UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

DESEMPENHO, SAÚDE E RESPOSTA IMUNE DE VACAS LEITEIRAS E DE BEZERROS SUPLEMENTADOS COM LEVEDURAS E EXTRATOS DE PAREDE CELULAR DE LEVEDURAS

Autor: Vanessa Jaime de Almeida Magalhães
Orientador: Prof. Dr. Antonio Ferriani Branco
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MARINGÁ
Estado do Paraná
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Tese apresentada, como parte das exigências para obtenção do título de DOUTOR EM ZOOTECNIA, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá - Área de Concentração Produção Animal.

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TITULAÇÃO: Doutora em Zootecnia – Área de Concentração Produção Animal

APROVADA em 19 de dezembro de 2007.

Prof. Dr. Paulo H. Mazza Rodrigues	Prof. Dr. Rodrigo de Almeida
Prof. Dr. Geraldo Tadeu dos Santos	Prof. Dr. Clóves Cabreira Jobim

Prof. Dr. Antonio Ferriani Branco
(Orientador)

"Tenha sempre presente
que a pele enruga,
o cabelo embranquece,
os dias convertem-se em anos...
mas o que é importante não muda;
a sua força e convicção não têm idade.
O seu espírito
é como qualquer teia de aranha,
atrás de cada linha de chegada,
há uma de partida.
Atrás de cada conquista,
vem um novo desafio.
Enquanto estiver vivo,
sinta-se vivo...
Se sentir saudades do que fazia,
volte a fazê-lo.
Não viva de fotografias amarelecidas...
Continue,
quando todos esperam que desista.
Não deixe que enferruje
o ferro que existe em você.
Faça com que em vez de pena,
tenham respeito por você.
Quando não conseguir mais
correr atrás dos anos, trote.
Quando não conseguir trotar,
caminhe.
Quando não conseguir mais caminhar,
use uma bengala,
MAS NUNCA SE DETENHA!!!"

Madre Teresa de Calcutá

(Agnes Gonxha Bojaxhiu)

Aos meus **pais e irmãos**,

pela base sólida de amor, cumplicidade e união,

sempre presentes na minha vida.

À minha **família de Arceburgo-MG**,

pelos inúmeros momentos de alegria que compartilhamos

e pelo amor e carinho com que sempre me trataram.

Aos **verdadeiros amigos**,

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"Quem tem amigo, tem tudo nessa vida!"

A **todos** que,

longe ou perto, sempre me incentivaram,

suavizando e aquecendo os meus dias, ao longo desta jornada.

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BIOGRAFIA

VANESSA JAIME DE ALMEIDA MAGALHÃES, filha de Marco Antônio de Almeida Magalhães e Marinur Jaime de Almeida Magalhães, nasceu em Araras, São Paulo, no dia 09 de fevereiro de 1974.

Em dezembro de 1998, concluiu o curso de Zootecnia pela Universidade do Oeste Paulista, Presidente Prudente – SP.

Em novembro de 2002, obteve o título de Mestre em Medicina Veterinária, pelo Programa de Pós-Graduação em Nutrição e Produção Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Pirassununga – SP.

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Em setembro de 2005, iniciou o estágio de doutorado no Centro de Pesquisa e Ensino de Medicina Veterinária (VMTRC) da Universidade da Califórnia - Davis, situado no município de Tulare, estado da Califórnia, Estados Unidos.

Em dezembro de 2007, submeteu-se à banca para defesa da Tese.

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RESUMO

Os estudos foram realizados com os objetivos de avaliar o desempenho animal, a saúde e a resposta imune de vacas e bezerros leiteiros suplementados com cultura de levedura de *Saccharomyces cerevisiae* enriquecida com extratos de parede celular. No primeiro experimento, foram utilizadas 333 vacas multíparas da raça Holandesa em início de lactação, distribuídas aleatoriamente no primeiro dia de lactação de acordo com os seguintes tratamentos: 1) 14 g/dia de levedura comercial (Diamond V XPC; CL = 159 vacas); 2) 14 g/dia da mesma levedura com adicional de 5 g/dia de extratos de parede celular (Diamond V XPC Plus; PCL = 174 vacas), adicionados sobre a dieta, na forma de ração total misturada, uma vez ao dia nos primeiros 100 dias em lactação. Uma sub-amostra de 80 vacas (40/tratamento) foi avaliada para resposta imune celular e humoral e para concentração de metabólitos no plasma. Não houve efeito dos tratamentos sobre a incidência de doenças pós-parto, embora um maior número de vacas do tratamento PCL tenha sido diagnosticado com pelo menos uma doença. O tratamento não afetou o escore de condição corporal, a concentração de metabólitos no plasma e a resposta humoral das vacas. A atividade fagocítica dos neutrófilos foi melhorada com o avanço da lactação, no entanto, os tratamentos não influenciaram a resposta imune celular. As vacas suplementadas com PCL tiveram menores produções de leite, de leite corrigido para 3,5% de gordura, de leite corrigido para energia, de gordura e de proteína verdadeira do leite, embora as concentrações de gordura, proteína verdadeira e energia líquida para lactação no leite não tenham sido influenciadas pelos tratamentos. A taxa de concepção à primeira inseminação pós-parto foi similar entre os tratamentos. Os resultados do presente estudo sugerem que a adição de extratos de parede celular à dieta de vacas leiteiras suplementadas com cultura de levedura não melhora a saúde, a

resposta imune ou a produção de leite. No segundo experimento, foram utilizados 512 bezerros da raça Holandesa com idade até 70 dias, distribuídos aleatoriamente aos 2 ± 1 dias de idade para receber cultura de levedura (CL, 218 fêmeas e 37 machos) ou controle (223 fêmeas e 34 machos). A cultura de levedura foi fornecida na proporção de 2% da MS da ração inicial. Todos os bezerros receberam colostro nas primeiras 24 h de vida e leite pasteurizado até 60 dias de idade. A ração inicial foi fornecida *ad libitum* nos primeiros 70 dias de idade. Os bezerros foram alojados em bezerreiros individuais e a ingestão de ração inicial foi medida em 5 dias/semana. Os bezerros foram pesados aos 5, 30 e 68 dias de idade, e os escores de atitude e de consistência fecal foram avaliados diariamente. A incidência e a duração dos problemas de saúde, bem como os tratamentos foram registrados. A atividade fagocítica dos neutrófilos e a resposta dos anticorpos à imunização com ovalbumina também foram medidas. Foram realizadas dosagens das concentrações de glicose e 3-hidroxibutirato no plasma. A ingestão de ração inicial não foi influenciada pelos tratamentos e a média foi de 908 g/dia durante o estudo. O ganho de peso vivo, as concentrações de glicose e de 3-hidroxibutirato não diferiram entre CL e controle. Foram observados efeitos menores sobre as funções dos neutrófilos, e tendência em aumentar o número de bactérias fagocitadas e bactérias fagocitadas mortas para o tratamento CL, mas a resposta imune humoral não foi influenciada. Os escores de atitude, relacionados ao comportamento dos bezerros, foram similares entre os tratamentos durante o estudo. Quase todos os bezerros apresentaram diarréia moderada durante o estudo, mas a suplementação com CL melhorou o escore fecal e diminuiu o número de dias com diarréia líquida, a incidência de febre e de diarréia e o risco de problemas de saúde. Por causa da alta incidência de diarréia, a mortalidade antes da desmama também foi alta, mas CL aumentou a sobrevivência dos bezerros com a diminuição da taxa de mortalidade após os 13 dias de idade. A renda líquida no final do estudo foi maior em $48/bezerro para aqueles que receberam CL. O fornecimento da ração inicial com cultura de levedura melhorou a saúde, minimizou a freqüência ou os tratamentos de doenças, e reduziu a morbidade e mortalidade em bezerros leiteiros.

Palavras-chave: bezerro, vaca leiteira, saúde, *Saccharomyces cerevisiae*, cultura de levedura, parede celular de levedura

ABSTRACT

These studies were carried out to evaluate animal performance, health and immune response of dairy cows and calves supplemented with yeast culture of *Saccharomyces cerevisiae* enriched with cell wall extracts. In the first trial were used 333 early postpartum multiparous Holstein cows, randomly assigned at 1 d in milk (DIM) to receive 14 g/d of a commercial yeast culture (Diamond V XPC; YC = 159 cows) or 14 g/d of the same yeast culture with additional 5 g/d of yeast cell wall extracts (Diamond V XPC Plus; YCCW = 174 cows), top-dressed onto the total mixed ration diet once a day for the first 100 DIM. A subset of 80 cows (40/treatment) was evaluated for cellular and humoral immune responses, and plasma metabolites concentrations. There were no effects of treatments on incidence of postpartum disorders, although more cows receiving YCCW were diagnosed with at least one disease. Treatments did not affect body condition score, plasma metabolites concentrations and humoral response of cows. Phagocytic and killing activities of neutrophils were improved with days postpartum, but treatments did not influence cellular immune response. Cows fed YCCW had lesser yields of milk, 3.5% fat-corrected milk, energy-corrected milk, milk fat and milk true protein, although concentrations of milk fat, milk true protein and net energy for lactation in milk were not influenced by treatments. Conception rate at first postpartum insemination was similar between treatments. These results suggest that the inclusion of yeast cell wall extracts to diets of cows fed yeast culture does not improve health, immune response or lactation performance. In the second trial were used 512 Holstein calves in the first 70 d of age, randomly assigned at 2 ± 1 d of age to yeast culture (YC, 218 females and 37 males) or control (223 females and 34 males). Yeast culture was fed at 2% of the grain DM. All calves received colostrum during the first 24 h, pasteurized

milk thereafter until 60 d of age, and grain was fed *ad libitum* for the first 70 d of age. Calves were housed in individual hutches and grain intake was measured 5 d/wk. Body weight was measured at 5, 30 and 68 d of age, and attitude and fecal consistency were scored daily. Incidence and duration of health disorders and treatments were recorded. Neutrophil phagocytic and killing activities and antibody response to immunization with ovalbumin were measured. Glucose and 3-hydroxybutyrate concentrations were measured in plasma. Grain intake did not differ between treatments and averaged 908 g/d throughout the study. Body weight change as well as glucose and 3-hydroxybutyrate concentrations did not differ between YC and control. Minor effects on neutrophil function were observed, and YC tended to increase the number of phagocytized bacteria and killing of phagocytized bacteria, but did not influence humoral immune response. Attitude scores, related to calves' behavior, were similar between treatments throughout the study. Almost all calves experienced mild diarrhea during the study, but feeding YC improved fecal scores, reduced days with watery feces, incidence of fever and diarrhea, and risk of health disorders. Because of the high incidence of diarrhea, mortality pre-weaning was also high, but YC improved survival of calves by decreasing mortality rate past 13 d of age. Net income at the end of the study was improved by $48/calf with YC. Feeding yeast culture in grain improved health, minimized frequency or health treatments, and reduced morbidity and mortality in dairy calves.

Key words: calf, dairy cow, health, *Saccharomyces cerevisiae*, yeast culture, yeast cell wall

I - INTRODUÇÃO

O uso de aditivos microbianos, fornecidos como suplementos alimentares, tem se tornado uma prática comum na nutrição de ruminantes nos últimos anos, em função da crescente preocupação com a saúde humana. Tem-se, hoje em dia, a idéia de que o uso de alguns aditivos, como os antibióticos, pode contribuir para a formação de populações de bactérias entéricas resistentes a certas drogas, causando riscos à saúde pública. Em função desta vertente, o uso destes aditivos (promotores de crescimento, antibióticos, entre outros) na nutrição animal vem sendo proibido em alguns países.

Frente a essa situação, diversos trabalhos de pesquisa têm mostrado que as leveduras vêm se tornando uma alternativa de "aditivos microbianos naturais", em substituição a estes produtos, sendo utilizadas como uma opção para modificar a fermentação ruminal e proporcionar maior ingestão de alimentos e melhoria no desempenho produtivo dos animais. Uma nova situação dentro desta linha investigativa tem sido a utilização dos componentes extraídos da parede celular das leveduras, como os oligossacarídeos, que possuem propriedades de estimular a resposta imune do animal.

Diversos trabalhos mostram que o uso desses aditivos microbianos apresenta maior eficácia quando utilizados na dieta de animais que se encontram em estresse mais

intenso, como vacas leiteiras no período de transição e no início da lactação, quando entram em balanço energético negativo. O modo de ação das leveduras irá favorecer o aumento do consumo alimentar e, conseqüentemente, a produção de leite.

Devido aos efeitos benéficos que as leveduras apresentam sobre a funcionalidade ruminal, estas também apresentam significativa relevância quando utilizadas na alimentação de bezerros, auxiliando na digestão de carboidratos e no desenvolvimento ruminal destes animais.

O projeto foi desenvolvido em dois experimentos realizados com o objetivo de estudar o uso de cultura de levedura e extrato de parede celular de levedura na dieta de vacas leiteiras e de bezerros. O primeiro experimento foi conduzido para verificar a hipótese de que a presença de extratos de parede celular em adição à cultura de levedura, na dieta de vacas leiteiras em início de lactação, melhora o desempenho produtivo e principalmente a saúde e resposta imune destes animais. Assim, comparou-se dois produtos comerciais contendo cultura de levedura, sendo um enriquecido com extratos de parede celular, e o outro não. Foi proposto também, num segundo experimento, avaliar o desempenho e saúde de bezerros jovens suplementados com um terceiro produto comercial contendo cultura de levedura, uma vez que muitos dados encontrados na literatura são contraditórios e se baseiam em consumo, em parâmetros ruminais e em desempenho animal, mas não na saúde ou resposta imunológica de bovinos.

Levedura (*Saccharomyces cerevisiae*)

As leveduras são fungos, unicelulares, extremamente benéficas para a humanidade por sua ampla utilização na produção de alimentos. *Saccharomyces cerevisiae* é a levedura objeto da grande maioria das pesquisas. É utilizada na produção de cerveja, vinho, pão, combustível e de uma variedade de agentes bioquímicos e terapêuticos (Stewart, 2002).

Na nutrição animal, as leveduras constituem um produto com alto número de células de *S. cerevisiae* vivas e sem a adição do meio de cultura (Lynch e Martin, 2002). Por serem uma excelente fonte de nutrientes, essas leveduras têm sido utilizadas há anos na nutrição animal, fornecendo proteína, carboidrato e vitaminas do complexo B (Quigley, 2005).

Outra alternativa de fornecimento desse aditivo é por meio da "cultura de levedura", que é um produto que contém células vivas e mortas de *S. cerevisiae*, além do meio utilizado para seu desenvolvimento. Geralmente a cultura é produzida pela fermentação de líquido selecionado e grão de cereal cru com leveduras de panificação, seguida da secagem de todo o meio de cultura sem destruir os componentes da levedura, como as vitaminas do complexo B e outros produtos da fermentação.

Na parede celular de *S. cerevisiae* destacam-se os componentes glucano e manano, presentes na proporção de 30 a 45% cada, dependendo das condições de preparo da cultura (Katohda et al., 1976; Reed e Nagodawithana, 1991). Estes oligossacarídeos protegem a parede celular do meio externo e promovem a integridade estrutural da célula. Os β-glucanos (**BG**) constituem a camada interna da parede da célula e os mananos ou mananoligossacarídeos (**MOS**), a camada externa, cobrindo a camada de BG (Van Der Vaart et al., 1995).

Utilização de culturas de leveduras na alimentação animal

Existem relatos de que os primeiros estudos utilizando as leveduras como aditivos na nutrição animal foram realizados com vacas leiteiras em 1925, como um suplemento protéico na dieta de ruminantes (Wallace e Newbold, 1995). Contudo, maior interesse no aumento da produtividade animal, com a inclusão de baixos níveis de levedura na dieta, somente foi despertado nas décadas de 40 e 50, após surgirem os primeiros relatos de melhoria no desempenho de bovinos. Beeson e Perry (1952) reportaram aumento de 6% no ganho médio diário de novilhos que receberam oito gramas/dia de levedura, e Renz (1954), aumento na produção leiteira com a inclusão de 50 g/d na dieta de vacas. Entretando, os resultados variavam, e muitos estudos reportaram pouco ou nenhum aumento na produção (Norton, 1945; Renz e Koch, 1956; Lassiter et al., 1958).

Os resultados científicos sobre a suplementação animal com leveduras ou culturas de leveduras continuam sendo muito variáveis. Estudos mais recentes relataram aumento na ingestão de matéria seca (Williams et al., 1991b; Wohlt et al., 1998; Dann et al., 2000), na produção de leite (Williams et al., 1991b; Wohlt et al., 1991; Piva et al., 1993; Putman et al., 1997; Wohlt et al., 1998) e alterações na composição do leite (Williams et al., 1991b; Piva et al., 1993; Putman et al., 1997) de vacas leiteiras alimentadas com culturas de leveduras. Todavia, outros estudos (Erdman e Sharma, 1989; Arambel e Kent, 1990; Robinson 1997; Soder e Holden, 1999) observaram ausência de respostas dos animais à suplementação com estes aditivos alimentares.

