was used in the diet; LS = low-starch diet; HS = high-starch diet.

²Amino Plus (Ag Processing Inc., Omaha, NE).

³F-100 Dairy Fat (Pro-Ag, Winnipeg, MB, Canada).

⁴Contained 99.0% of dry ground corn grain and 1.0% of canola oil, providing 0 g/d of SCFP.

⁵Contained 91.1% of dry ground corn grain, 7.9% of SCFP, and 1.0% of canola oil, providing 19 g/cow per d of SCFP (as-fed basis) based on expected DMI of cows (11 kg/cow per d before calving; 20 kg/cow per d after calving).

⁶Contained 1.2% of Rumensin (Elanco Animal Health, Greenfield, IN), 97.5% of dry ground barley grain, and 1.3% of canola oil.

⁷Contained 11.0% Ca, 2.78% Na, 14.2% Cl, 11.8% Mg, 7.68% S, 16.2 mg/kg of Mn, 4.88 mg/kg of I, 1,212 kIU/kg of vitamin A, 121 kIU/kg of vitamin D, and 4,040 IU/kg of vitamin E for close-up diet; contained 0.71% Ca, 24.3% Na, 39.4% Cl, 13.0% Mg, 0.13% S, 2,427 mg/kg of Cu, 4,244 mg/kg of Mn, 174 mg/kg of Co, 4,583 mg/kg of Zn, 10.0 mg/kg of Se, 85.0 mg/kg of I, 543 kIU/kg of vitamin A, 155 kIU/kg of vitamin D, and 5,118 IU/kg of vitamin E for postpartum diets.

⁸Estimated from NRC (2001).

Table 3. Primer sequences for genes measured for mRNA abundance

Gene	Accession no. ¹	Forward primer (5'-3')	Reverse primer (5'-3')	bp	Source
<i>TLR4</i>	NM_174198.6	TGCGTACAGGTTGTTCTTAACATT	TAGTTAAAGCTCAGGTCCAGCATCT	110	Moyes et al., 2010
<i>IRAK1</i>	NM_001040555.1	CCTCAGCGACTGGACATCCT	GGACGTTGGAACCTTTGACATCT	103	Moyes et al., 2010
<i>NFKB1</i>	NM_001076409.1	TTCAACCGGAGATGCCACTAC	ACACACGTAAACGGAACGAAATC	95	Moyes et al., 2014
<i>OCLN</i>	NM_001082433.2	GCCATTTTCGCCCTGTGTG	CCAAAGGCACTTCCTGCATAA	101	Mimiti et al., 2015
<i>CLDN1</i>	NM_001001854	GGCATCCTGCTGGGACTAATAG	CAGCCATCCGCGATCTTCTGT	100	Mimiti et al., 2015
<i>CLDN4</i>	NM_205801	CCCGGCCCTCATCGTCAATC	GTTGGCGGACGAGACACCG	185	Malmuthuge et al., 2013
<i>TJP1</i>	NM_002696650.3	GCACATAGGATCCCTGAACCA	TGCTTCCGGTAGTACTCCTCATC	107	Mimiti et al., 2015

¹Gene accession number from the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>).

procedure of JMP (version 13.1.0, SAS Institute Inc., Cary, NC) using the following model:

$$Y_{ij} = \mu + T_i + S_j + TS_{ij} + e_{ij},$$

where Y_{ij} is the dependent variable, μ is the overall mean, T_i is the fixed effect of SCFP supplementation, S_j is the fixed effect of fresh diet starch content, TS_{ij} is the interaction of SCFP supplementation by fresh diet starch content, and e_{ij} is the residual. Starch content of fresh diets and its interactions with other parameters were not included in the model for analysis of data from d -10 relative to calving.