Algumas das razões para o aparecimento ou não de efeitos positivos do uso desses aditivos podem ser: baixa viabilidade de certas culturas utilizadas, doses empregadas, diferenças entre cepas e interação das leveduras com nutrientes e outros microingredientes presentes na dieta. Além disso, outros fatores referentes aos animais que utilizam esses aditivos na alimentação também podem afetar a resposta, como a fase

do ciclo de lactação, o tipo de forragem fornecida, a estratégia de alimentação e a proporção de volumoso e concentrado da dieta (Piva et al., 1993).

Portanto, para avaliar o valor da inclusão de culturas de leveduras na dieta de vacas leiteiras, alguns autores sugerem que pode ser mais vantajoso iniciar o fornecimento antes do parto, quando ocorre diminuição da ingestão de matéria seca, extendendo até o pico de lactação (Wohlt et al., 1991). Outros autores reforçam que estes aditivos microbianos parecem ser mais eficazes quando fornecidos para vacas em início de lactação, proporcionando maior resposta produtiva (Kellems et al., 1990).

Wohlt et al. (1991) observaram que o pico de lactação ocorreu mais cedo, e que a produção de leite foi maior para vacas da raça Holandesa primíparas suplementadas com cultura de levedura dos 30 dias pré-parto até 18 semanas de lactação. Entretanto, Robinson (1997) não encontrou efeito da cultura de levedura sobre a produção ou composição de leite quando suplementou vacas leiteiras por um período mais curto, ou seja, dos 14 dias antes do parto aos 14 dias após o parto. Dann et al. (2000) demonstraram que o fornecimento de cultura de levedura para vacas Jersey, primíparas e multíparas, dos últimos 21 dias pré-parto até os primeiros 140 dias pós-parto, aumentou a ingestão de matéria seca nos últimos sete dias de gestação e nos primeiros 42 dias de lactação e resultou em menor perda de peso e menor utilização da reserva energética corporal para produção de leite durante o início da lactação. Estes autores também observaram que as vacas suplementadas atingiram o pico da lactação mais cedo, embora sem aumento na produção ou alteração na composição do leite. Robinson e Garrett (1999) e Wang et al. (2001) também observaram que a suplementação com culturas de *Saccharomyces cerevisiae* proporcionou menores perdas de peso corporal e escore de condição corporal quando fornecida para vacas de leite do pré-parto ao pós-parto.

Entretanto, outros achados (Wohlt et al., 1998; Soder e Holden, 1999) não encontraram efeito desta suplementação sobre o escore de condição corporal de vacas em lactação. O fornecimento de extratos da parede celular de levedura para vacas Holandesas em início de lactação não promoveu melhoras no desempenho corporal e produtivo destes animais (Bolt, 2002).

As mudanças físicas e metabólicas que ocorrem no período de transição e início da lactação associadas com a alta produção de leite geram um período de grande estresse para vacas leiteiras. A baixa ingestão de matéria seca por animais sob condições de estresse não proporciona à população bacteriana fatores de crescimento suficientes, como ácidos orgânicos, vitamina B e aminoácidos.

Alguns autores (Phillips e von Tungelin, 1985; Arambel e Kent, 1990) sugerem que a suplementação com culturas de leveduras tem maior eficácia num período de estresse para o animal, por estimular o crescimento de bactérias celulolíticas (Dawson et al., 1990) e proteolíticas (Yoon e Stern, 1996) no rúmen, alterar as concentrações de ácidos graxos voláteis (Callaway e Martin, 1997) e melhorar a digestibilidade da fração fibrosa (Carro et al., 1992), otimizando assim o metabolismo ruminal e, conseqüentemente, a ingestão de matéria seca e a produção de leite (Piva et al., 1993; Adams et al., 1995; Robinson e Garrett, 1999).

Robinson e Erasmus (2007) revisando 21 trabalhos publicados verificaram que as respostas ao uso de produtos contendo *S. cerevisiae* na alimentação de vacas Holandesas em lactação dependem da composição da dieta. Estes autores observaram que vacas suplementadas com produtos contendo *S. cerevisiae* tiveram redução na produção de leite à medida que ocorria aumento no teor de FDN ou FDA, enquanto em dietas com alto teor de amido o impacto sobre a produção de leite ou ganho de peso foi inexpressivo.

O modo de ação das leveduras no rúmen ainda não está completamente elucidado. Várias pesquisas (Wiedmeier et al., 1987; Harrison et al., 1988; Williams et al., 1991b) reportaram que as culturas de leveduras podem causar uma variedade de efeitos no rúmen, como aumento no pH, alteração nas concentrações de AGV, diminuição na produção de metano, aumento do número de bactérias celulolíticas e aumento na taxa e extensão da digestão da fibra no rúmen.

Entre as diversas propostas de ações associadas às culturas de leveduras, Wallace (1994) demonstrou que as leveduras teriam um papel importante na remoção do oxigênio do ambiente ruminal, o que implicaria no aumento da viabilidade bacteriana, pois apesar de ser considerado um meio totalmente anaeróbio, o gás produzido no rúmen contém de 0,5 a 1% de oxigênio (McArthur e Multimore, 1962). Por ser tóxico para as bactérias ruminais, o oxigênio inibe o crescimento bacteriano e a adesão das bactérias celulolíticas à fibra (Roger et al., 1990), o que diminui a eficiência do processo digestivo. Assim, quando culturas de *S. cerevisiae* são adicionadas à dieta de ruminantes, o número de bactérias no rúmen é aumentado, provavelmente devido a atividade das leveduras, que protege as bactérias anaeróbias contra danos causados pelo oxigênio e proporciona uma melhor fermentação ruminal (Newbold et al., 1996).

Outra possível forma de ação das leveduras no rúmen é a redução da concentração de ácido lático, o que promoveria melhor manutenção do pH e ambiente ruminal mais estabilizado. Por este ácido não ser utilizado como substrato para o crescimento da *S. cerevisiae*, supõe-se que a levedura reduz a concentração de lactato inibindo a produção do mesmo, ou estimulando a utilização de lactato pelas bactérias ruminais (Williams et al., 1991b; Callaway e Martin, 1997). Em um ambiente ruminal adequado, com manutenção do pH mais alto, o crescimento bacteriano será favorecido, principalmente o das bactérias celulolíticas, (Harrison et al., 1988; Dawson et al., 1990; Yoon e Stern,

1996), proporcionando aumento na digestão da fibra e, por conseqüência, no consumo e na produtividade animal.

O efeito benéfico das culturas de leveduras sobre a ingestão de alimentos em bezerros também pode estar relacionado com a manutenção de pH mais elevado, via redução nas concentrações de ácido lático (Fallon e Harte, 1987; Hughes, 1988). Williams et al. (1985) reportaram que o baixo pH ruminal foi um fator limitante do apetite de bezerros jovens que consumiram dietas com alta concentração de amido rapidamente fermentável.

As culturas de *S. cerevisiae* demonstram acelerar as atividades microbianas no rúmen, o que potencialmente favorece a transição de uma dieta líquida para uma dieta sólida nos animais pré-ruminantes (Chaucheyras-Durand e Fonty, 2001). Esta melhora da atividade microbiana pode facilitar a transição da dieta, resultando num maior consumo de concentrado por bezerros mais jovens, o que explica, em parte, o melhor desenvolvimento de animais suplementados com estas culturas. Estudos demonstram aumento na ingestão de matéria seca e no ganho de peso, bem como melhoria na eficiência alimentar de bezerros em aleitamento suplementados com cultura de levedura (Lesmeister et al., 2004) ou produto contendo leveduras vivas (Galvão et al., 2005), embora achados de Seymour et al. (1995) contestem esses efeitos.

A parede celular da *S. cerevisiae* contém oligossacarídeos que, de modo geral, parecem ser pouco degradados no rúmen pelo fato de as bactérias predominantes neste ambiente não serem capazes de utilizá-los para seu crescimento (Chandler e Newman, 1994). Entretanto, acredita-se que pelo menos uma porção dos componentes da parede celular é degradada pelas bactérias do rúmen, uma vez que, após a lise da parede, a célula ficaria aberta para ser degradada; promovendo alterações na fermentação ruminal (Callaway e Martin, 1997; Sullivan e Martin, 1999). A porção que permanece íntegra,

não sofrendo ações da fermentação ruminal, atingirá o cólon do sistema digestivo do animal, onde provavelmente será fermentada pela microflora intestinal (Quigley, 2005).

As bactérias intestinais são componentes do sistema imune do intestino, promovendo o equilíbrio entre absorção, secreção e controlando patógenos. Uma microflora intestinal normal é essencial para manter a saúde do animal. Quando ocorre um desequilíbrio entre a flora benéfica e a prejudicial, os animais podem apresentar inflamações, infecções, diarréia e outras doenças. Os principais mecanismos de defesa contra infecções causadas por microrganismos enteropatogênicos são a mucosa intestinal intacta e o sistema imunológico eficiente.

Um dos mecanismos mais comuns de danos ao trato digestório por microrganismos ocorre por meio de uma interação específica ou fixação entre as bactérias e as células epiteliais da parede intestinal. Esse mecanismo é utilizado pelas bactérias Gram negativas, como as *Salmonellas*, que possuem em sua superfície estruturas conhecidas como fímbrias. As fímbrias servem como suporte para a ligação entre as lectinas, presentes em sua superfície, e o receptor no epitélio. As lectinas são proteínas capazes de reconhecer resíduos de açúcares que formam as glicoproteínas (Edens, 2003).

Para muitos microrganismos, a habilidade de aderir ao epitélio intestinal é essencial para sua permanência e desenvolvimento, evitando serem removidos com os movimentos peristálticos. Portanto, um método para prevenir a colonização do intestino por patógenos é saturar os sítios receptores do epitélio. Os oligossacarídeos, MOS e BG, apresentam uma alta afinidade ligante e podem aderir às fímbrias bacterianas, bloqueando a adesão das bactérias à superfície intestinal (Menten, 2001).

Alguns microrganismos podem reconhecer sítios de ligação nesses açúcares como sendo da mucosa intestinal, reduzindo a colonização do intestino por bactérias

patógenas Gram negativas, que apresentam a fímbria tipo 1, específica para oligossacarídeos, como o MOS. Com isso, além da menor incidência de infecções, tem-se a mucosa inteiramente apta as suas funções de secreção, digestão e absorção de nutrientes (Iji e Tivey, 1998). O princípio de funcionamento desse mecanismo consiste na ocupação física dos sítios de ligação do epitélio intestinal pelas bactérias benéficas, formando uma barreira física contra as bactérias patogênicas. Estas bactérias, ao se ligarem ao MOS, não se ligam a sítios de ligação dos enterócitos e movem-se com o bolo fecal, o que impede a colonização do trato intestinal (Newman, 1994).

Ainda, os oligossacarídeos, principalmente os BG, agem como imuno-estimuladores, por terem a habilidade de estimular células do sistema imune (Williams et al., 1991a), como os neutrófilos e os macrófagos. Estas células contêm receptores de BG e MOS e, ao se ligarem a estes oligossacarídeos, são estimulados, aumentando a atividade imunológica (Vetivicka et al., 1996; Davis et al., 2004). Ao influenciarem diferentes funções de macrófagos e outras funções celulares, os oligossacarídeos promovem rápida resposta de defesa (Quigley, 2005). Portanto, estes componentes da parede celular da levedura podem beneficiar a resposta imune local ou sistêmica (Newman, 1994), modulando o sistema imune e resultando em aumento na resposta de neutrófilos e células T (White et al., 2002).

Assim, estimulando o crescimento das bactérias benéficas pela adição de extratos da parede celular ou de cultura de levedura na dieta animal, ocorrerá maior crescimento dos microrganismos benéficos em detrimento dos patogênicos. A escassez dos nutrientes que possam ser metabolizados pelas bactérias patógenas na luz intestinal acaba se tornando um fator limitante para a manutenção do crescimento destas no trato digestório.

Desta forma, a inclusão de cultura de levedura ou extratos de parede celular na dieta animal poderá alterar a microbiota intestinal, aumentando o número de microrganismos benéficos e diminuindo as bactérias patogênicas, modulando o sistema imune e melhorando a saúde do animal (Spring et al., 2000; Burkey et al., 2004; Davis et al., 2004). A obtenção destes benefícios, no entanto, implicará na capacidade de sobrevivência das leveduras e manutenção da integridade dos componentes da parede celular durante o processo de fermentação ruminal, determinando assim a eficiência destes produtos.

Os efeitos benéficos das culturas de leveduras sobre a saúde animal podem reduzir a morbidade e a mortalidade de bezerros, além de proporcionar melhor desempenho animal. As infecções gastrointestinais e subseqüentes diarréias e desidratação são as maiores causas dos problemas de saúde que afetam bezerros no período de aleitamento, sendo também as principais razões de morte e baixo desenvolvimento nos primeiros meses de idade (Davis e Drackley, 1999). Quando o animal se encontra sob condições de estresse, devido a manejo e ambiente inadequados ou má alimentação, o crescimento de patógenos é favorecido, levando ao aumento no risco de doenças.

Galvão et al. (2005) suplementaram bezerros com leveduras vivas e notaram que os animais apresentaram menor número de dias com diarréia. Embora dados sobre os efeitos dos produtos de leveduras na saúde de ruminantes sejam mais limitados, em outras espécies a utilização destes produtos têm mostrado benefícios. Spring et al. (2000) observaram que a adição de MOS à dieta de frangos reduziu a presença de *Salmonella typhimurium* no ceco das aves. Leitões suplementados com leveduras, como fonte de MOS, tiveram aumento nas concentrações de IgA e IgG do soro, menor colonização do intestino por coliformes e redução dos coliformes fecais, o que preveniu a ocorrência de diarréia, indicando os possíveis benefícios dos produtos da parede

celular sobre a imunidade do intestino (White et al., 2002). White et al. (2002) e LeMieux et al. (2003) constataram aumento na produção de anticorpos, ativação de macrófagos e proliferação de células T com o uso dos oligossacarídeos, o que melhorou a resposta imune de leitões desmamados e diminuiu o risco de infecções. Davis et al. (2004) verificaram melhora na atividade fagocítica de macrófagos em leitões desmamados que recebiam mananoligossacarídeo fosforilado na dieta, o que sugeriu ser um resultado da exposição de macrófagos à manose presente no trato intestinal.

Achados de Dann et al. (2000) mostraram que a adição de cultura de levedura à dieta de vacas leiteiras não afetou a ocorrência de retenção de placenta, cetose, febre do leite ou mastite.

Entretanto, o estímulo da atividade imunológica de ruminantes suplementados com culturas de leveduras ainda é uma questão muito pouco estudada, não havendo nenhum tipo de constatação conclusiva na literatura.

Devido aos resultados positivos de várias pesquisas e também dos resultados contraditórios encontrados na literatura com o uso das culturas de leveduras, sugere-se que estas sejam melhor investigadas, pois podem ser consideradas uma nova alternativa da nutrição animal, que potencialmente proporciona benefícios econômicos por influenciar na produtividade dos animais e auxiliar na proteção contra enfermidades, sem deixar resíduos nos alimentos e respeitando o meio ambiente.

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II - OBJETIVOS GERAIS

O projeto foi realizado no sentido de determinar os efeitos do fornecimento de cultura de levedura enriquecida com extratos de parede celular de levedura, comparado com a mesma cultura de levedura não enriquecida, para vacas de alta produção em início de lactação, e também os efeitos da adição de cultura de levedura na ração inicial de bezerros leiteiros durante os primeiros 70 dias de idade sobre a saúde, as medidas da imunocompetência celular e humoral e o desempenho destes animais.

III – Effect of Feeding Yeast Culture Enriched with Cell Wall Extracts on Lactation, Health and Immune Responses of Dairy Cows

ABSTRACT

Objectives were to determine effects of feeding yeast culture of *Saccharomyces cerevisiae* enriched with cell wall extracts on lactation, health and immune response of early postpartum Holstein cows. Multiparous cows, totalizing 333, were randomly assigned at 1 d in milk (DIM) to receive 14 g/d of a commercial yeast culture (Diamond V XPC; YC = 159 cows) or 14 g/d of the same yeast culture with additional 5 g/d of yeast cell wall extracts (Diamond V XPC Plus; YCCW = 174 cows), top-dressed onto the total mixed ration diet once a day for the first 100 DIM. A subset of 80 cows (40/treatment) was evaluated for cellular and humoral immune responses, and plasma metabolites concentrations. There were no effects of treatments on incidence of postpartum disorders, although more cows receiving YCCW were diagnosed with at least one disease. Treatments did not affect body condition score, plasma metabolites concentrations and humoral response of cows. Phagocytic and killing activities of neutrophils were improved with days postpartum, but treatments did not influence cellular immune response. Cows fed YCCW had lesser yields of milk, 3.5% fat-corrected milk, energy-corrected milk, milk fat and milk true protein, although concentrations of fat, true protein and net energy for lactation in milk were not influenced by treatments. Conception rate at first postpartum insemination was similar between treatments. These results suggest that the inclusion of yeast cell wall extracts to diets of cows fed yeast culture does not improve health, immune response or lactation performance.

Key words: dairy cow, health, yeast culture, yeast cell wall

INTRODUCTION

Yeast products are known to be better utilized by animals that are under stress (Phillips and von Tungelin, 1985). Early lactation dairy cows do not consume enough feed to meet their energy requirements to support high milk production, resulting in negative energy balance, which makes it a stressful period for these animals. Yeast cultures have been used as supplement in diets of high producing dairy cows based on the hypothesis that its stimulate the growth of rumen cellulolytic (Dawson et al., 1990) and proteolytic bacteria (Yoon and Stern, 1996), change volatile fatty acids concentration (Callaway and Martin, 1997; Miller-Webster et al., 2002) and improve fiber digestibility (Carro et al., 1992; Dann et al., 2000), which may optimize rumen metabolism and, consequently, feed intake and milk production (Piva et al., 1993; Robinson and Garrett, 1999). Despite of these positive findings, responses to yeast cultures supplementation have been variable, and in other studies (Arambel and Kent, 1990; Swartz et al., 1994) were not found any beneficial response.

Cell wall of yeast *Saccharomyces cerevisiae* contains, depending on the culture conditions, at about 30 to 45% of two types of carbohydrates, beta-glucans and mannan-oligosaccharides (Katohda et al., 1976; Reed and Nagodawithana, 1991), which may provide immunological benefits to the animal, because of their biological activity (Burkey et al., 2004). These oligosaccharides protect the cell wall from outside environment providing structural integrity to the cell. Beta-glucans (**BG**) constitutes the inner layer of the cell wall and mannan-oligosaccharides (**MOS**), the outer layer, covering the BG layer (Van Der Vaart et al., 1995).

When fed to the cows, the yeast survival or the yeast cell wall integrity to rumen fermentation determines the effectiveness of these products. It is suggested that at least

a portion of cell wall components will be degraded by ruminal bacteria providing changes in ruminal fermentation (Callaway and Martin, 1997; Sullivan and Martin, 1999). Chandler and Newman (1994) noted that the predominant ruminal bacteria are unable to grow on MOS, indicating that MOS is poorly degraded in the rumen. The portion that remained viable through the rumen will not be absorbed or digested in the small intestine, and reaches the colon, where it will be fermented by the large intestinal flora (Quigley, 2005).

The inclusion of yeast cell wall in the diet, therefore, may change the intestinal flora increasing the number of beneficial microorganisms and decreasing the presence of pathogenic bacteria, which improves the immune response and animal health (Spring et al., 2000; Burkey et al., 2004; Davis et al., 2004).

Mannan-oligosaccharides bind to pathogenic bacteria cell wall, which prevents bacteria from attaching to intestinal epithelial cells, inducing an antibody response that improves the immune response and reduces the risk of infection (White et al., 2002; LeMieux et al., 2003). Analyzing isolated jejunum intraepithelial lymphocytes samples from lamina propria of leukocytes in weaned pigs fed phosphorylated mannans in the diet, Davis et al. (2004) observed an improvement of phagocytic activities, even when the percentage of macrophages remained the same, which suggests to be a result of exposure to mannose in the enteric environment.

Beta-glucans seem to have an ability to stimulate the immune system (Williams et al., 1991) binding to some components of this system, affecting different functions of macrophages and other cellular functions. Thus, BG is processed by macrophages into components that neutrophils recognize, providing a quickly response (Quigley, 2005). They also stimulate phagocytes activities of the reticuloendothelial system (Auclair,

2001) and may have some effect on T and B cells (Tsukada et al., 2003), therefore, BG act as an immunostimulator enhancing the immune response.

Majority of the results found in the literature have shown effects of yeast cultures on rumen function and performance of ruminants, nevertheless, studies have not been conducted evaluating effects of yeast cell wall components, associated or not to yeast cultures, and the influence of these products on health and immune system of dairy cows. Therefore, we hypothesize that *S. cerevisiae* culture with additional yeast cell wall extracts as a diet supplement would improve performance and immune response of early lactation dairy cows. Objectives of the current study were to determine the effects of feeding a yeast culture of *Saccharomyces cerevisiae* enriched with cell wall extracts compared with the same yeast culture non-enriched on lactation, health, and cellular and humoral immune responses of early postpartum high producing dairy cows.

MATERIAL AND METHODS

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

Animals, Housing and Treatments

Three-hundred and thirty-three early lactation multiparous Holstein cows were randomly assigned at 1 DIM to receive 14 g/d of a commercial yeast culture (Diamond V XPC; **YC** = 159 cows) or 14 g/d of the same yeast culture with additional 5 g/d of yeast cell wall extracts (Diamond V XPC Plus; **YCCW** = 174 cows), top-dressed onto the total mixed ration diet (**TMR**) once a day for the first 100 DIM. Both yeast cultures

were diluted with 52 g of corn distiller's grain as carrier, allowing each cow to receive a total of 66 and 71 g/d of the YC and YCCW, respectively.

The experiment was conducted from November 2005 to June 2006. Cows were housed in free-stall barns and individual pens were virtually identical in design, size, and number of animals housed, and similar milking and feeding times. They were milked three times per day at 0500, 1300 and 2100 h and milk yield and composition were recorded for individual cows once a month during the official California Dairy Herd Improvement Association test.

Diets, Diet Sampling and Nutrient Analyses

Cows were fed twice daily at 0500 and 1100 h, and all cows received the same TMR (Table 1) to meet the nutrient requirements for lactating Holstein cows weighing 680 kg, consuming 26 kg of DM, and producing 51 kg/d of 3.5% FCM (NRC, 2001) during the first 100 DIM. Diet ingredients and the TMR were sampled monthly and weekly, respectively, dried at 55 ºC for 48 h in an air circulating oven, ground with Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 2 mm screen, followed by a cyclone mill (Udy Co., Fort Collins, CO) to pass a 1 mm screen. Samples of ingredients were then composited for every 3 month periods and samples of TMR for 2 month periods and analyzed for DM, OM (AOAC, 2000), ADF and NDF (Van Soest et al., 1991), N using a N analyzer (FP-528 Nitrogen Determinator, LECO Corporation, St. Joseph, MI). The CP was estimated as N percentage multiplied by 6.25. Minerals were analyzed at the Dairyland Laboratory (Arcadia, WI) using an inductively-coupled plasma emission spectrometer (Thermo Garrell Ash, Franklin, MA).

Body Condition Scoring

Body condition (**BCS**) of all cows was scored at study enrollment, d 14, 35, 66 and 100 postpartum. Cows were scored for body condition in a scale of 1 (emaciated) to 5 (obese), with 0.25 unit increments as described by Ferguson et al. (1994).

Incidence of Health Disorders

Incidence of health disorders were daily recorded for individual cows by herd personnel and they included retained placenta, postpartum fever, metritis, ketosis, mastitis, lameness, displaced abomasum, and other miscellaneous digestive problems. Cases of retained placenta were characterized when the cows retained the fetal membranes longer than 24 h. Postpartum fever was diagnosed when rectal temperature > 39.5^{o}C. Metritis was characterized by watery fetid vaginal discharge of brown or red color in the first 3 weeks postpartum in cows having or not fevers symptoms. Ketosis was diagnosed based on the lack of appetite and ketonuria (Ketostix®, Bayer Co., Pittsburgh, PA). Clinical mastitis cases were characterized by the presence of abnormal milk, or by signs of inflammation in one or more quarter, and were treated by intramammary infusion of antibiotics according to treatment protocols established by the herd veterinarian. A new case of mastitis was defined for the same cow when a different quarter was affected or a period of 21 d had elapsed since the previous clinical mastitis diagnosis. The diagnosis of lameness was based on visual observation of cows when walking from the milking parlor back to the free-stall barns, and further confirmed by visual inspection of the hoof on a trimming table by the hoof trimmer. Diagnosis of displacement of the abomasum was based upon clinical signs presented by the affected cow, which included reduced milk production, rumen atony, ketonuria (Ketostix®, Bayer Co., Pittsburgh, PA), diarrhea, and presence of an acute ping sound at

auscultation and percussion on the left or right side of the abdomen. All cows sold or that died during the study were recorded.

Reproductive Management and Pregnancy at First AI

Starting at 39 d postpartum, all cows received an intramuscular injection with 25 mg of $PGF_{2\alpha}$ (Lutalyse®, dinoprost tromethamine; Pfizer Animal Health, New York, NY) every 14 d. Estrus was detected once daily, in the morning, by removal of tail chalk which was applied daily using paintsticks (All-weather Paintstik, LA-CO Industries, Chicago, IL). Cows observed in estrus were inseminated in the same morning, and inseminated cows were evaluated for pregnancy by palpation per rectum 35 d after AI.

Blood Sample Collection

Blood samples were collected from a subset of 80 cows (40/treatment) on d 2, 14, 35 and 66 postpartum, by puncture of the median coccygeal vein or artery using evacuated tubes (Becton Dickinson, Franklin Lakes, NJ) without additive for serum separation, and with K_2 EDTA for whole blood or plasma separation. Samples were immediately placed on ice and transported to the laboratory within 2 h from the collection. Plasma and serum were obtained after tubes centrifugation at 2000 x *g* for 15 min, and then frozen at -25º C until analyses.

Concentration of Plasma Metabolites

Plasma samples collected on d 14, 35, and 66 postpartum were analyzed for concentrations of glucose using a YSI Model 2700 SELECT Biochemistry Analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH), nonesterified fatty acids

(**NEFA**) according to Johnson and Peters (1993) using a commercial kit (NEFA C, Wako Chemicals USA Inc., Richmond, VA), and beta-hydroxybutyrate (**BHBA**) using a commercial kit (RANBUT, D-3-Hydroxibutyrate, Randox, Laboratories Ltd, United Kingdom) based on the enzymatic oxidation of BHBA to acetoacetate and concomitant reduction of NAD+ to NADH (Williamson et al., 1962).

Evaluation of Humoral and Cellular Immune Responses

Ovalbumin (**OVA**) solution was prepared by dissolving 1 mg of OVA (Type VII, Sigma Chemical Co., St. Louis, MO) in 1 mL of phosphate buffered saline (PBS 0.1 M, pH = 7.4) and emulsified in 0.5 mg of adjuvant Quil-A (Accurate Chemical, Westbury, NY) diluted in 1 mL of PBS. The same subset of 80 cows (40/treatment) from which blood was analyzed for metabolites concentrations received an intramuscular injection of 2 mL containing 1 mg of OVA on d 2, 14 and 35 postpartum. Blood was sampled immediately prior to each injection and again on d 66 postpartum for measurements of serum antibody concentration to OVA. Concentrations of IgG to OVA were measured by ELISA as described by Wagter et al. (2000).

Neutrophil phagocytosis and intracellular kill of *E. coli* ATCC 25922 were evaluated. The assays were performed using the blood of the same subset of 80 cows (40/treatment) on d 20 and 39 postpartum.

Neutrophils were collected and bacteria were prepared as described by Hogan et al. (1992). Neutrophils were harvested from 50 mL of median coccygeal blood and bacteria were prepared as described by Hogan et al. (1992). Suspensions of neutrophils and opsonized bacteria were incubated in a 1:3 at 37 ºC and 100 rpm for 90 min in water bath. Following incubation, 50 µL of bacteria-neutrophil samples were mixed with 25 µL of acridine orange solution and 25 µL of crystal violet solution. Wet mount

slides were prepared and neutrophils were evaluated under an epifluorescent microscope. Bacteria present in the cytoplasm of neutrophils were stained in either red, when dead, or green, when live, and number of neutrophils phagocytizing bacteria, number of dead and live bacteria was counted in the first 50 neutrophils visible under the microscope.

Statistical Analyses

Data such as incidence retained placenta, fever, metritis, ketosis, displaced abomasum, digestive problems, lameness, mastitis, overall incidence of diseases and conception rate were binomially distributed and analyzed by logistic regression using the LOGISTIC procedure of SAS (2001). A backward stepwise regression model was utilized (Allison, 1999), and all models included the effects of treatments, parity (second lactation vs. third or greater lactation), interaction between treatment and parity, and other explanatory variables as found to be appropriate for the outcome analyzed. Variables were continuously removed from the model by the Wald's statistic criterion if the significance was greater than 0.10. Adjusted odds ratio (**AOR**) and the 95% confidence interval (**CI**) were calculated.

Concentrations of plasma metabolites (glucose, NEFA and BHBA), yields of milk and milk components, BCS, percentage of neutrophils phagocytizing bacteria, mean number of phagocytized bacteria per neutrophil, percentage of phagocytized bacteria being killed, total number of phagocytized bacteria, percentage of neutrophils killing bacteria, and IgG anti-ova concentrations were analyzed by ANOVA for repeated measures (Littell et al., 2002) using the MIXED procedure (SAS, 2001). All models included the effects of treatments, parity, days in milk, interaction between treatment and days in milk, and other explanatory variables were included as appropriate. Cow

nested within treatment was used as the random effect in the model, and the covariance structure was chosen based on the Schwartz's Bayesian criterion (Littell et al., 2002).

Treatment differences with $P \leq 0.05$ were considered significant and $0.05 < P \leq 0.10$ were designated as tendency.

RESULTS

Treatment YCCW did not influence postpartum BCS of cows from 2 to 100 days in milk (Figure 1). Days in milk, however, had effect ($P < 0.001$) on BCS of the cows, but no interaction between treatment and days in milk was observed.

Cows fed YC tended to have less digestive problems (diarrhea or bloat; $P = 0.07$) and incidence of diseases (cows experiencing one or more of the diseases evaluated; $P = 0.06$), than cows fed YCCW (Table 2). There was no effect of treatments for others postpartum disorders.

Yields of milk, FCM, ECM, milk fat, milk true protein, and SCC were higher ($P < 0.02$) for YC than for YCCW cows, whereas yield of NE_L in milk and concentrations of milk fat and milk true protein were not altered by treatments (Table 3).

Glucose, NEFA and BHBA concentrations measured in plasma of cows at 14, 35 and 66 days postpartum were similar between YC and YCCW diets, but different ($P < 0.001$) with days postpartum (Figure 2). Interaction between treatments and days in milk was not observed.

Treatments did not affect serum titers for IgG anti-ovalbumin of cows in the first 66 days postpartum (Figure 3). Concentrations of IgG anti-ovalbumin in serum differed ($P < 0.001$) with days postpartum, but no interaction was observed between treatment and days in milk on serum IgG anti-ovalbumin titers.

Phagocytic and bactericidal activities of neutrophils harvested from cows at 20 and 39 days postpartum were not changed by the YC or YCCW (Table 4). Cows at 20 days postpartum had smaller ($P < 0.02$) percentage of neutrophils phagocytizing bacteria, average number of phagocytized bacteria per neutrophil, number of phagocytized bacteria, and percentage of neutrophils killing at least one bacterium, compared with cows at 39 days postpartum. There was interaction between treatments and days in milk on number of phagocytized bacteria ($P < 0.03$) and tendency ($P = 0.08$) on the average number of phagocytized bacteria per neutrophil.

The conception rate at first postpartum AI of cows from the treatments YC and YCCW were 30.2% and 24.7%, respectively. This difference, however, were not significant (Figure 4).

DISCUSSION

A feeding strategy that prevents decrease in dry matter intake and, consequently, supports higher animal performance is one of the main goals of ruminant nutritionists. It has been hypothesized that *Saccharomyces cerevisiae* used as a supplement in dairy cow diets may have properties to improve production and reproduction performance, because enhances ruminal metabolism. Besides that, yeast cell wall composition would further provide benefits stimulating the immune response and, as a result, the animal health.

In the present study, both groups of cows sustained very low and similar body condition losses, maybe because of good pre-partum conditions, and treatments had no effect on BCS. The lowest BCS coincided with peak lactation, and YCCW promoted a slight numerical earlier BCS recovery after 66 days postpartum compared with YC

(Figure 1). Other authors (Robinson and Garrett, 1999; Dann et al., 2000; Wang et al., 2001) also found that dairy cows supplemented with *Saccharomyces cerevisiae* from pre-partum to postpartum showed only numerically lower losses of BW and BCS in comparison to unsupplemented cows. Soder and Holden (1999) reported no effect on BCS after parturition for dairy cows supplemented with yeast culture. Additionally, early lactation Holstein cows fed yeast cell walls (YCW) top-dressed onto a TMR did not improve BW or BCS compared with cows not supplemented (Bolt, 2002). On the other hand, Robinson (1997) found a numerically earlier BCS recovery, started around 21 DIM for lactating cows supplemented with yeast culture.