The other data were analyzed using the following model with MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). The covariance structure with the smallest Akaike's information criterion was selected (Littell et al., 1996):

$$Y_{ijk} = \mu + T_i + S_j + D_k + TS_{ij} + TD_{ik} + SD_{jk} + TSD_{ijk} + e_{ijk},$$

where Y_{ijk} is the dependent variable; μ is the overall mean; T_i is the fixed effect of SCFP supplementation; S_j is the fixed effect of fresh diet starch content; D_k is the fixed effect of day as a repeated measure; TS_{ij} is the interaction of SCFP supplementation by fresh diet starch content; TD_{ik} is the interaction of SCFP supplementation by day; SD_{jk} is the interaction of fresh diet starch content by day; TSD_{ijk} is the interaction of SCFP supplementation, fresh diet starch content, and day; and e_{ijk} is the residual. When significant SCFP $\times$ starch interactions were detected, Student's t -test was used to separate means and characterize the nature of interactions. As all cows were fed high-starch diets, data for d 42 reflect carryover effects of fresh diets differing in starch content. Starch content of fresh diets and its interactions with other parameters were not included in the model for analysis of data from d -10 and 1 relative to calving.

Initially, treatment by parity (primiparous vs. multiparous cows) interactions were evaluated for all response variables, but few significant interactions were observed and results are not presented separately by parity. Significance was declared at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Gene Expression in Rumen Epithelium

Tight Junctions. No differences were observed among treatments for mRNA expression of tight junc-

Table 4. The effect of supplementation of a *Saccharomyces cerevisiae* fermentation product (SCFP) on relative mRNA abundance in rumen papillae on d -10 ± 3 relative to expected calving date

Gene	Treatment ¹		SEM	P-value
	CON	SCFP		SCFP
<i>TLR4</i>	1.53	1.12	0.221	0.22
<i>IRAK1</i>	1.35	1.15	0.205	0.52
<i>NFKB1</i>	1.04	1.12	0.144	0.70
<i>OCLDN</i>	1.29	1.33	0.175	0.88
<i>CLDN1</i>	0.84	0.86	0.105	0.91
<i>CLDN4</i>	1.70	2.01	0.485	0.67
<i>TJP1</i>	0.97	1.00	0.072	0.75

¹CON = control diet, no SCFP supplementation; SCFP = SCFP-supplemented diet.

tion genes at d -10 (Table 4) or d 21 (Table 5). The change from a high-forage close-up diet to a high-concentrate fresh diet increases the risk of SARA (Zebeli et al., 2010), and evidence indicates that SARA can lead to tight junction failure (Plaizier et al., 2008; Steele et al., 2011). Decreased mRNA abundance of *OCLN* and *TJP1* is associated with reduced barrier function (Fanning et al., 1998; Feldman et al., 2005). When tight junctions are compromised, microbes and LPS endotoxin can translocate into the blood stream and induce an immune response (Plaizier et al., 2008). The differences in rumen fermentation in the current study found by Shi et al. (2018) may not have been drastic enough to cause a postpartum difference in tight junction protein gene expression or the sample size may not have been large enough to provide adequate statistical power for the gene expression responses, or postpartum mRNA abundance changes may have occurred before the sampling date.

Immune System Activation. The SCFP treatment had no effect on relative mRNA abundance of immune system activation genes in rumen papillae on d -10 (Table 4). On d 21 after calving, feeding LS

diets significantly reduced relative mRNA abundance of *IRAK1* in rumen tissue compared with HS diets (Table 5). Multiple immune proteins are present in the rumen epithelium including *TLR4*, *IRAK1*, and *NFKB1* (Minuti et al., 2015), and *TLR4* is activated by LPS (Ulevitch and Tobias, 1995). This activation leads to the phosphorylation of *IRAK1* and the activation of the NFKB pathway (Gottipati et al., 2008). The NFKB pathway leads to the transcription of genes for cytokines such as IL-6, IL-1 β , and TNF- α (Wolf et al., 2001). These cytokines are involved in recruiting immune cells and activating an acute phase response (Gruys et al., 2005). Because *IRAK1* is involved in the chain of events to activate the immune system response, reduced mRNA abundance of *IRAK1* suggests immune system activation in rumen papillae may have been reduced on d 21 after calving.

During bouts of SARA, LPS concentrations in rumen fluid are typically increased. Li et al. (2016) compared a SARA challenge diet with a control diet and found a greater concentration of LPS in rumen fluid 6 h after feeding when feeding the SARA challenge compared with the control diet. Although not measured in the current study, we speculated that LS diets might have decreased ruminal concentrations of LPS, and this may be related to reduced activation of *TLR4* and therefore reduced mRNA abundance of *IRAK1*, the latter found in the current study.