The potential of using yeast culture or yeast cell walls to improve animal health and productivity has been recognized, especially in animals under severe stress because of high production demands. Data from the present study indicated a tendency of cows fed YC to have less digestive problems and incidence of diseases than cows fed YCCW. Although not significant, a lower percentage of cows fed YC had fever, ketosis, and displaced abomasum, lameness and mastitis, compared with cows fed YCCW. Dann et al. (2000) demonstrated that the inclusion of yeast culture to diet of pre and postpartum dairy cows did not affect the occurrence of retained placenta, ketosis, displaced abomasum, milk fever, or mastitis. Also, results from Nocek and Kautz (2006) indicated that feeding direct-fed microbial (DFM; mix of yeast and bacteria) to multiparous Holstein cows on prepartum and early lactation had no effect on retained placenta and incidences of metritis, ketosis, and displaced abomasum.

Results from the present study indicated higher yields of milk and its components, as well as for 3.5% FCM and ECM when cows were supplemented with YC compared with cows supplemented with YCCW; possibly justified by the higher percentage of cows with digestive problems when fed YCCW. *Saccharomyces cerevisiae* have been

added to diets of dry and lactating dairy cows to challenge ruminal fermentation, and potentially increasing dry matter intake and milk production. Some reports have demonstrated certain responses from dairy cows supplemented with yeast culture. Piva et al. (1993) observed increase on yields of milk, 4% FCM and milk fat when cows in mid-lactation received YC. Furthermore, Robinson and Garrett (1999) observed a trend of improvement on milk production from primiparous and multiparous cows fed with YC from 21 d prepartum to 56 d postpartum. In addition, milk yield or milk composition, as well as SCC of Jersey cows supplemented with YC on pre and postpartum until 140 DIM were not affected, but these cows reached peak milk yield earlier than cows fed control (Dann et al., 2000). On the other hand, Wohlt et al. (1991) observed that primiparous Holstein cows fed YC peaked milk yield earlier and had greater milk production. Other several studies (Robinson, 1997; Soder and Holden, 1999; Wang et al., 2001) showed that the addition of YC to the diet of early postpartum dairy cows have not enhanced lactational performance. Moreover, a study with early lactation Holstein cows fed YCW reported no effect on the yields of milk, milk components and 4% FCM (Bolt, 2002).

High BHBA levels suggest that cows may be in negative energy balance and high plasma glucose may reflect that cows are in a positive energy balance. Therefore, an optimal situation to meet postpartum energy requirements is elevating concentration of blood glucose and reducing BHBA and NEFA; thus providing reduction of fatty acid mobilization, better oxidation of fatty acids and increasing energy derived from dietary carbohydrate sources. However, in this work no effects of treatments on plasma metabolites were found, even with a slight numerically better condition for YCCW blood metabolites, which suggested that YC could be supporting the situation and minimizing any effect from additional cell wall.

Bovine polymorphonuclear leukocytes produce reactive oxygen species to kill harmful bacteria (Mehrzad et al., 2001), being an important defense mechanism against Gram-negative and Gram-positive bacteria (Burvenich et al., 1994). High producing dairy cows around parturition and during early lactation are under severe stress condition and it suppress blood polymorphonuclear cells, decreasing the production of reactive oxygen species and leading to an overall impair bactericidal capacity of the blood polymorphonuclear cells (Mehrzad et al., 2001). Beta-glucans, polysaccharides extracted from the cell wall of yeast, fungi, algae, and oats have been recognized to enhance the activities of the immune system, stimulating neutrophil, macrophage, and natural killer cells (Vetivicka et al., 1996); these cells contain specific BG receptor sites that is stimulated by BG when bound, increasing the immunological activity (Vetivicka et al., 1996). Neutrophils are one of the defense cells responsible for the phagocytosis of many pathogenic microorganisms and its overall activity are determined by their number and function. Moreover, neutrophils are involved in the synthesis and release of cytokines, which influence T and B cells activities (Murphy et al., 2007), and therefore, protecting the organism from bacterial challenges.

The supplementation with YC or YCCW, did not affect immune response in the present study; it was observed improvement on neutrophil functions and antibody response from 20 DIM to 39 DIM. This can be explained by the great hormonal and physic changes that took place after parturition, causing extreme stress to the animal and decreasing its immunity. In addition to that, the nutritional YCCW support, obtained mainly from BG and MOS promoted earlier cellular immune response by the animal, compared with YC, and showed interaction between treatment and days in milk on neutrophil phagocytic activity. Perhaps, if the supplementation with yeast cell wall had started in the prepartum, would be possible to observe some improvement right in the

beginning of lactation. Wohlt et al. (1991) affirmed to be necessary start the yeast culture supplementation before parturition and extending through peak lactation to obtain maximum benefices on cows lactation, and corroborating, Hutjens (2005) suggested that optimal time to supplement dairy cows with YC should be from two weeks prepartum to four weeks postpartum to support a better stabilization of the rumen environment and to attenuate the stress when the dry cows change from a lower energy diet to a high energy lactational nutrition. Murphy et al. (2007) observed increase in neutrophil functions when fed oat BG to mice, with increase in endogenous neutrophil burst activity and number of cells. Also derived from *S. cerevisiae* cell wall, mannan-oligosaccharides may affect the microbes in the intestinal tract by adsorbing and preventing bacteria from attaching to the gut wall, therefore reducing intestinal pathogen colonization (Newman, 1994; Spring 2000). Several bacteria contain in their surface specific lectins for mannose and, when MOS is a source in the intestine, bacterial lectins bind to receptors containing D-mannose blocking the lectins and inhibiting the pathogens to adhere in the intestinal epithelium (Newman, 1994; Spring 2000). Additionally, MOS may also influence the immune response because of the presence of mannose receptors in the natural immune defense cells (Davis et al., 2004). Some findings indicated that oral administration of *S. cerevisiae* may increases IgA and secretory component of immunoglobulins in growing rats (Buts et al., 1990), and feeding MOS may promote overall health and growth performance in pigs (Newman, 1994; Davis et al., 2004).

The effects of yeast cell wall on reproductive performance of dairy cows still a puzzle and the few findings are not consistent. For instance, Dann et al. (2000) showed that days to first breeding averaged 74.9 and was not affected when cows were supplemented with *Saccharomyces cerevisiae*. Other study (Wang et al., 2001)

demonstrated that services per conception were 1.8 and 2.5, and days open were 91 and 137, for cows fed YC and without YC, respectively; however the authors suggested that those results were questionable. In this experiment was observed a numerical minor conception rate for cows fed YCCW. Cows maintained BCS, but milk yield and conception rate of YCCW cows were smaller. Lucy (2001) affirmed that early postpartum dairy cows utilize first partition of metabolizable energy toward milk production and body condition gain before reproductive functions.

The lack of effect of treatment in this study, may be also explained by the feeding procedure in which the yeast products were top-dressed on cow's TMR along the pen at time of feeding rather than being mixed into the ration, or yet by the improvement with only the supplementation with yeast culture, that could be enough to provide certain benefices; hence, the addition of yeast cell wall extracts to yeast culture would be not necessary. Because we did not evaluate a group of cows feeding TMR without yeast, however, it is unclear.

CONCLUSIONS

The addition of 5 g of cell wall extracts to a yeast culture of *Saccharomyces cerevisiae* top-dressed onto the TMR of dairy cows did not influence BCS, blood metabolites, humoral immune response and conception rate at first AI. Incidence of diseases and digestive problems tended to be higher, and milk yield and its components were lower for cows supplemented with additional cell wall extracts. There was an interaction between treatments and days in milk where YCCW slightly enhanced the cellular immune response. These results indicated that the incorporation of cell wall extracts to yeast culture as diet supplement for cows in the begin of lactation was not

benefic for the animals because of decrease in performance and increase in health disorders.

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Table 1. Ingredient composition of diets

	Treatment[1]	
	YC	YCCW
Ingredients	(% of DM)	
Corn silage	22.9	22.9
Alfalfa hay	16.2	16.2
Alfalfa haylage	3.5	3.5
Steam-flaked corn	15.0	15.0
Whole cottonseed	9.3	9.3
Soybean hulls	5.0	5.0
Canola meal	19.9	19.9
Almond hulls	4.7	4.7
Minerals and vitamins[2]	2.0	2.0
Ca salts of fatty acids[3]	1.5	1.5
YC[4] (top-dressed), g/cow/d	66	---
YCCW[5] (top-dressed), g/cow/d	---	71

[1] YC = yeast culture; YCCW = yeast culture with cell wall extracts.
[2] 9 g of Zinpro 4-Plex (mixture of Zn methionine, Mn methionine, Cu lysine, and Co glucoheptonate; Zinpro Co., Eden Prairie, MN), 6% Ca, 1% P, 3.8% Mg, 0.4% K, 0.7% S, 16% Na, 8% Cl; and (per kg) 1719 mg Zn, 227 mg Cu, 590 mg Mn, 41 mg Co, 24 mg I, 3 mg Se, 180,000 IU of vitamin A, 40,000 IU of vitamin D, and 800 IU of vitamin E.
[3] Ener GII® calcium salts of fatty acids (Virtus Nutrition, LLC, Fairlawn, OH).
[4] Diamond V XPC Yeast Culture (Diamond V Mills, Inc., Cedar Rapids, IA).
[5] Diamond V XPC Plus Yeast Culture (Diamond V Mills, Inc., Cedar Rapids, IA).

Table 2. Effect of yeast culture containing cell wall extracts on incidence of postpartum disorders

	Treatment[1]				
	YC	YCCW	AOR[2]	95% CI[3]	*P* value
	[% (no./no.)]				
Retained placenta	6.3 (10/159)	5.8 (10/174)	1.12	0.45, 2.79	0.80
Fever	6.3 (10/159)	9.2 (16/174)	0.66	0.29, 1.51	0.33
Metritis	15.1 (24/159)	12.1 (21/174)	1.30	0.69, 2.45	0.41
Ketosis	8.8 (14/159)	10.3 (18/174)	0.85	0.41, 1.77	0.66
DA[4]	1.3 (2/159)	1.7 (3/174)	0.70	0.12, 4.29	0.70
Digestive problems[5]	2.5 (4/159)	6.9 (12/174)	0.35	0.11, 1.10	0.07
Lame	1.9 (3/159)	2.9 (5/174)	0.66	0.15, 2.81	0.57
Mastitis	13.2 (21/159)	17.8 (31/174)	0.75	0.40, 1.39	0.36
Diseases[6]	38.4 (61/159)	49.4 (86/174)	0.64	0.40, 1.02	0.06
Left Study[7]	8.8 (14/159)	9.8 (17/174)	0.64	0.21, 1.92	0.55

[1] YC = yeast culture; YCCW = yeast culture with cell wall extracts.
[2] AOR = adjusted odds radio; YCCW as the reference value.
[3] CI = confidence internal.
[4] DA = displaced abomasum.
[5] Cows diagnosed with diarrhea or bloat.
[6] Cows experiencing one or more of the diseases evaluated (retained placenta, fever, metritis, ketosis, displaced abomasum, digestive problems, lameness, or mastitis)
[7] Cows that left study because of culling or death.

Table 3. Effect of yeast culture containing cell wall extracts on lactation performance (LSM ± SEM)

	Treatment[1]		
	YC	YCCW	*P* value
Milk, kg/d	51.4 ± 0.7	48.3 ± 0.7	0.002
3.5% FCM[2], kg/d	52.5 ± 0.7	48.9 ± 0.7	< 0.001
ECM[3], kg/d	47.4 ± 0.6	44.1 ± 0.6	< 0.001
Milk NE_L[4], Mcal/kg	0.69 ± 0.003	0.69 ± 0.003	0.64
Milk fat			
%	3.65 ± 0.04	3.63 ± 0.03	0.70
kg/d	1.87 ± 0.03	1.73 ± 0.02	< 0.001
Milk true protein			
%	2.84 ± 0.02	2.83 ± 0.02	0.56
kg/d	1.45 ± 0.02	1.36 ± 0.02	< 0.001
SCS[5]	2.86 ± 0.15	3.60 ± 0.14	< 0.001
SCC[6], x1000 cells/mL	375.2 ± 73.2	648.2 ± 69.8	---

[1] YC = yeast culture; YCCW = yeast culture with cell wall extracts.
[2] FCM = fat-corrected milk.
[3] ECM = energy-corrected milk.
[4] Milk NE_L = net energy for lactation in milk.
[5] SCS = somatic cell score.
[6] SCC = somatic cell count.

Table 4. Effect of yeast culture containing cell wall extracts on neutrophil function (LSM ± SEM)

	Treatment[1]						
	YC		YCCW		*P* value[2]		
Days in milk	20	39	20	39	TRT	DIM	TRT*DIM
Neutrophils phagocytizing,[3] %	64.2 ± 2.6	70.9 ± 2.7	63.2 ± 2.7	76.5 ± 2.7	0.47	< 0.001	0.13
Intracellular bacteria/neutrophil,[4] n	8.0 ± 0.4	9.4 ± 0.5	7.3 ± 0.4	10.2 ± 0.5	0.89	< 0.001	0.06
Bacteria killed,[5] %	83.6 ± 1.8	86.3 ± 1.8	82.8 ± 1.8	85.7 ± 1.8	0.70	0.12	0.96
Bacteria phagocytized, n	261.2 ± 23.0	348.5 ± 23.2	240.3 ± 23.1	401.7 ± 23.3	0.57	< 0.001	0.03
Neutrophils killing bacteria,[6] %	97.3 ± 0.5	98.2 ± 0.5	97.4 ± 0.5	98.6 ± 0.5	0.63	0.02	0.73

[1] YC = yeast culture; YCCW = yeast culture with cell wall extracts.
[2] TRT = treatments; DIM = days in milk; TRT*DIM = interaction between TRT and DIM.
[3] Percentage of neutrophils containing at least one intracellular bacterium (alive or dead).
[4] Number of bacteria phagocytized/number of neutrophils phagocytizing at least one bacterium.
[5] Number of dead, phagocytized bacteria/(number of alive + dead phagocytized bacteria)*100.
[6] Number of neutrophils containing at least one dead bacterium/number of neutrophils phagocytizing at least one bacterium.

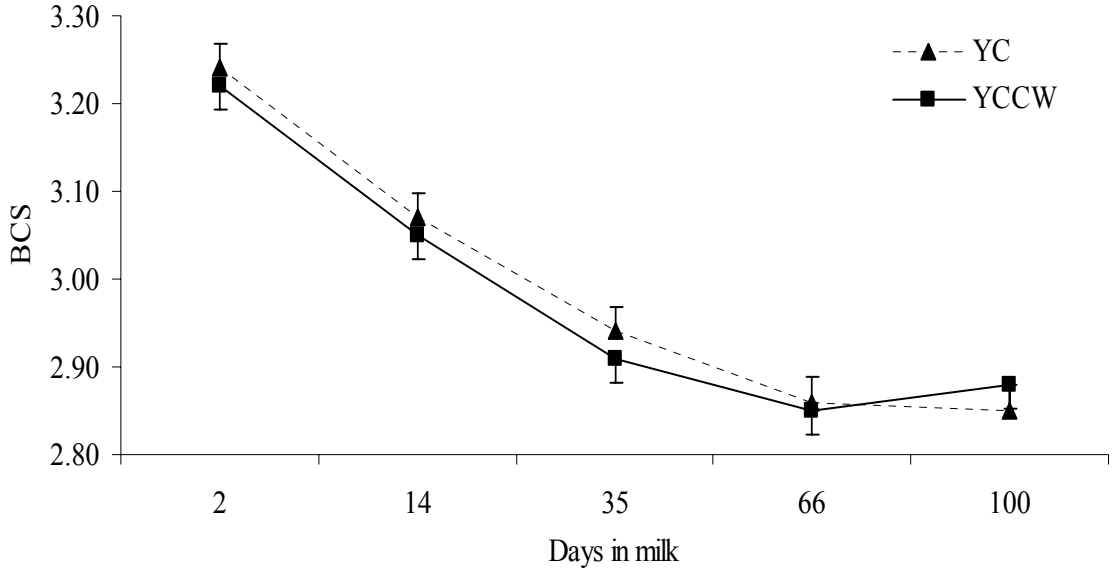

Figure 1. Body condition score (BCS) of dairy cows fed yeast culture (YC) or yeast culture with cell wall extracts (YCCW). Effect of treatment ($P = 0.76$), days in milk ($P < 0.001$) and interaction between treatment and days in milk ($P = 0.61$).