Acute Phase Response

No interaction was observed between dietary starch content and SCFP supplementation. Serum amyloid-A concentrations were not affected by SCFP (Figure 1A) nor starch content of fresh diets (Figure 2A), but a significant effect of day was present such that SAA concentrations were greater on d 1 and 7 than other time points (d -10 , 21, and 42; Figure 1A). Concen-

Table 5. The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product (SCFP) and reducing dietary starch content on relative mRNA abundance in rumen papillae on d 21 ± 3 after calving

Gene	Treatment ¹				SEM	P-value ²		
	LS		HS			S	SCFP	S × SCFP
	CON	SCFP	CON	SCFP				
<i>TLR4</i>	1.06	0.63	1.01	1.15	0.227	0.32	0.53	0.23
<i>IRAK1</i>	1.02	0.87	1.50	1.25	0.185	0.04	0.30	0.81
<i>NFKB1</i>	0.86	0.74	0.88	0.96	0.095	0.23	0.88	0.32
<i>OCLDN</i>	1.25	1.23	1.30	1.41	0.189	0.55	0.83	0.76
<i>CLDN1</i>	0.73	0.86	0.93	0.80	0.081	0.38	0.99	0.12
<i>CLDN4</i>	1.99	4.13	3.13	3.55	1.818	0.88	0.50	0.64
<i>TJP1</i>	0.99	1.06	0.97	0.92	0.116	0.49	0.92	0.60

¹LS = low-starch fresh diet; HS = high-starch fresh diet; CON = control diet, no SCFP supplementation; SCFP = SCFP-supplemented diet.

²S = starch content; SCFP = SCFP supplementation; S $\times$ SCFP = interaction between starch and SCFP.

trations of SAA were above acute concentrations that indicate inflammation (Cecilian et al., 2012). These results indicate that regardless of treatment all cows experienced inflammation during and after calving. In previous studies, SCFP supplementation affected SAA concentration; Zaworski et al. (2014) found that SCFP supplementation resulted in lower concentrations of SAA 1 d before calving and greater concentrations of SAA 1 d after calving, compared with control, with no difference after 14 d after calving. This suggests that SCFP supplementation may affect concentrations of SAA around the calving time. The lack of response with SCFP seen in the current study may be due to the sampling time. In addition, the effects of SAA are short lived; concentrations increase and fall rapidly within 24 h without additional stimulation (Wang et al., 2001). Sampling for the current study, unlike sampling in Zaworski et al. (2014), may not have been frequent enough to detect a treatment effect of SCFP supplementation or dietary starch content on SAA concentrations.

Similarly to SAA, no interaction was observed between starch content of the fresh diet and SCFP supplementation on serum concentrations of Hp, and no effect was observed of dietary starch content on serum concentrations of Hp (Figure 2B). However, interaction of day by SCFP was significant ($P < 0.01$) where animals not supplemented with SCFP had greater serum concentrations of Hp than animals supplemented with SCFP on d 7 after calving (Figure 1B). This suggests that cows not receiving SCFP had greater inflammation on d 7 after calving. Inflammation results in the signs of sickness, which includes fever, depression, and depressed feed intake (Gruys et al., 2005). The initiation of these signs is controlled by the same cytokines that induce an acute phase response (Gruys et al., 2005). In addition, inflammation indicated by increased serum concentrations of Hp was associated with health disorders after calving such as retained placenta (Qu et al., 2014), and Cheong et al. (2017) reported that cows classified as nonovulatory had elevated serum concentrations of Hp during the calving transition. In a companion paper for the present study, Shi et al. (2019) reported that SCFP supplementation increased DMI on d 1 and 5 after calving and resulted in a more consistent increase in DMI after calving. The decrease in serum Hp concentration due to SCFP supplementation may also be an indicator of reduced inflammation, which may have led to increased feed intake after calving by cows supplemented with SCFP.

Oxidative Status

Plasma concentrations of ROM were not different between treatments (Table 6). Cows supplemented

with SCFP had greater plasma concentrations of MDA on d 21 than cows not fed SCFP. Plasma concentrations of TAOC tended to decrease on d 1 and 7 due to SCFP supplementation. Supplementation of SCFP and dietary starch content tended to interact to affect plasma concentrations of TAOC on d 21 after calving. Supplementation of SCFP decreased plasma concentrations of TAOC for cows fed LS diets, but not for those fed HS diets.

Figure 1. The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product (SCFP) on serum concentrations of serum amyloid-A (A) and haptoglobin (B). (A) Effect of day, $P < 0.01$; effect of SCFP supplementation, $P = 0.48$; interaction of day by SCFP supplementation, $P = 0.46$. Day means with different letters differ significantly ($P < 0.05$). (B) Effect of day, $P < 0.01$; effect of SCFP supplementation, $P = 0.07$; interaction of day by SCFP supplementation, $P < 0.01$. Treatment means with different letters (a,b) differ significantly ($P < 0.05$). CON = control diet, no SCFP supplementation. Data shown are LSM $\pm$ SE.

Reactive oxygen metabolites are released during many processes in the body, including oxidative burst by immune cells and during respiratory processes such

Figure 2. The effects of fresh diet starch content and supplementation of a *Saccharomyces cerevisiae* fermentation product (SCFP) on serum concentrations of serum amyloid-A (A) and haptoglobin (B). (A) Effect of day, $P < 0.01$; effect of starch content of fresh diets, $P = 0.76$; interaction of day by starch content, $P = 0.91$; effect of SCFP supplementation, $P = 0.67$; interaction of starch content by SCFP supplementation, $P = 0.35$. Day means with different letters differ significantly ($P < 0.05$). (B) Effect of day, $P < 0.01$; effect of starch content of fresh diets, $P = 0.91$; interaction of day by starch content, $P = 0.97$; effect of SCFP supplementation, $P = 0.05$; interaction of starch content by SCFP supplementation, $P = 0.55$. Day means with different letters differ significantly ($P < 0.05$). Data shown are LSM $\pm$ SE.

as β -oxidation of free fatty acids in the liver (Miller et al., 1993). If not neutralized, ROM can break down tissues. Malondialdehyde is a marker of lipid peroxidation, or the breakdown of lipids such as the lipid membrane of tissues by oxidants (Halliwell and Chirico, 1993). While plasma concentrations of TAOC tended to be lower for SCFP cows on d 1 and 7, this may indicate that antioxidants are being used to neutralize oxidants (Abuelo et al., 2015). No differences were observed between treatments in plasma concentrations of ROM or MDA on d 1 and 7, suggesting that the reduction in TAOC was not associated with oxidative stress, but rather with the effective neutralization of oxidants.

Plasma MDA concentrations were greater for SCFP-supplemented cows compared with those not supplemented with SCFP at d 21. Oxidative stress is the imbalance between oxidants and antioxidants, and an increase in the plasma concentration of a marker of oxidative damage could be suggestive of oxidative stress. Sordillo and Raphael (2013) suggested that greater plasma concentration of free fatty acids contributes to oxidative stress, which is associated with greater plasma MDA concentration. However, plasma free fatty acid concentration was not affected by SCFP treatment in the current study (Shi et al., 2019), and the reason for greater plasma MDA concentration for cows fed SCFP is unknown. Although SCFP contains antioxidants (Jensen et al., 2008), SCFP supplementation did not enhance antioxidant capacity of animals in the current study.

The effect of dietary starch content on indices of oxidative stress has not been explored previously, but may have an indirect effect on oxidative status. Multiple in vitro studies and reviews have implicated free fatty acids as a cause of oxidative stress because of β -oxidation of free fatty acids in the liver and the release of oxidants (Sordillo and Raphael, 2013). Diets fed postcalving may have an effect on the metabolic status of the animal, but results are inconclusive. A low-starch diet increased plasma concentrations of free fatty acids and BHB after calving (McCarthy et al., 2015). But another study found no difference between high- or low-starch diets on concentrations of free fatty acids (Dias et al., 2018). Dietary starch content might not have an effect on indices of oxidative stress in the current study because of the limited effect on plasma free fatty acid concentrations.