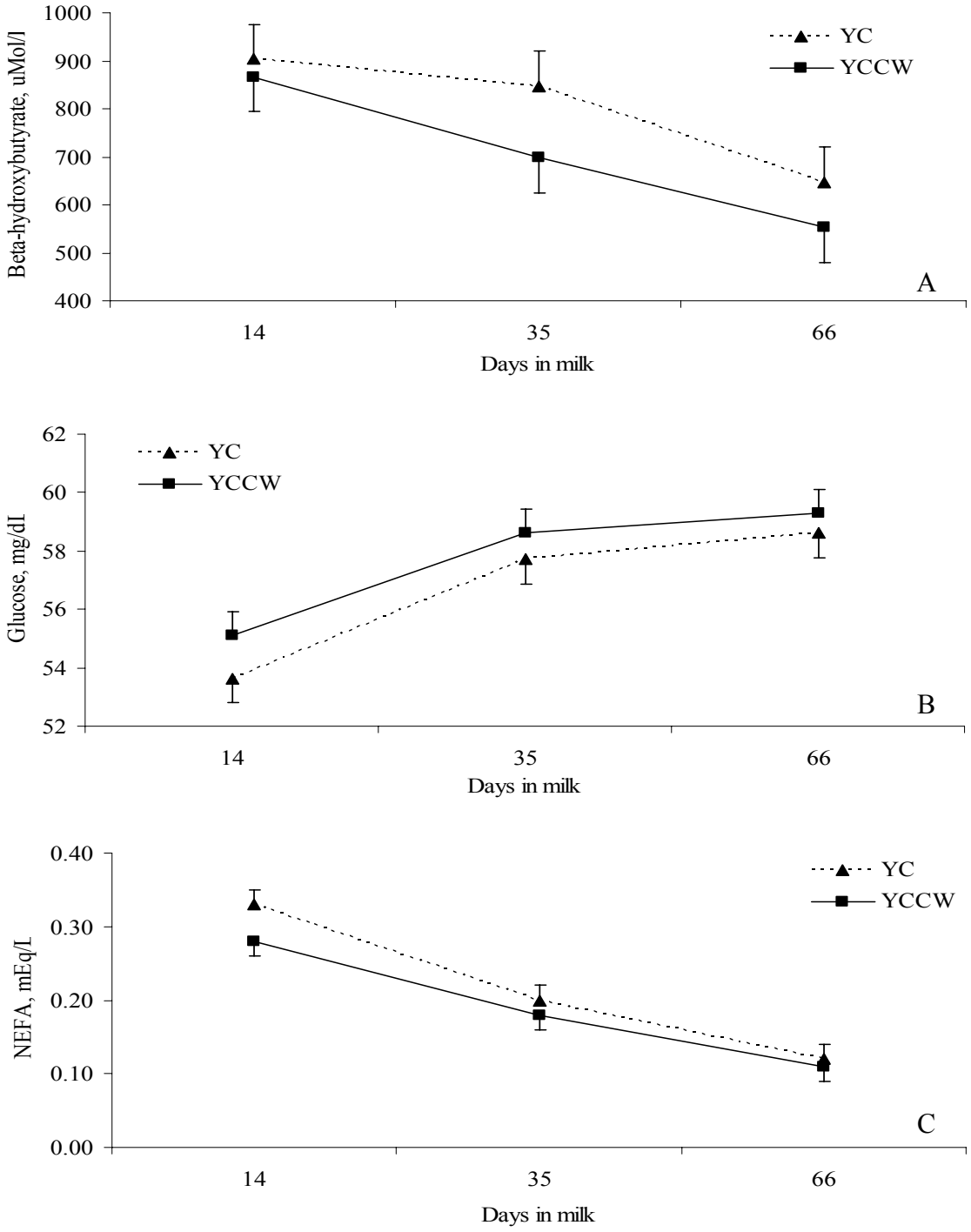

Figure 2. Plasma concentrations of beta-hydroxybutyrate (A; uMol/L), glucose (B; mg/dL) and NEFA (C; nonesterified fatty acids, mEq/L) of dairy cows fed yeast culture (YC) or yeast culture with cell wall extracts (YCCW). For plasma beta-hydroxybutyrate concentrations: treatment ($P = 0.22$), days in milk ($P < 0.001$) and interaction between treatment and days in milk ($P = 0.67$). For plasma glucose concentrations: treatment ($P = 0.25$), days in milk ($P < 0.001$) and interaction between treatment and days in milk ($P = 0.80$). For plasma NEFA concentrations: treatment ($P = 0.27$), days in milk ($P < 0.001$) and interaction between treatment and days in milk ($P = 0.50$).

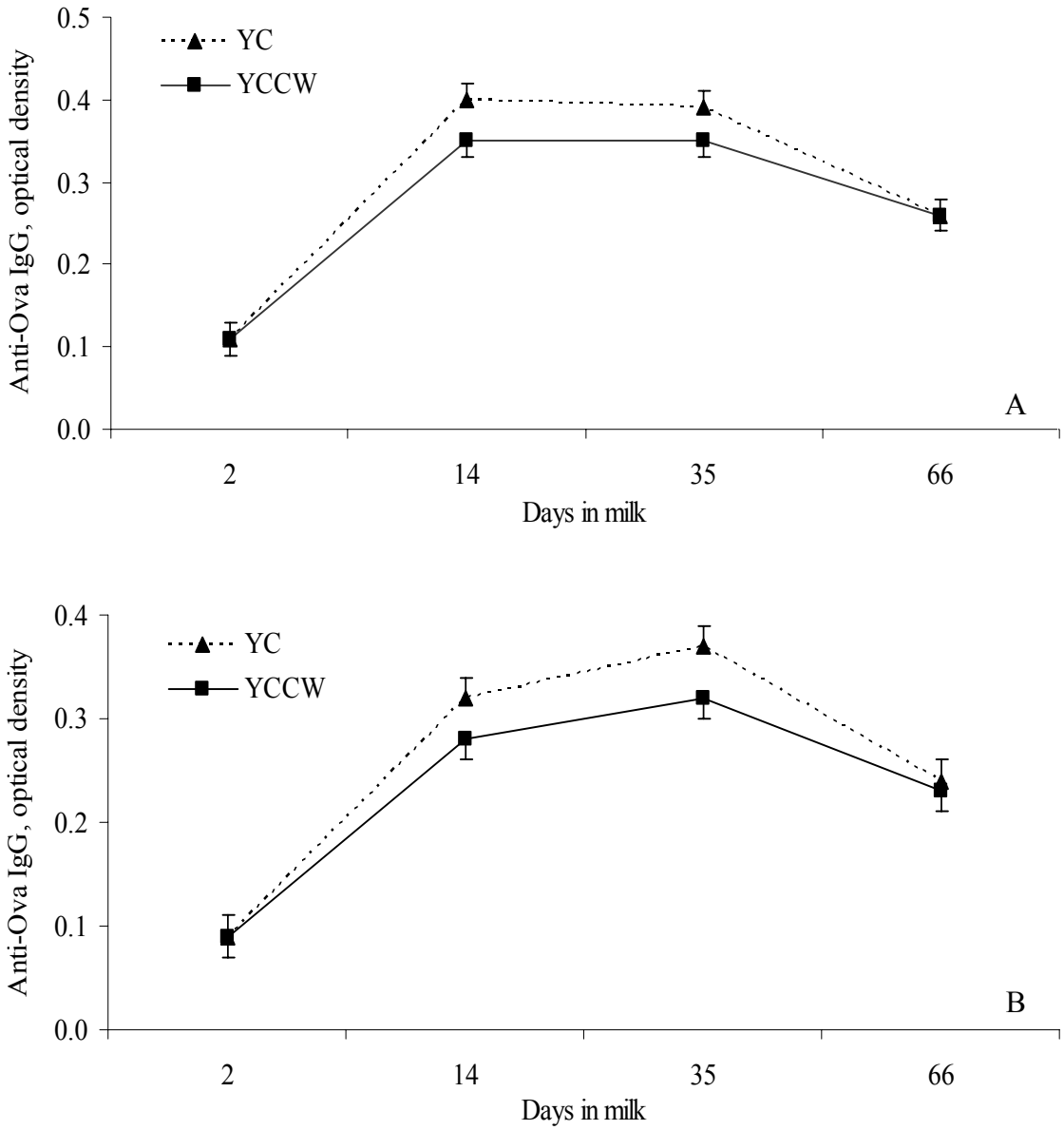

Figure 3. Anti-ovalbumin IgG titers in serum (optical density) of dairy cows fed yeast culture (YC) or yeast culture with cell wall extracts (YCCW). For serum diluted in wash buffer 1:50 (A): treatment (P = 0.23), days in milk (P < 0.001) and interaction between treatment and days in milk (P = 0.52). For serum diluted in wash buffer 1:200 (B): treatment (P = 0.17), days in milk (P < 0.001) and interaction between treatment and days in milk (P = 0.49).

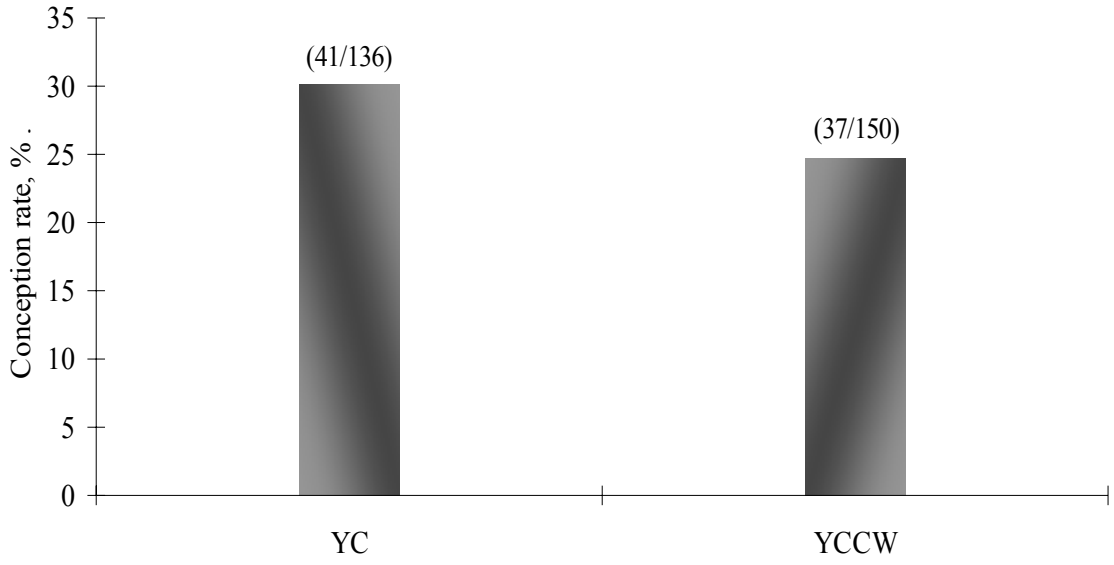

Figure 4. Conception rate at first postpartum artificial insemination of dairy cows fed yeast culture (YC) or yeast culture with cell wall extracts (YCCW). Effect of treatment (P = 0.30); Adjusted odds ratio = 1.32; 95% confidence interval = 0.78, 2.22.

IV – Effect of Feeding Yeast Culture on Performance, Health, and Immunocompetence of Dairy Calves

ABSTRACT

Objectives were to determine effects of feeding a culture of *Saccharomyces cerevisiae* on performance, health and immunocompetence of calves in the first 70 d of age. Holstein calves (n = 512), at 2 ± 1 d of age were randomly assigned to yeast culture (YC, 218 females and 37 males) or control (223 females and 34 males). Yeast culture was fed at 2% of the grain DM. All calves received colostrum during the first 24 h, pasteurized milk thereafter until 60 d of age, and grain was fed *ad libitum* for the first 70 d of age. Calves were housed in individual hutches and grain intake was measured 5 d/wk. Body weight was measured at 5, 30 and 68 d of age, and attitude and fecal consistency were scored daily. Incidence and duration of health disorders and treatments were recorded. Neutrophil phagocytic and killing activities and antibody response to immunization with ovalbumin were measured. Glucose and beta-hydroxybutyrate concentrations were measured in plasma. Grain intake did not differ between treatments and averaged 908 g/d throughout the study. Body weight change as well as glucose and beta-hydroxybutyrate concentrations did not differ between YC and control. Minor effects on neutrophil function were observed, and YC tended to increase the number of phagocytized bacteria and killing of phagocytized bacteria, but did not influence humoral immune response. Attitude scores, related to calves' behavior, were similar between treatments throughout the study. Almost all calves experienced mild diarrhea during the study, but feeding YC improved fecal scores, reduced days with watery feces, incidence of fever and diarrhea, and risk of health disorders. Because of the high incidence of diarrhea, mortality pre-weaning was also high, but YC improved survival

of calves by decreasing mortality rate past 13 d of age. Income at the end of the study was improved by $48/calf with YC. Feeding yeast culture in grain improved health, minimized frequency of health treatments, and reduced risk of morbidity and mortality in dairy calves.

Key words: calf, health, *Saccharomyces cerevisiae*, yeast culture

INTRODUCTION

Incorporation of microbial additives such as culture of *Saccharomyces cerevisiae* to the diet has become a common practice in ruminant nutrition. Various *S. cerevisiae* based products have been shown to impact DM intake, rumen pH and nutrient digestibility (Callaway and Martin, 1997; Dann et al., 2000; Kumar et al., 1997), but most studies have been conducted with lactating cows or *in vitro*. *In vitro* and *in vivo* studies have shown that yeasts and yeast cultures stimulate growth of rumen cellulolytic bacteria (Callaway and Martin, 1997), which is critical for carbohydrate digestion and rumen development in newborn calves. Some strains of *S. cerevisiae* have been shown to favor the establishment of fibrolytic bacteria in the digestive tract of gnotobiotically-reared lambs, which accelerated microbial activities in the rumen, thereby potentially favoring the transition from a liquid to a solid diet in pre-ruminant animals (Chaucheyras-Durand and Fonty, 2001). This improvement in rumen microbial activities might partially explain the improvements observed in calf growth when yeast or yeast culture was incorporated into the diet in some studies (Galvão et al., 2005; Lesmeister et al., 2004).

As the young calf matures and shifts from a liquid diet to a diet based on cereal grains and forages, the risk for diarrhea tends to decline (Davis and Drackley, 1998).

Gastrointestinal infections and subsequent diarrhea and dehydration account for the majority of health problems affecting calves during the pre-weaning period, and are the primary reason for death and poor development in the first 60 d of age (Davis and Drackley, 1998; NAHMS, 2007). In the most recent report of the National Animal Health Monitoring System (NAHMS, 2007), 7.8% of the unweaned heifers died in dairy farms in the US.

Oligosaccharides present in the cell wall of *S. cerevisiae* such as glucan and mannan (Reed and Nagodawithana, 1991) have been shown to impact the immune system and to influence host-pathogen interactions in the digestive tract of humans and animals. In laboratory animals, consumption of glucan from oats improved neutrophil function as measured by killing activity (Murphy et al., 2007), which might have ramifications to defense against pathogens. This may be particularly important in young calves, which are typically affected by a range of bacterial, viral and protozoal pathogens that cause disease of the digestive tract, some potentially leading to systemic infections. In monogastric animals, use of yeast cultures and yeast cell wall extract products is a common practice in an attempt to minimize the risk of digestive diseases (White et al., 2002). In young calves, incorporating live yeast into the grain reduced the number of days with diarrhea (Galvão et al., 2005). Feeding yeast culture to calves reduced the incidence of elevated body temperature and antibiotic treatments from birth to 46 d of age (Seymour et al., 1995). In addition, soluble products present in yeast culture have been shown to inhibit microbial growth and activity (Jensen et al., 2007a) and modulate the immune system (Jensen et al., 2007b). Collectively, these results indicate potential benefits to animal health, which are not necessarily accompanied by improvements in growth performance.

Potential improvements on performance of young calves fed yeast culture might reflect increased feed intake and improved energy status, enhanced immune function, or reduced incidence of diseases. Thus, it was hypothesized that the inclusion of culture of *S. cerevisiae* into calf grain might accelerate consumption of dry feed, improve animal health, and reduce the risk of morbidity and mortality. Objectives were to determine the effects of feeding a yeast culture of *S. cerevisiae* incorporated into the grain during the first 70 d of age on performance, health, and measures of humoral and innate immunocompetence of dairy calves.

MATERIALS AND METHODS

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

Animals, Housing and Feeding

The study was conducted in a commercial farm with approximately 5,300 milking cows located in central California. Holstein calves (441 females and 71 males), at 2 ± 1 d of age (day 1 = day of birth) were assigned to the study in 41 daily cohorts of 4 to 22 calves. Calves were housed in individual hutches located at approximately 60 cm apart and assigned in sequence of 3 per treatment.

All calves received 3 feedings of 1.9 L of frozen-thawed colostrum each in the first 24 h of life, and were fed non-saleable pasteurized whole milk (Table 1) thereafter, originated from recently calved cows or cows in the hospital pen. Milk was collected twice daily and pasteurized once using a continuous flow commercial calf milk pasteurizer (Terminator T1000, Goodnature Products Inc., Orchard Park, NY) by flash

pasteurization, in which milk temperature was elevated and held at 72 ^{o}C for 15 to 30 sec and then quickly cooled to 35 ^{o}C. Milk was fed twice daily at 07:00 and 14:00 h and calves were offered 1.9 L in bottles at each feeding during the first 55 d of age, and then once a day until 60 d of age, when calves were weaned from milk.

All calves were fed the same mixture of grains (Table 2) to meet or exceed nutrient requirements for a pre and early weaned Holstein calf to achieve adequate growth as suggested by the NRC (2001) and others (Davis and Drackley, 1998). Grain was fed once a day in the morning, immediately after milk feeding, for *ad libitum* intake during the first 70 d of age.