Adaptive Immune Response

The ovalbumin challenge was successful; day had an effect on serum IgG concentrations (Figure 3). Serum IgG concentrations were lowest at d 7, and increased

Table 6. The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product (SCFP) and reducing dietary starch content on plasma concentrations of reactive oxygen metabolites (ROM), malondialdehyde (MDA), and total antioxidant capacity (TAOC)

Item	Treatment ¹				SEM	P-value ²		
	LS		HS			S	SCFP	S × SCFP
	CON	SCFP	CON	SCFP				
ROM, U CARR ³								
d -10 ⁴	132	138			14.1		0.70	
d 1 ⁴	112	122			9.0		0.41	
d 7	117	131	116	114	12.8	0.50	0.60	0.52
d 21	103	113	116	101	7.8	0.94	0.79	0.11
d 42 ⁵	131	153	135	126	10.7	0.28	0.49	0.15
MDA, μM								
d -10	17.5	17.8			1.11		0.84	
d 1	16.7	16.9			1.11		0.87	
d 7	17.3	16.2	15.2	19.3	1.63	0.79	0.35	0.12
d 21	18.9	19.6	16.0	20.2	1.19	0.33	0.04	0.13
d 42	21.1	24.4	22.7	22.7	1.48	0.99	0.26	0.26
TAOC, mM								
d -10	0.75	0.73			0.06		0.83	
d 1	0.99	0.80			0.07		0.06	
d 7	1.07	0.75	0.93	0.87	0.11	0.93	0.07	0.22
d 21	1.04 ^a	0.74 ^b	0.93 ^{ab}	0.95 ^{ab}	0.09	0.58	0.13	0.09
d 42	0.94	0.81	0.83	0.92	0.08	0.99	0.77	0.17

^{a,b}Treatment means with different superscripts in a row differ significantly ($P < 0.05$).

¹LS = low-starch fresh diet; HS = high-starch fresh diet; CON = control diet, no SCFP supplementation; SCFP = SCFP-supplemented diet.

²S = starch content; SCFP = SCFP supplementation; S × SCFP = interaction between starch and SCFP.

³1 U CARR is equivalent to 0.08 mg/100 mL of H₂O₂.

⁴Starch was not a treatment on d -10 and 1 because cows were receiving the close-up diets.

⁵All animals were fed high-starch diets after d 21. Carryover effects of fresh diets are of interest.

from d 7 to 21 and from d 21 to 35. An ovalbumin solution was injected on d 7 and again on d 21, and the increase in serum IgG concentration from d 7 to 21 suggests an adaptive immune response occurred after the first injection.

We had expected that SCFP supplementation would affect adaptive immune response. Because one of the primary components of *S. cerevisiae* is β -glucans in the yeast cell wall, SCFP has been shown to increase B-cell activation in vitro (Jensen et al., 2008). β -Glucan inclusion in diets has been shown to increase humoral response to vaccination in broilers (Muthusamy et al., 2011) and to an ovalbumin challenge in pigs (Li et al., 2005). However, an ovalbumin challenge conducted in preweaning calves also resulted in no difference between SCFP-supplemented and control calves (Magalhães et al., 2008). In the current study, treatment had no effect on serum concentrations of IgG, and effects of SCFP on adaptive immune responses were not confirmed.

To our knowledge, no studies have been conducted to evaluate the effects of dietary starch content on the adaptive immune response. However, feeding low-starch fresh diets may increase plasma free fatty acid concentrations (McCarthy et al., 2015), and in vitro studies

have shown that increasing free fatty acid concentrations may reduce lymphocyte function (Lacetera et al., 2004, 2005). In the present study a low-starch diet increased concentrations of free fatty acids (Shi et al., 2019), but treatment had no effect on response to the ovalbumin challenge. The results from in vitro studies may not be applicable to in vivo situations. The rate of free fatty acid inclusion in vitro may be higher than what is seen in vivo, and in vitro applications may not account for the interactions and fates of free fatty acids other than the effect on immune cells.

CONCLUSIONS

All cows experienced a degree of apparent inflammation after calving, but cows supplemented with SCFP had reduced inflammation at d 7 after calving, compared with control. There were no differences between diets for tight junction gene expression, but a low-starch fresh diet reduced mRNA abundance of IRAK1 in rumen tissue at d 21 after calving. Supplementing SCFP and reducing dietary starch content after calving may reduce inflammation and improve health status of the animal after calving.

Figure 3. The effects of supplementation of a *Saccharomyces cerevisiae* fermentation product (SCFP) and decreasing dietary starch content [low starch (LS) vs. high starch (HS)] on serum concentrations of IgG in response to an ovalbumin challenge. On d 7 and 21 after calving, animals underwent an ovalbumin challenge. Effect of day, $P < 0.01$; effect of SCFP supplementation, $P = 0.81$; effect of fresh diet starch content, $P = 0.83$; interaction of day by SCFP supplementation, $P = 0.55$; interaction of day by fresh diet starch content, $P = 0.86$; interaction of day by SCFP supplementation and fresh diet starch content, $P = 0.85$. Day means with different letters differ significantly ($P < 0.05$). Data shown are LSM $\pm$ SE.

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REFERENCES

- Abuelo, A., J. Hernández, J. L. Benedito, and C. Castillo. 2015. The importance of the oxidative status of dairy cattle in the periparturient period: Revisiting antioxidant supplementation. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 99:1003–1016.
- Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. *J. Dairy Sci.* 88:2017–2026.
- Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. *J. Dairy Sci.* 91:3300–3310.
- Bionaz, M., E. Trevisi, L. Calamari, F. Librandi, A. Ferrari, and G. Bertoni. 2007. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. *J. Dairy Sci.* 90:1740–1750.
- Bouwstra, R. J., R. M. A. Goselink, P. Dobbelaar, M. Nielen, J. R. Newbold, and T. van Werven. 2008. The relationship between oxidative damage and vitamin E concentration in blood, milk, and liver tissue from vitamin E supplemented and nonsupplemented periparturient heifers. *J. Dairy Sci.* 91:977–987.
- Canadian Council on Animal Care. 2009. Guidelines on the care and use of farm animals in research, teaching and testing. Canadian Council on Animal Care, Ottawa, ON.
- Cecilian, F., J. J. Ceron, P. d. Eckersall, and H. Sauerwein. 2012. Acute phase proteins in ruminants. *J. Proteomics* 75:4207–4231.
- Cheong, S. H., O. G. Sá Filho, V. A. Absalon-Medina, A. Schneider, W. R. Butler, and R. O. Gilbert. 2017. Uterine and systemic inflammation influences ovarian follicular function in postpartum dairy cows. *PLoS One* 12:e0177356.
- Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction. *Anal. Biochem.* 162:156–159.