Treatments, Measurements of Grain DM Intake and Body Weight

Calves were randomly assigned to one of two treatments, yeast culture (**YC**, n = 255, 218 females, Diamond V XP^{TM} Yeast Culture, Diamond V Mills, Inc., Cedar Rapids, IA) or control (n = 257, 223 females). Yeast culture was incorporated daily into the grain at 2% of DM immediately prior to feeding. Grain was offered once daily to allow for 5% orts and amounts offered and refused were measured 5 d/wk for individual calves to calculate intake. Weekly averages of grain DM intake were generated based on the 5 d of grain intake measurements.

All calves were weighed the day after study enrollment, and again at 30 and 68 d of age. To facilitate activities during the study, body weight (**BW**) of calves was measured twice weekly for the respective cohort of animals according to age. Because of that, the median and mean (± SD) age of calves when BW was measured were, respectively, 5 and 5.5 ± 2.4 d for study enrollment, 30 and 29.9 ± 2.5 d for d 30, and 68 and 68.4 ± 1.2 d for d 68. Nevertheless, age of calves at each measurement of BW did not differ ($P > 0.70$) between treatments.

Grain and Milk Sampling, and Nutrient Analyses

Grain was sampled once a week, dried at 55ºC for 48 h and moisture content was recorded. Dried samples were ground to pass a 1 mm screen and samples were then composited for two-month periods and analyzed for contents of DM, OM, and ether extract (AOAC, 2000), ADF and NDF (Van Soest et al., 1991). The N content of samples was analyzed using an N analyzer (FP-528 Nitrogen Determinator, LECO Corporation, St. Joseph, MI), and CP was calculated by multiplying the N content by 6.25. Mineral content was analyzed at the Dairyland Laboratory (Arcadia, WI) using an inductively coupled plasma mass spectrometer (Thermo Jarrell-Ash, Franklin, MA).

Samples of pasteurized milk were collected weekly throughout the study and analyzed for concentrations of total solids, ash, lactose, fat and true protein (Foss 303 Milk-O-Scan®; Foss Foods, Inc.; Eden Prairie, MN) at the DHIA Laboratory in Tulare, CA. Solids not-fat were calculated by difference between total solids and fat. Composition of pasteurized milk (Table 1) was used to estimate the energy concentration in milk using NRC (2001) calculations. Although amount of milk consumed by each calf was fixed at 3.8 L/d, composition of milk varied throughout the study, which resulted in calves of different ages consuming different quantities of milk solids at different time points in the study. Therefore, weekly milk composition was used to determine total nutrient intake and ME intake of calves throughout the study.

Samples of colostrum and milk post-pasteurization were sampled in the last 2 wk of enrollment, transported to the Milk Quality Laboratory at the Veterinary Medicine Teaching and Research Center and cultured to determine number of CFU/mL and presence of *Salmonella spp.*

Attitude and Fecal Consistency Scoring

Attitude and fecal consistency were scored daily on the morning milk feeding using a 1 to 4 scale. For attitude, calves were categorized as 1 when alert and responsive, 2 when non-active, 3 when depressed, and 4 when moribund. Fecal consistency was scored as 1 when firm, 2 when soft or of moderate consistency, 3 when runny or mild diarrhea, and 4 when watery and profuse diarrhea. Weekly averages of attitude and fecal scores were generated for individual calves for statistical analyses. Calves with fecal score > 2 were used for analysis of incidence of diarrhea.

Incidence of Health Disorders, Treatments and Costs Associated with Treatments

Incidence of health disorders was recorded daily for individual calves. Rectal temperature was measured for calves displaying signs of any disease and those with temperature > 39.5 ^{o}C were considered to be febrile. Febrile calves were evaluated for diarrhea, which was characterized by presence of watery feces using fecal score > 2, and for pneumonia based on presence of respiratory distress, increased respiratory frequency and nasal discharge. Day when disease was first diagnosed was recorded and duration of each illness event was determined. Number of fever, diarrhea, and pneumonia episodes was determined. To distinguish between different episodes, an interval of 4, 4, and 10 d between diagnoses of fever, diarrhea and pneumonia, respectively, had to elapse to characterize a new event. Calves with digestive and respiratory problems were treated by farm personnel according to protocols established by the herd veterinarian. Medication used (antibiotics, anti-inflammatory and anti-diarrheic products), dosage, and duration of treatments were recorded for individual calves. Costs associated with health treatments were calculated based on currents costs for each product, daily dosage for each medication for individual calves, which was

administered based on BW of animals, estimated time spent by personnel with individual treatments, and respective personnel wages.

Calves that died after d 15 were subjected to a post-mortem examination and specimens were collected for diagnostics performed by the California Animal Health and Food Safety System laboratory in Tulare, CA. Risk of morbidity and mortality were evaluated. At 60 ± 3 d of age, calves received a dose of modified live vaccine for viral diseases including a bacterin against several serovars of *Lepstopira spp* (Vista 5 L5, Intervet Inc., Millsboro, DE).

Economic Analysis of Calf Raising

An economic analysis of cost of raising calves was performed and the analysis considered cost of grain consumed by each calf, cost of milk consumed by each calf, treatment costs for health problems, labor costs associated with feeding calves, vaccination costs, and the value of a calf that survived at the end of the study. The input values in the calculation were as follows: $0.28/kg of grain DM; $0.15/L of pasteurized milk considering $0.11/L for non-saleable milk and $0.04/L for transportation and pasteurization costs; lost opportunity cost when a calf died considering a loss of $500/heifer and $100/bull calf, which corresponded to market values for a newborn of the respective genders; and value of a live calf at 70 d of age and 80 kg of BW of $750/heifer and $300/bull calf. Labor cost was computed at actual local cost of $9.00/h, with approximately 3 min/d spent per calf for daily activities such as feeding milk and grain, replacing water, bedding the calf hutches, and other activities, but excluded labor costs associated with health treatments and preparation of grain and pasteurization of milk, which were considered in costs of grain, milk and health treatments. For YC, cost associated with treatment was considered based on market price for the product at

$0.9/kg. For calves that survived past 60 d, an additional cost for vaccination of $1.5/calf was added to account for the vaccine and labor per application.

Blood Collection

Blood was sampled by puncture of the jugular vein using evacuated tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) containing either no anticoagulant for serum separation or K2 EDTA for plasma separation. Blood tubes were placed on ice immediately after collection and later centrifuged at 2,000 x g for 15 min in a refrigerated centrifuge at 6 ºC for separation of serum and plasma. Serum and plasma were frozen at -20 ºC for later analyses.

Analyses of Total Protein in Serum and Metabolites in Plasma

Concentration of total protein was measured in serum from all calves on enrollment day using a clinical refractometer. Plasma collected from a subset of 60 female calves (30 YC and 30 control) at 30 and 60 d of age were analyzed for concentrations of glucose by direct measurement using the YSI Model 2700 SELECT Biochemistry Analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH, USA), and beta-hydroxybutyrate (**BHBA**) using a commercial kit (RANBUT, D-3-Hydroxibutyrate, Randox, Laboratories Ltd, UK) based on the enzymatic oxidation of BHBA to acetoacetate and concomitant reduction of NAD^+ to NADH (Williamson et al., 1962).

Evaluation of Humoral and Innate Immune Responses

Ovalbumin (**OVA**) solution was prepared by dissolving 0.5 mg of OVA (Type VII, Sigma Chemical Co., St. Louis, MO) in 0.5 mL of phosphate buffered saline (PBS 0.1

M, pH = 7.4) and emulsified in 0.5 mg of adjuvant Quil-A (Accurate Chemical, Westbury, NY) diluted in 0.5 mL of PBS. The same subset of 60 female calves (30/treatment) from which blood was analyzed for metabolites concentrations received an i.m. injection of 1 mL containing 0.5 mg of OVA at 3, 21 and 42 d of age. Blood was sampled immediately prior to each injection and again at 56 d of age for measurements of serum antibody concentration to OVA. Concentrations of IgG to OVA were measured by ELISA as described by Wagter et al. (2000).

Neutrophil phagocytosis and intracellular kill of two *Escherichia coli* bacteria were evaluated. The assays were performed using the blood of the same subset of 60 female calves (30/treatment) on d 25 ± 3 of age, with *E. coli* ATCC 25922 and with a field strain of pathogenic *E. coli* collected from a case of clinical mastitis in a dairy cow.

Neutrophils were harvested from 50 mL of jugular blood and bacteria were prepared as described by Hogan et al. (1992). Suspensions of neutrophils and opsonized bacteria were incubated in a 1:3 at 37 ºC and at 100 rpm for 90 min in water bath. Following incubation, 50 µL of bacteria-neutrophil samples were mixed with 25 µL of acridine orange solution and 25 µL of crystal violet solution. Wet mount slides were prepared and neutrophils were evaluated under an epifluorescent microscope. Bacteria present in the cytoplasm of neutrophils were stained in either red, when dead, or green, when live, and number of neutrophils phagocytizing bacteria, number of dead and live bacteria was counted in the first 50 neutrophils visible under the microscope.

Experimental Design and Statistical Analyses

The experimental design was a randomized complete block design. Daily, a cohort of 4 to 22 calves at 2 d of age were blocked according to gender and day of birth and, within each block, randomly assigned to treatments. A sample size calculation was

performed assuming that risk for diarrhea would be reduced with addition of yeast culture. Baseline values were 40% of the calves affected with diarrhea based on farm data. To detect a decrease in risk for diarrhea from 40% to 32%, 250 calves per treatment were required ($\alpha = 0.05$ and $\beta = 0.20$; two-tailed test).

Continuous variables were analyzed by ANOVA using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Variables with a single measurement during the study were analyzed with the fixed effects of treatment and gender of calf. For neutrophil phagocytic and killing activities, the concentrations of serum total protein were used as covariates in statistical models. For BW changes, initial BW at study enrollment was used as covariate. Variables with repeated measurements within the same calf were analyzed with the fixed effects of treatment, time of measurement (day or week), interaction between treatment and time, gender of calf, and the random effect of calf nested within treatment. Other covariates were included in specific analyses when found appropriate. The repeated statement was included and the covariance structure was chosen based on the smallest Schwartz's Bayesian criterion.

Daily fecal and attitude scores were analyzed in two ways. First, daily results were analyzed by the GENMOD procedure of SAS (SAS Inst. Inc., Cary, NC) fitting a Poisson distribution and log transformation function with repeated measures for count data. The model included the effects of treatment, day, interaction between treatment and day, and gender of calf. A second analysis was performed by averaging daily results into weekly means to normalize the data. Weekly means were then analyzed by the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with a model that included the effects of treatment, week, interaction between treatment and week, and gender of calf, with calf nested within treatment as the random error. For the latter model, the covariance structure that best fitted the data was chosen based on the smallest

Schwartz's Bayesian criterion. In addition, the proportion of days with scores either low or high scores were analyzed by the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with the effects of treatment and gender of calf.

Binomially distributed data were all analyzed by logistic regression using the LOGISTIC procedure of SAS (SAS Inst. Inc., Cary, NC). The models included the effects of treatment, gender of calf, serum total protein concentration and interaction between treatment and serum total protein concentration. Adjusted odds ratio (**AOR**) and the 95% confidence interval (**CI**) were calculated. Number of cases of health disorders per calf was analyzed by the Kruskal-Wallis nonparametric method to test equality of medians between treatments. Medians and mean rank were generated using MINITAB (Minitab Inc., State College, PA). Number of cases per 1000 calf days at risk was calculated for each calf and analyzed for each treatment. Survival time was evaluated using the product limit method of the Kaplan-Meier model by the LIFETEST procedure of SAS (SAS Inst. Inc., Cary, NC). Calves that survived the entire study period were censored at 70 d of age.

Treatment differences with $P \leq 0.05$ were considered significant and $0.05 < P \leq 0.10$ were designated as tendency.

RESULTS

Serum total protein concentration on the day of study enrollment was similar between control and YC and were 6.18 ± 0.05 and 6.14 ± 0.05 g/dL, respectively. Using serum total protein ≤ 5.2 g/dL as the cut-off for failure of passive transfer, 8.6 and 9.4% of control and YC calves, respectively, did not receive adequate transfer of colostral antibodies and proportions were similar ($P = 0.74$) between treatments.

Microbiological analyses of 12 colostrum and 12 post-pasteurized milk samples resulted in a mean (± SD) of 15,727 ± 16,416 CFU/mL, with a median of 11,600 CFU/mL and a range of 400 to 44,000 for colostrum, with 2 samples positive for *S. newport* and 1 sample positive for *S. dublin*. For post-pasteurized milk, the mean (± SD) was 467 ± 599 CFU/mL, with a median of 200 CFU/mL, and a range of 0 to 1,600, and all samples negative for *Salmonella spp*.

Grain intake was less than 40 g/d in the first 15 d of study. A marginal increase (15 g/d) in grain intake ($P = 0.05$) was observed between wk 1 and 4 for calves receiving the control compared with YC treatment, but no differences in intake were observed after 4 wk of age and averaged 1420 g/d (Table 3). Likewise, grain intake throughout the study was similar between treatments and averaged 908 g/d. Nutrient intake from grain and milk was similar between treatments, and throughout the study calves consumed an average of 252 g of protein/d and 4.65 Mcal of ME/d. Due to similar nutrient intake, BW gain was similar between treatments for wk 1 to 4, and wk 5 to 10 of age. Similarly, efficiency of grain utilization did not differ between treatments throughout the study.

Glucose and BHBA concentrations in plasma did not differ between control and YC (Table 3). No interaction ($P > 0.10$) between treatment and age of calves was observed for glucose or BHBA concentrations. BHBA concentrations increased ($P < 0.01$) with age of calves, but those of glucose remained similar throughout the study.

Treatment did not affect serum titers for anti-OVA IgG of calves (Figure 1). Concentrations of anti-OVA IgG in serum differed ($P < 0.01$) with age and sequential immunizations of calves with OVA, but no interaction was observed between treatment and age. Similar to humoral responses, treatment with YC did not affect measures of neutrophil function of calves at 25 d of age when neutrophils were incubated with *E.*

coli ATCC (Table 4). The proportion of neutrophils phagocytizing bacteria, number of phagocytized bacteria per neutrophil, proportion of phagocytized bacteria killed, and proportion of neutrophils killing bacteria were all similar between control and YC; however, when a pathogenic *E. coli* was incubated with neutrophils, the number of bacteria phagocytized and proportion of phagocytized bacteria killed tended ($P < 0.10$) to increase in neutrophils from calves fed YC compared with those fedthe control.

Incidence of diseases, particularly diarrhea was greater than initially anticipated. Almost all calves experienced at least 2 d of fecal score > 2. Attitude scores were similar between treatments throughout the study; however, calves fed YC tended ($P < 0.08$) to have smaller fecal score and experienced fewer ($P < 0.01$) days with mild or watery diarrhea (Table 5). Feeding YC tended ($P < 0.08$) to reduce the incidence of fever and diarrhea, but had no impact on the proportion of calves affected by respiratory disease (Table 6). Median number of cases of fever per calf tended ($P = 0.10$) to be reduced by feeding YC, but cases per calf day at risk was similar between treatments (Table 7). Yeast culture also reduced ($P = 0.02$) the median number of cases of diarrhea and cases per calf day at risk ($P = 0.01$). Median number of cases of health disorders and cases per calf day at risk were both reduced ($P < 0.02$) by feeding YC. Because of the reduced incidence of health disorders, the proportion of calves treated with anti-inflammatory and anti-diarrheic products was reduced ($P < 0.04$), and that of calves treated with antibiotics tended ($P < 0.06$) to be reduced for YC compared with control (Table 8). Although differences in frequency of treatments were observed, costs associated with treatment of diseases were generally not influenced by dietary treatments and averaged US $ 2.7/calf in the first 70 d of age. Calves fed YC experienced increased ($P = 0.01$) raising costs of approximately $7/calf (Table 9), which were associated with increased ($P < 0.01$) costs associated with milk, labor,

vaccination, and yeast culture. Nevertheless, net income at the end of the study was numerically greater, approximately $48, for calves fed YC because of the numerical improvement in income with calf value.

Feeding YC reduced (P = 0.05) the proportion of calves that died during the study (Table 6) and mortality rate was markedly decreased (P = 0.05) with YC after 13 d of age (Figure 2). In fact, risk of death before d 13 was similar (P = 0.88) between YC and control and the proportions affected were 6.3 and 6.6%, respectively; however, past d 13, risk of mortality was 6-fold greater (P = 0.008) for control than YC (AOR = 6.0; 95% CI = 1.6, 22.6), and the proportions affected were 1.3 and 5.8% for YC and control, respectively.

Of the calves subjected to postmortem diagnosis, one was diagnosed with *Cryptosporidium parvum* and death was caused by dehydration and transmural necrosis of the small intestine; 4 calves died because of septicemia caused by *S. dublin*; 2 calves died because of septicemia by *E. coli*; and 2 died because of bronchopneumonia caused by mixed bacteria and enteritis associated with *S. newport*. No clear differences in cause of death were observed between treatments.