- Dias, A. L. G., J. A. Freitas, B. Micai, R. A. Azevedo, L. F. Greco, and J. E. P. Santos. 2018. Effect of supplemental yeast culture and dietary starch content on rumen fermentation and digestion in dairy cows. *J. Dairy Sci.* 101:201–221.
- Erasmus, L. J., P. H. Robinson, A. Ahmadi, R. Hinders, and J. E. Garrett. 2005. Influence of prepartum and postpartum supplementation of a yeast culture and monensin, or both, on ruminal fermentation and performance of multiparous dairy cows. *Anim. Feed Sci. Technol.* 122:219–239.
- Fanning, A. S., B. J. Jameson, L. A. Jesaitis, and J. M. Anderson. 1998. The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. *J. Biol. Chem.* 273:29745–29753.
- Feldman, G. J., J. M. Mullin, and M. P. Ryan. 2005. Occludin: Structure, function and regulation. *Adv. Drug Deliv. Rev.* 57:883–917.
- Gottipati, S., N. L. Rao, and W. P. Fung-Leung. 2008. IRAK1: A critical signaling mediator of innate immunity. *Cell. Signal.* 20:269–276.
- Gruys, E., M. J. M. Toussaint, T. A. Niewold, and S. J. Koopmans. 2005. Acute phase reaction and acute phase proteins. *J. Zhejiang Univ. Sci. B* 6:1045–1056.
- Halliwell, B., and S. Chirico. 1993. Lipid peroxidation: Its mechanism, measurement, and significance. *Am. J. Clin. Nutr.* 57(Suppl):715S–724S.
- Harrison, G. A., R. W. Hemken, K. A. Dawson, R. J. Harmon, and K. B. Baker. 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial population. *J. Dairy Sci.* 71:2967–2975.
- Herr, M., H. Bostedt, and K. Failing. 2011. IgG and IgM levels in dairy cows during the periparturient period. *Theriogenology* 75:377–385.
- Jensen, G. S., K. M. Patterson, and I. Yoon. 2008. Yeast culture has anti-inflammatory effects and specifically activates NK cells. *Comp. Immunol. Microbiol. Infect. Dis.* 31:487–500.
- Lacetera, N., D. Scalia, U. Bernabucci, B. Ronchi, D. Pirazzi, and A. Nardone. 2005. Lymphocyte functions in overconditioned cows around parturition. *J. Dairy Sci.* 88:2010–2016.
- Lacetera, N., D. Scalia, O. Franci, U. Bernabucci, B. Ronchi, and A. Nardone. 2004. *Short communication:* Effects of nonesterified fatty acids on lymphocyte function in dairy heifers. *J. Dairy Sci.* 87:1012–1014.
- LeBlanc, S. J. 2008. Postpartum uterine disease and dairy herd reproductive performance: A review. *Vet. J.* 176:102–114.
- Li, J., J. Xing, D. Li, X. Wang, L. Zhao, S. Lv, and D. Huang. 2005. Effects of β -glucan extracted from *Saccharomyces cerevisiae* on humoral and cellular immunity in weaned piglets. *Arch. Anim. Nutr.* 59:303–312.
- Li, S., I. Yoon, M. Scott, E. Khafipour, and J. C. Plaizier. 2016. Impact of *Saccharomyces cerevisiae* fermentation product and subacute ruminal acidosis on production, inflammation, and fermentation in the rumen and hindgut of dairy cows. *Anim. Feed Sci. Technol.* 211:50–60.
- Littell, C. R., G. A. Milliken, W. W. Stroup, and F. D. Wolfinger. 1996. SAS System for Mixed Model. SAS Inst. Inc., Cary, NC.

- Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* 25:402–408.
- Magalhães, V. J. A., F. Susca, F. S. Lima, A. F. Branco, I. Yoon, and J. E. P. Santos. 2008. Effect of feeding yeast culture on performance, health, and immunocompetence of dairy calves. *J. Dairy Sci.* 91:1497–1509.
- Mallard, B. A., J. C. Dekkers, M. J. Ireland, K. E. Leslie, S. Sharif, C. Lacey Vankampen, L. Wagter, and B. N. Wilkie. 1998. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. *J. Dairy Sci.* 81:585–595.
- Malmuthuge, N., M. Li, L. A. Goonewardene, M. Oba, and L. L. Guan. 2013. Effect of calf starter feeding on gut microbial diversity and expression of genes involved in host immune responses and tight junctions in dairy calves during weaning transition. *J. Dairy Sci.* 96:3189–3200.
- McCarthy, M. M., T. Yasui, C. M. Ryan, S. H. Pelton, G. D. Mechor, and T. R. Overton. 2015. Metabolism of early-lactation dairy cows as affected by dietary starch and monensin supplementation. *J. Dairy Sci.* 98:3351–3365.
- Miller, J. K., E. Brzezinska-Slebodzinska, and F. C. Madsen. 1993. Oxidative stress, antioxidants, and animal function. *J. Dairy Sci.* 76:2812–2823.
- Minuti, A., A. Palladino, M. J. Khan, S. Alqarni, A. Agrawal, F. Piccoli-Capelli, F. Hidalgo, F. C. Cardoso, E. Trevisi, and J. J. Loor. 2015. Abundance of ruminal bacteria, epithelial gene expression, and systemic biomarkers of metabolism and inflammation are altered during the periparturient period in dairy cows. *J. Dairy Sci.* 98:8940–8951.
- Moyes, K. M., J. K. Drackley, D. E. Morin, and J. J. Loor. 2010. Greater expression of TLR2, TLR4, and IL6 due to negative energy balance is associated with lower expression of HLA-DRA and HLA-A in bovine blood neutrophils after intramammary mastitis challenge with *Streptococcus uberis*. *Funct. Integr. Genomics* 10:53–61.
- Moyes, K. M., D. E. Graugnard, M. J. Khan, M. Mukesh, and J. J. Loor. 2014. Postpartal immunometabolic gene network expression and function in blood neutrophils are altered in response to prepartal energy intake and postpartal intramammary inflammatory challenge. *J. Dairy Sci.* 97:2165–2177.
- Mulligan, F. J., and M. L. Doherty. 2008. Production diseases of the transition cow. *Vet. J.* 176:3–9.
- Muthusamy, N., S. Haldar, T. K. Ghosh, and M. R. Bedford. 2011. Effects of hydrolyzed *Saccharomyces cerevisiae* and yeast cell wall components on live performance, intestinal histo-morphology and humoral immune response of broilers. *Br. Poult. Sci.* 52:694–703.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. *Vet. J.* 176:21–31.
- Qu, Y., A. N. Fadden, M. G. Traber, and G. Bobe. 2014. Potential risk indicators of retained placenta and other diseases in multiparous cows. *J. Dairy Sci.* 97:4151–4165.
- Shi, W., C. E. Knoblock, K. V. Murphy, T. C. Bruinje, I. Yoon, D. J. Ambrose, and M. Oba. 2019. Effects of supplementing a *Saccharomyces cerevisiae* fermentation product during periparturient period on performance of dairy cows fed fresh diets differing in starch content. *J. Dairy Sci.* 102:3082–3096.
- Shi, W., C. E. Knoblock, I. Yoon, and M. Oba. 2018. Effects of *Saccharomyces cerevisiae* fermentation product supplementation during the periparturient period on rumen pH of dairy cows fed postpartum diets differing in starch content. *J. Dairy Sci.* 101(Suppl. 2):T275.
- Sordillo, L. M., and W. Raphael. 2013. Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. *Vet. Clin. North Am. Food Anim. Pract.* 29:267–278.
- Steele, M. A., J. Croom, M. Kahler, O. AlZahal, S. E. Hook, K. Plaizier, and B. W. McBride. 2011. Bovine rumen epithelium undergoes rapid structural adaptation during grain-induced subacute ruminal acidosis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 300:R1515–R1523.
- Ulevitch, R. J., and P. S. Tobias. 1995. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu. Rev. Immunol.* 13:437–457.
- Volman, J. J., J. d. Ramakers, and J. Plat. 2008. Dietary modulation of immune function by β -glucans. *Physiol. Behav.* 94:276–284.
- Wang, Y., E. Kinzie, F. G. Berger, S. K. Lim, and H. Baumann. 2001. Haptoglobin, an inflammation-inducible plasma protein. *Redox Rep.* 6:379–385.
- Wolf, J. S., Z. Chen, G. Dong, J. B. Sunwoo, C. C. Bancroft, D. E. Capo, N. T. Yeh, N. Mukaida, and C. V. Waes. 2001. IL (Interleukin)-1 α promotes Nuclear Factor- κ B and AP-1- induced IL-8 expression, cell survival, and proliferation in head and neck squamous cell carcinomas. *Clin. Cancer Res.* 7:1812–1820.
- Yuan, K., J. K. Farney, L. K. Mamedova, L. M. Sordillo, and B. J. Bradford. 2013. TNF α altered inflammatory responses, impaired health and productivity, but did not affect glucose or lipid metabolism in early-lactation dairy cows. *PLoS One* 8:e80316.
- Zaworski, E. M., C. M. Shriver-Munsch, N. A. Fadden, W. K. Sanchez, I. Yoon, and G. Bobe. 2014. Effects of feeding various dosages of *Saccharomyces cerevisiae* fermentation product in transition dairy cows. *J. Dairy Sci.* 97:3081–3098.
- Zebeli, Q., D. Mansmann, H. Steingass, and B. N. Ametaj. 2010. Balancing diets for physically effective fiber and ruminally degradable starch: A key to lower the risk of sub-acute rumen acidosis and improve productivity of dairy cattle. *Livest. Sci.* 127:1–10.