DISCUSSION

Alternatives to non-antimicrobial feed additives that enhance health and performance of young calves are continuously being evaluated as methods to minimize the need for antimicrobial additives (Heinrichs et al., 2003). Yeast culture contains yeast cells and compounds of the fermentation activity such as B vitamins, polyphenols, and organic acids, and both might be responsible for the positive effects on performance and health when incorporated into the diet of animals. Components of the yeast cell wall

such as the oligosaccharides glucan and mannan might benefit the local and systemic immune responses (Murphy et al., 2007; Newman, 1994). Furthermore, metabolites produced by *S. cerevisiae* in culture might have antimicrobial activities against pathogens (Jensen et al., 2007a) and modulatory effects on the immune system (Jensen et al., 2007b). Nevertheless, data on benefits to feeding yeast and yeast culture on calf health are scarce and not conclusive (Cole et al., 1992; Galvão et al., 2005; Seymour et al., 1995).

Response to feeding yeast products on performance of young calves has been variable. Lesmeister et al. (2004) reported that addition of 1% of yeast culture to the grain of young calves did not affect performance, but when included at 2%, yeast culture improved DM intake, BW gain and feed efficiency compared with a diet without yeast culture. In agreement, Galvão et al. (2005) also observed improvements in grain intake, BW gain, and blood parameters of calves when fed live yeast incorporated into the grain during the pre-weaning, but not during the post-weaning period. Quigley et al. (1992) observed no differences in intake, BW gain or efficiency of gain in calves fed yeast culture for 12 wk, and suggested that the high incidence of health problems and low grain intake might have masked response to treatments (Quigley et al., 1992). On the other hand, addition of yeast culture to the grain increased rumen pH and rumen VFA concentration (Quigley et al., 1992; Kumar et al., 1997), which might benefit rumen health and development.

Results from the current study indicate YC did not increase grain intake and, as a result, the BW change did not differ between treatments. The smaller grain intake in the first month of age for YC calves is unlikely to be of any importance as the difference was negligible, approximately 16 g/d (11% of YC intake), and it did not influence the overall energy and protein consumption by the calves. Another report (Seymour et al.,

1995) also observed a decrease in DM intake in the first weeks for calves fed brewer's yeast, with no influence on the overall DM intake, growth rate and feed efficiency. Collectively, these studies suggest that, with few exceptions (Lesmeister et al., 2004), addition of yeast products to the grain generally does not influence intake and BW gain of calves in the first 70 d of age.

In the first weeks after birth, in systems in which milk is fed at restricted amounts, calves usually maintain or gain little BW (Davis and Drackley, 1998; Galvão et al., 2005). Because most of the nutrient intake in the first 30 d of the current study was supported by milk consumption, and less by grain intake, calculated efficiency of grain conversion into BW was high. After 30 d, the increased DM intake of grain, and the relatively smaller contribution of nutrients from milk, resulted in efficiency of grain conversion into BW similar to observed by others (Galvão et al., 2005; Lesmeister et al., 2004). The lack of effects of YC on feed efficiency is similar to results observed by others (Galvão et al., 2005; Lesmeister et al., 2004; Quigley et al., 1992).

Glucose concentration in plasma was not affected by treatment, possibly because of a lack of effect on overall nutrient intake. Concentrations of glucose in plasma are influenced by energy consumption resulting in greater glucose absorption, but also by utilization by tissues. In young calves, increased glucose availability is expected to result in improved BW gain. Because YC and control calves experienced similar intake of milk and grain ME and protein, and also experienced similar BW changes, it is unlikely that glucose availability was altered by treatment, which was reflected by the similar plasma concentrations. When grain intake increased with feeding yeast, so did concentrations of glucose in plasma (Galvão et al., 2005). On the other hand, feeding yeast culture to Jersey calves in the first 12 wk of age did not influence intake, BW gain, and glucose concentrations (Quigley et al., 1992).

Glucose is the main source of energy for calves before ruminal development, but as intake of dry feeds increases and the rumen starts development, the contribution of VFA to the energy needs of calves also increases and it results in elevated concentrations of BHBA in plasma (Galvão et al., 2005; Quigley et al., 1991). Quigley et al. (1991) suggested that the increased concentrations in plasma of BHBA resulted from the increased ketogenesis in the rumen, which is influenced by the rapid increase in intake of solid feeds after weaning. In young calves, concentrations of BHBA in plasma shifted abruptly by 4 h after feeding (Quigley et al., 1992), supporting the concept that ruminal production of BHBA is the main source of ketones in calves with adequate energy intake. Similar to the findings of others (Galvão et al., 2005; Lesmeister et al., 2004; Quigley et al., 1991), plasma BHBA increased with age of calves, which paralleled the increased grain intake; however, because no difference in grain intake was observed between treatments, feeding YC did not affect BHBA concentrations. Furthermore, because feeding different concentrations of yeast culture resulted in marginal nonsignificant effects on measures of structural rumen development of calves (Lesmeister et al., 2004), it is unlikely that addition of yeast culture to the grain promotes changes in functional rumen development.

Yeast culture contains yeast cells and soluble products with potential antimicrobial effects. Incubation of *E. coli*, *Staphylococcus aureus*, *Candida tropicalis*, or healthy oral microbial flora with soluble metabolites of yeast culture extract suppressed the growth of *C. tropicalis* and *E. coli*, but not of *S. aureus* or oral flora obtained from healthy human saliva (Jensen et al., 2007a). The same authors observed that these soluble products not only inhibited the growth of *E. coli*, but also suppressed the activity of the bacteria. Furthermore, metabolites present in the yeast culture have been shown to modulate immune response *in vitro*, with reduction in inflammatory response

and oxidative stress (Jensen et al., 2007b). This can be important in diseases in which inflammatory response exacerbates the deleterious effects of the illness such as in chronic processes or in infections associated with gut pathogens like enterotoxigenic *E. coli*. These effects are expected to improve gut health and might explain the benefits to fecal scores and diarrhea observed in the current study when calves were fed YC.

Yeast cell wall also contains approximately 35% mannan and 30% glucan (Reed and Nagodawithana, 1991), which are normally not digested or absorbed in the small intestine (Newman, 1994), and their presence in the gut might enhance immune response and prevent colonization by pathogens. Mannan and glucan may bind to receptors on a variety of defense cells of the gut, which activates immune defenses such as phagocytosis (Murphy et al., 2007). Moreover, mannan present in the cell wall of yeasts might block bacterial attachment to the intestinal epithelium (Newman, 1994), which might explain the similar efficacy of mannan to improve fecal scores in calves compared with antibiotics (Heinrichs et al., 2003).

Although immune response of the gut was not evaluated in the current study, measures of innate and humoral immune responses were generally not influenced by feeding YC. Serum titers for OVA were similar between treatments. Likewise, measures of phagocytosis and intracellular killing activity of neutrophils were unaffected when cells were incubated with a non-pathogenic strain of *E. coli*. On the other hand, when neutrophils were incubated with a pathogenic strain of *E. coli*, the mean number of phagocytized bacteria and the proportion of phagocytized bacteria killed tended to increase for YC compared with control. It is possible that under a greater challenge, such as when a pathogenic strain of *E. coli* was used to evaluate neutrophil function, feeding YC improved some of the responses. Nevertheless, these data suggest that feeding YC had minor effects on the measures of immune response

evaluated, and only when neutrophils were incubated with pathogenic bacteria, differences were observed.

In spite of the small differences in immunocompetence of calves, fecal score and susceptibility to digestive disease were markedly reduced by feeding YC. It is important to indicate that in the current study the population of calves suffered from more diarrhea than initially expected, with 98% of the calves experiencing fecal score > 2 for at least 2 consecutive days. Because colostrum contained high CFU counts and *Salmonella spp* was present in some samples, it is possible that the excessive bacterial load present in colostrum favored the high incidence of gastrointestinal diseases observed in the current study. Although incidence of diarrhea was high, less than 15% of all the fecal scores were > 2 and diarrhea affected 3.1% of the calf days at risk in the pre-weaning period. Under those circumstances, feeding YC reduced the risk of fever and diarrhea suggesting protective effects of YC in calves subjected to high risk of morbidity. Differences in risk of enteric diseases are important as diarrhea associated with dehydration is the major cause of death of young calves in the first weeks of life (Davis and Drackley, 1998; NAHMS, 2007). In fact, calves fed YC not only experienced reduced risk for gastrointestinal diseases, but they also experienced reduced risk of morbidity and lesser mortality. The reduced mortality rate was observed after 13 d of age (Figure 2), which might be related to increased grain intake with age and, therefore, intake of yeast culture.

Diarrhea in calves can be caused by pathogenic bacteria that attach and may or may not invade the host's intestinal cells. Of the calves subjected to postmortem diagnosis, *Salmonella spp* and *E. coli* were the predominant pathogens. It is possible that feeding YC might have decreased the risk of diarrhea by reducing the attachment and invasion of intestinal cells by these pathogens as they might bind to

oligosaccharides present in the yeast cell wall (Newman, 1994; Pérez-Sotelo et al., 2005; White et al. 2002), minimizing the growth of enteric pathogens by the metabolites present in yeast culture (Jensen et al., 2007a), or reducing inflammatory response in the gut because of the metabolites of yeast culture (Jensen et al., 2007b). *In vitro*, the majority of *Salmonella spp* isolates adhered to the cell wall of *S. cerevisiae* (Pérez-Sotelo et al., 2005), which might prevent attachment and invasion of intestinal cells. In fact, Rodrigues et al. (1996) observed a protective effect of *S. cerevisiae* against *S. typhimurium* and *Shiguella flexneri* in mice.

Although data on the effects of yeast culture and yeast cell wall components on risk of enteric diseases is more common in monogastrics (Newman, 1994; White et al., 2002), others have also observed a decrease in days with diarrhea in calves fed yeast (Galvão et al., 2005) and improved fecal scores with feeding mannan-oligosaccharides (Heinrichs et al., 2003). Similarly, feeding brewer's yeast reduced the incidence of fever and frequency of antibiotic treatments during the pre-weaning period in calves (Seymour et al., 1995). In a series of studies with stressed beef calves, Cole et al. (1992) demonstrated that feeding yeast culture reduced duration of disease and improved intake in morbid calves. Adding mannan-oligosaccharides to milk replacer fed to calves improved fecal sores in a similar manner to feeding antibiotics in milk (Heinrichs et al., 2003). Because of the positive effects of yeast culture on enteric health, morbidity and mortality were reduced with feeding YC, in spite of the similar risk of respiratory diseases. Frequency of health treatments were also reduced with YC. These data suggest that feeding YC might improve overall gut health of calves in spite similar measures of systemic immunity.

Costs associated with treatments were generally lesser for calves fed YC, although raising costs increased for YC compared with control. The increased raising costs of

approximately $7/calf for those fed YC was caused by increased feeding and labor costs as a result of improved survival of calves. As more calves fed YC survived, total consumption of milk, number of doses of vaccine, and labor needed to feed and care for calves also increased. In spite of the increased raising costs, feeding YC resulted in a numerical improvement in net income at the end of the study of approximately $48/calf, or 14.6% over the net income of calves fed control. Results of the present study indicate that incorporation of yeast culture at 2% of the grain DM has the potential to improve health of calves by reducing risk of morbidity and, ultimately, mortality and also to minimize the frequency of health treatments in the first 70 d of life.

CONCLUSIONS

Incorporation of yeast culture at 2% of the grain fed to dairy calves from 2 to 70 d of age did not alter DM, protein and ME intake, feed efficiency and BW gain. Measure of humoral immune response was not influenced by dietary treatment, but some improvements in neutrophil function were observed with supplemental yeast culture when cells were incubated with pathogenic *E. coli*. Although reduction of costs for health treatments were not significant, calves fed yeast culture had decreased frequency of medical treatments because of reduced incidence of fever and diarrhea and reduced overall morbidity. The improvement in health of calves was consequent to reduction in enteric diseases and culminated with reduced mortality, particularly after 13 d of age, which probably reflected the increased intake of grain and yeast culture after 2 wk of age. Improvements in survival of calves resulted in numerical improvement in net income of $48/calf fed yeast culture at the end of the study. Under the conditions of the current study, in which incidence of diarrhea was high, these data support the concept

that yeast culture improves health of the digestive tract of young calves and reduce morbidity and mortality. Further studies are needed to determine the exact components of yeast culture and respective mechanisms that elicit these positive effects on animal health.

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Table 1. Nutrient composition (mean ± SD) of pasteurized milk fed to calves[1]

Nutrient composition	As is	DM basis
DM, %	10.89 ± 3.01	----
Fat, %	3.37 ± 0.98	32.35 ± 8.02
True protein, %	2.67 ± 0.57	25.67 ± 3.98
Lactose, %	3.77 ± 1.23	35.18 ± 5.98
Solids not fat, %	7.16 ± 1.86	67.65 ± 8.02
Ash, %	0.73 ± 0.22	6.80 ± 0.96
ME,[2] Mcal/kg	0.60	5.51

[1] Average of 16 samples collected weekly throughout the study.
[2] ME = metabolizable energy calculated according to NRC (2001) based on the composition of milk using average values for DM, fat, and true protein.

Table 2. Ingredient composition of the calf grain and nutrient content of grain and yeast culture

Ingredient composition	---------- DM basis ----------	
Steam-rolled corn, %	30.0	
Steam-rolled barley, %	18.5	
Dried beet pulp, shreds %	20.0	
Pellet[1], %	25.0	
Cane molasses, %	6.5	
Nutrient composition	(mean ± SD)	
	Calf grain[2]	Yeast culture
DM, %	84.84 ± 0.54	92.69
	---------- DM basis ----------	
ME,[3] Mcal/kg	3.10	---
OM, %	92.65 ± 0.07	94.38
CP, %	20.90 ± 0.16	15.69
NDF, %	18.39 ± 1.05	23.99
ADF, %	10.03 ± 0.67	5.10
ADF insoluble CP, %	3.16 ± 1.48	---
Lignin, %	0.56 ± 0.13	---
Nonfiber carbohydrates,[3] %	52.15 ± 3.05	47.61
Fat, %	4.38 ± 0.29	7.15
Ca, %	1.25 ± 0.01	0.25
P, %	0.74 ± 0.01	0.80
K, %	1.14 ± 0.01	0.29
Mg, %	0.41 ± 0.01	0.38
Na, %	0.16 ± 0.01	0.23
Cl, %	0.31 ± 0.01	0.20
S, %	0.37 ± 0.01	0.44
Zn, mg/kg	153.0 ± 9.9	68.0
Cu, mg/kg	18.5 ± 3.5	8.0
Mn, mg/kg	130.0 ± 4.2	35.0
Monensin, mg/kg	30	---

[1] Pellet contained: 18.20% Pro-Lak (blend of marine and animal by-products; H. J. Baker & Bro., Inc., Stamford, CT), 65.00% solvent extract soybean meal, 9.10% corn distiller's grains, 1.80% calcium carbonate, 4.10% dicalcium phosphate, 0.55% sodium chloride, 0.55% magnesium oxide, 0.09% Zinpro 4-Plex (Zinpro Corporation, Eden Prairie, MN), 0.018% zinc sulfate, 0.01% manganese sulfate, 0.0003% sodium selenite, 0.40% of a mixture of iodine and vitamins A, D and E, and 0.07% Rumensin 80 (176 mg/kg of monensin; Elanco Animal Health, Indianapolis, IN).

[2] Average of 3 composited samples collected weekly.

[3] ME = metabolizable energy according to NRC (2001) for a young calf consuming 1.0 kg of DM.

[3] Calculated according to the following formula: OM – (CP + NDF + Fat – NDF insoluble CP).

Table 3. Least square means for the effect of feeding a yeast culture (YC) in grain on DM and nutrient intakes, body weight changes, and plasma concentrations of metabolites in dairy calves

	Treatment[1]			
	Control	YC	SEM	*P* value
Grain intake, g/d				
Week 1 to 4	153.5	139.1	6.6	0.05
Week 5 to 10	1428.7	1409.6	34.1	0.59
Week 1 to 10	916.5	900.1	23.8	0.54
Grain nutrient intake				
Protein, g/d	157.5	154.4	3.3	0.41
ME, Mcal/d	2.54	2.50	0.05	0.40
Milk nutrient intake				
Total solids, g/d	448.6	448.2	1.4	0.78
Protein, g/d	106.1	106.1	0.3	0.94
ME, Mcal/d	2.37	2.36	0.01	0.86
Total nutrient intake				
Protein, g/d	253.2	250.0	3.4	0.41
ME, Mcal/d	4.67	4.62	0.05	0.39
Body weight change, g/d				
Day 5 to 30	229	212	11	0.15
Day 30 to 68	780	772	16	0.67
Final body weight at d 68, kg	76.4	76.7	0.5	0.39
Efficiency of grain utilization[2]				
Week 1 to 4	1.50	1.22	0.21	0.22
Week 5 to 10	0.56	0.57	0.01	0.28
Glucose, mg/dL	75.9	76.1	1.5	0.92
Beta-hydroxybutyrate, µMol/L	344.7	342.5	15.7	0.92

[1] Addition of 0 (control) or 2% yeast culture (YC) to grain.
[2] Body weight change (kg/d)/grain intake (kg/d).

Table 4. Least square means and medians for the effect of feeding a yeast culture (YC) in grain on neutrophil function of dairy calves

	Treatment[1]			
	Control	YC	SEM	*P* value
Escherichia coli ATCC				
Neutrophils phagocytizing,[2] %	53.6	54.0	2.5	0.96
Intracellular bacteria/neutrophil,[3] n				
Mean	4.5	4.5	0.3	0.98
Median	4.6	4.7	---	0.78
Phagocytized bacteria killed,[4] %	92.9	93.8	1.4	0.67
Bacteria phagocytized, n				
Mean	124.2	134.3	8.6	0.68
Median	108	122	---	0.92
Neutrophils killing bacteria,[5] %				
Neutrophils phagocytizing	98.9	99.6	0.5	0.37
All neutrophils	52.9	53.7	2.4	0.94
E. coli (mastitis agent)				
Neutrophils phagocytizing,[2] %	17.4	20.6	2.8	0.39
Intracellular bacteria/neutrophil,[3] n				
Mean	1.4	1.5	0.1	0.35
Median	1.0	1.3	---	0.26
Phagocytized bacteria killed,[4] %	96.4	99.8	1.4	0.10
Bacteria phagocytized, n				
Mean	11.8	17.2	2.2	0.08
Median	8.0	10.0	---	0.23
Neutrophils killing bacteria,[5] %				
Neutrophils phagocytizing	99.6	97.8	1.1	0.26
All neutrophils	17.3	20.2	2.6	0.21

[1] Addition of 0 (control) or 2% yeast culture (YC) to grain.
[2] Percentage of neutrophils containing at least one intracellular bacterium (live or dead).
[3] Number of bacteria phagocytized / number of neutrophils phagocytizing at least one bacterium.
[4] Number of dead, phagocytized bacteria / (number of live + dead phagocytized bacteria) x 100.
[5] Number of neutrophils containing at least one dead bacterium divided either by the number of neutrophils phagocytizing at least one bacterium or by the total number of neutrophils.

Table 5. Least square means and medians for the effect of feeding a yeast culture (YC) in grain on attitude and fecal scores of dairy calves

	Treatment[1]			
	Control	YC	SEM	*P* value
Attitude score[2] (1 to 4)				
Mean	1.06	1.05	0.01	0.76
Median	1.0	1.0	---	0.37
Proportion of days = 1	94.9	95.6	0.6	0.37
Fecal score[3] (1 to 4)				
Mean	1.47	1.44	0.01	0.06
Median	1.3	1.2	---	0.08
Proportion of days > 2	15.9	13.5	0.8	0.006
Proportion of days = 4	5.9	4.6	0.4	0.004

[1] Addition of 0 (control) or 2% yeast culture (YC) to grain.
[2] Score 1 = alert and responsive, score 2 = non-active, score 3 = depressed, and score 4 = moribund.
[3] Score 1 = firm, score 2 = soft or of moderate consistency, score 3 = runny or mild diarrhea, and score 4 = watery and profuse diarrhea.

Table 6. Effect of feeding a yeast culture (YC) in grain on incidence of health disorders and mortality in dairy calves

	Treatment[1]				
	Control	YC	AOR[2]	95% CI[3]	*P* value
	% (no./no.)				
Fever[4]	41.6 (107/257)	34.1 (87/255)	1.38	0.96 – 1.97	0.08
Diarrhea[5]	99.6 (256/257)	97.3 (248/255)	7.22	0.88 – 59.58	0.07
Respiratory disease[6]	14.0 (36/257)	13.7 (35/255)	1.03	0.62 – 1.69	0.92
Mortality	12.1 (31/257)	7.5 (19/255)	1.79	0.98 – 3.32	0.05

[1] Addition of 0 (control) or 2% yeast culture (YC) to grain.
[2] AOR = adjusted odds ratio (YC served as referent for comparison).
[3] CI = confidence interval.
[4] Rectal temperature > 39.5 ^{o}C.
[5] Presence of watery feces for at least 2 d during the study.
[6] Calves with signs of respiratory distress and fever.

Table 7. Effect of feeding a yeast culture (YC) in grain on risk of health disorders

	Treatment[1]		
	Control	YC	*P* value
Fever[2]			
Median number of cases (mean rank)[3]	0 (265.5)	0 (247.4)	0.10
Cases/1000 calf days at risk[4] (LSM ± SEM)	13.0 ± 1.8	11.2 ± 1.8	0.40
Diarrhea[5]			
Median number of cases (mean rank)	1.0 (268.4)	1.0 (244.5)	0.02
Cases/1000 calf days at risk (LSM ± SEM)	34.5 ± 2.4	27.6 ±2.4	0.01
Respiratory disease[6]			
Median number of cases (mean rank)	0 (256.6)	0 (256.4)	0.98
Cases/1000 calf days at risk (LSM ± SEM)	2.5 ± 0.5	2.8 ± 0.5	0.57
Any health disorder[7]			
Median number of cases (mean rank)	2.0 (270.7)	1.0 (242.2)	0.02
Cases/1000 calf days at risk (LSM ± SEM)	48.6 ± 3.6	39.9 ± 3.6	0.03

[1] Addition of 0 (control) or 2% yeast culture (YC) to grain.
[2] Rectal temperature > 39.5 ^{o}C. A new case was characterized when 4 d had elapsed between episodes.
[3] Mean rank generated by the Kruskal-Wallis nonparametric test to evaluate differences between median values.
[4] Calculated as (number of cases per calf/number of calf days at risk) x 1000 d.
[5] Presence of watery feces at least 2 d during the study. A new case was characterized when at least 4 d had elapsed between episodes.
[6] Calves with fever and increased respiratory frequency. A new case was characterized when at least 10 d had elapsed between episodes.
[7] Includes fever, diarrhea, pneumonia, bloat and keratoconjunctivitis.

Table 8. Effect of feeding a yeast culture (YC) in grain on frequency and costs associated with health treatments

	Treatment[1]		
	Control	YC	*P* value
Anti-inflammatory			
Calves treated, % (no./no.)	26.1 (67/257)	16.5 (42/255)	0.008
Treatment days/calf in study			
Mean (± SEM)	0.39 ± 0.05	0.28 ± 0.05	0.07
Median (mean rank)[2]	0 (268.4)	0 (244.5)	0.01
Cost, US $/calf (mean ± SEM)	0.13 ± 0.02	0.10 ± 0.02	0.09
Anti-diarrheic			
Calves treated, % (no./no.)	96.9 (249/257)	92.9 (237/255)	0.04
Treatment days/calf in study			
Mean (± SEM)	3.05 ± 0.13	2.99 ± 0.13	0.66
Median (mean rank)	3 (260.7)	3 (252.3)	0.51
Cost, US $/calf (mean ± SEM)	0.93 ± 0.04	0.91 ± 0.04	0.67
Injectable antibiotics			
Calves treated, % (no./no.)	61.1 (157/257)	52.9 (135/255)	0.06
Treatment days/calf in study			
Mean (± SEM)	1.64 ± 0.13	1.51 ± 0.13	0.40
Median (mean rank)	1 (269.6)	1 (243.3)	0.03
Cost, US $/calf (mean ± SEM)	1.70 ± 0.15	1.63 ± 0.15	0.69
Total treatment cost/calf, US $	2.77 ± 0.17	2.64 ± 0.17	0.51

[1] Addition of 0 (control) or 2% yeast culture (YC) to grain.
[2] Mean rank of the data generated by the Kruskal-Wallis nonparametric test to evaluate differences between medians.

Table 9. Least square means for the effect of feeding a yeast culture (YC) in grain on raising costs and income

	Treatment[1]			
	Control	YC	SEM	*P* value
Raising costs,[2] $/calf				
Pasteurized milk	28.5	31.1	0.7	0.01
Grain	14.4	15.6	0.6	0.19
YC	0	1.0	0.02	<0.001
Labor for feeding	26.2	28.5	0.7	0.01
Vaccination	1.18	1.32	0.04	0.01
Health treatment	2.77	2.64	0.17	0.51
Total raising costs, $/calf	71.8	78.9	1.9	0.01
Income with calf value,[3] $/calf	402.9	458.2	26.9	0.14
Net income, $/calf	331.1	379.4	25.0	0.17

[1] Addition of 0 (control) or 2% yeast culture (YC) to grain.
[2] Costs associated with each of items listed.
[3] A dead calf represented a lost opportunity of $500/heifer and $100/bull, which corresponded to the market price of newborns. A live calf at 70 d of age represented a net gain of $750/heifer and $300/bull.

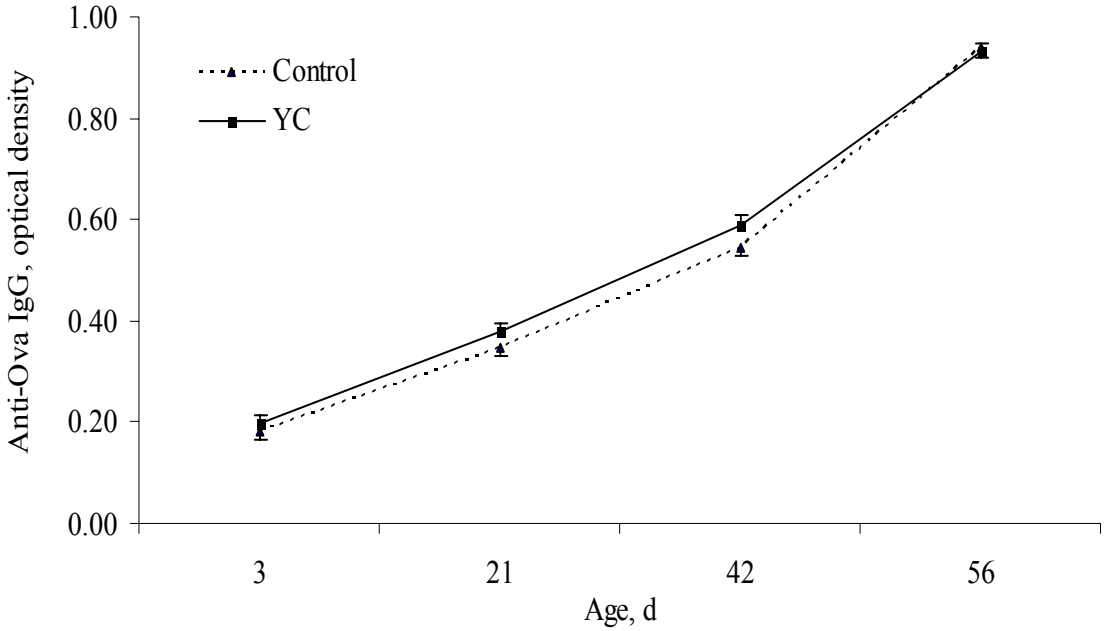

Figure 1. Effect of feeding yeast culture (YC) in grain on IgG anti-ovalbumin serum titers in response to ovalbumin immunization in calves. The LSM ± SEM for the entire study period were 0.50 ± 0.01 and 0.53 ± 0.01 for control and YC, respectively. Effect of treatment (P = 0.13), age (P < 0.001) and interaction between treatment and age (P = 0.29).

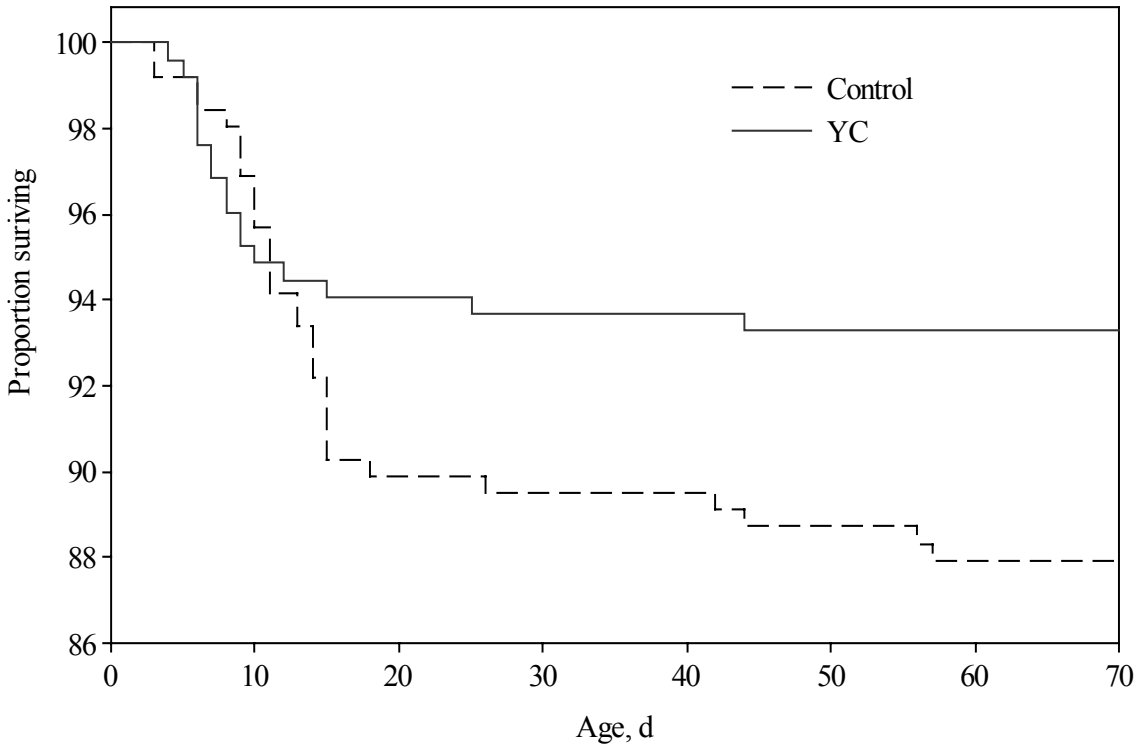

Figure 2. Effect of feeding yeast culture (YC) in grain on survival of calves during the first 70 d of age. Calves that survived were censored at 70 d of age. For control and YC, the mean ± SEM days to death were 63.6 ± 1.1 and 66.0 ± 1.0, respectively. Effect of treatment (P = 0.05).

V - CONSIDERAÇÕES FINAIS

O fornecimento de cultura de *Saccharomyces cerevisiae* mais 5 g de extratos de parede celular de levedura adicionada sobre a ração total misturada de vacas leiteiras em início de lactação não teve influência sobre o escore de condição corporal, os metabólitos do sangue, a resposta imune humoral e a taxa de concepção à primeira inseminação.

Dentro das condições do presente experimento, a incorporação de extratos de parede celular à cultura de levedura como suplemento da dieta de vacas em início de lactação não se apresentou vantajosa, uma vez que estes animais tiveram aumento das desordens metabólicas e menor desempenho produtivo.

Em função de poucos dados disponíveis na literatura sobre a ação de extratos de parede celular associados à cultura de levedura com relação ao sistema imune e saúde de ruminantes, mais trabalhos devem ser desenvolvidos, principalmente iniciando o fornecimento no pré-parto e o mantendo até o pico de lactação, a fim de amenizar o efeito do estresse pós-parto e de auxiliar num melhor desempenho animal.

Outros estudos são necessários também para determinar níveis de suplementação com extratos de parede celular de levedura, associados ou não à cultura de levedura, sobre o desempenho e imunidade dos animais.

A incorporação de cultura de levedura a 2% da matéria seca da ração inicial fornecida a bezerros leiteiros de 2 a 70 dias de idade não alterou a ingestão de matéria seca, proteína e energia metabolizável, nem a eficiência alimentar e o ganho de peso.

As medidas de resposta imune humoral não foram influenciadas pelo tratamento com cultura de levedura, mas melhoria nas funções dos neutrófilos foi observada com esta suplementação quando as células foram incubadas com a *E. coli* patogênica.

Embora a redução dos custos por tratamentos de doenças não tenha sido significante, os bezerros alimentados com cultura de levedura tiveram diminuição na frequência destes tratamentos, em virtude da diminuição da incidência de febre e diarréia, bem como redução geral da morbidade.

A melhora na saúde dos bezerros foi conseqüência da redução das doenças entéricas e culminou com a redução da mortalidade, principalmente depois dos 13 dias de idade, o que provavelmente refletiu no aumento da ingestão de ração inicial e cultura de levedura após a segunda semana de idade.

Melhoras na sobrevivência dos bezerros resultaram em melhora na renda líquida, que foi de $48/bezerro suplementado com cultura de levedura no final do estudo.

Nas condições do presente estudo, no qual a incidência de diarréia foi alta, os resultados suportam o conceito de que a cultura de levedura melhora a saúde do trato digestivo de bezerros jovens e ainda reduz a morbidade e a mortalidade.

Mais estudos são necessários para determinar os componentes exatos da cultura de levedura e os respectivos mecanismos que promovem estes efeitos positivos na saúde de bezerros jovens.