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Original article

Effect of feeding live yeast products to calves with failure of passive transfer on performance and patterns of antibiotic resistance in fecal *Escherichia coli*

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Abstract – Fifty-two newborn Holstein calves with serum IgG concentrations less than 0.73 g·dL^{-1} were randomly assigned to one of four treatments: no added live yeast (control), 0.5 g of live yeast added to the grain for 84 d (SC; *Saccharomyces cerevisiae*), 0.5 g of live yeast added to the milk for 42 d (SB; *S. cerevisiae,* spp. *boulardii*), and 0.5 g of live yeast added to the grain for 84 d and to the milk for 42 d (SCSB). Calves were offered 440 g of milk replacer DM for the first 42 d and grain for ad libitum intake throughout the study. Plasma was analyzed weekly for concentrations of glucose and β-hydroxybutyrate. *Escherichia coli* isolated from fecal samples collected every 2 weeks were used for determination of antibiotic resistance patterns. Calves receiving SC consumed more grain DM, had increased weight gain prior to weaning, and increased plasma glucose concentrations compared to controls. Days with diarrhea were reduced by feeding live yeast to calves. Antibiotic resistance in fecal *E. coli* was associated with the age of calves with highest levels of resistance observed in the 3 d calves. While calves receiving SCSB had higher levels of antibiotic resistance than controls, this effect was not associated with any of the other treatments. Improvements in performance of calves with failure of passive transfer were observed when live yeast was added only to the grain.

***Saccharomyces cerevisia* / yeast / failure of passive transfer**

1. INTRODUCTION

During the last decades, microbial additives (probiotics) such as yeasts (*Saccharomyces cerevisiae*), or fungi (*Aspergilus oryzae*) have been widely used in ruminant nutrition to improve growth, lactation, and health because of their effects on dry matter (DM) intake, rumen pH, and nutrient digestibility [1]. However, few studies have evaluated the effects of feeding yeast products to the diet of young calves.

Passive immunity in calves is provided when maternal colostrum antibodies are fed within the first few hours of life to assure adequate absorption, which results in

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increased concentrations of serum IgG and total protein [2]. Inadequate supply of good quality colostrum to newborns puts them at greater risk for diseases, especially bacterial infections of the digestive tract [3]. Immune-suppressed calves or those at increased risk for infections are often fed sub-therapeutic doses of antimicrobials in the diet to minimize prevalence of infections early in life. However, addition of penicillin to milk fed to calves increased resistance to antibiotics by gut bacteria [4], and sub-therapeutic use of antibiotics in food animals can potentially increase the risks for human infections caused by antibiotic-resistant bacteria [5].

An alternative method to improve animal performance is to provide non-antimicrobial feed additives that minimize pathogenic bacteria colonization of the digestive tract. A subspecies of *Saccharomyces cerevisiae*, *S. cerevisiae boulardii,* has been extensively used as both a preventive and therapeutic agent for the treatment of a variety of intestinal diseases in humans and other monogastrics [6]. Gedek [7] found that *S. cerevisiae boulardii* can be irreversibly bound to pathogenic bacteria such as some strains of entero-hemorrhagic *Escherichia coli* and the multiple antibiotic-resistant *Salmonella typhimurium* DT 104 due to the presence of lectin sites for mannose-sensitive adhesion in the outer membrane of the yeast cell.

A functional microflora must be established in the developing rumen prior to weaning as it controls the animal's intake potential, and therefore production performance [3, 8]. Feeding of a yeast culture from *S. cerevisiae* to newborn calves improved grain intake and slightly influenced rumen development [9]. Furthermore, when a dried live yeast was fed to young lambs, establishment of cellulolytic bacteria [8, 10] ciliate protozoa [11], which might partially explain the improved DM intake and weight gain in young calves fed *S. cerevisiae* [9, 12, 13].

Although some studies have indicated positive effects of yeast products on calf performance, no study has evaluated the effect of feeding a live yeast product incorporated into the grain or milk replacer on performance and patterns of antibiotic resistance in bacteria from gut of calves with failure of passive transfer. We hypothesize that: feeding *S. cerevisiae* incorporated to the grain of Holstein calves with failure of passive transfer would enhance production performance by improving DM intake and body weight gain; feeding *S. cerevisiae boulardii* added to the milk replacer would improve performance of calves by reducing diarrhea; and feeding *S. cerevisiae* incorporated to the grain and *S. cerevisiae boulardii* to the milk replacer would have additive effects on calf performance. We also hypothesized that the addition of live yeast containing *S. cerevisiae boulardii* could reduce the level of antibiotic resistance in commensal intestinal bacteria. Therefore, the objectives of this study were to determine the effects of a live yeast product added to the grain (SC; *S. cerevisiae*), milk replacer (SB; *S. cerevisiae,* spp. *boulardii*), or both (SCSB) on performance, some health parameters, and patterns of antibiotic resistance in calves with failure of passive transfer.

2. MATERIALS AND METHODS

2.1. Animals, housing, and feeding

All procedures involving animals were approved by the University of California Davis Institutional Animal Care and Use Committee. Fifty-two Holstein bull calves in the first week of age (5 ± 2 d of age) were randomly assigned to one of the four treatments (13/treatment). All calves originated from a single dairy and were transported to the study site by truck. All calves received 4 L of a solution containing electrolytes and were only offered milk replacer and grain 12 h later. Milk replacer was fed in bottles for the first week, and then offered in buckets afterwards. In the morning following arrival, a blood sample was collected and

serum separated and analyzed for concentrations of total protein using a refractometer, and total IgG using a single radial immunodiffusion assay according to the manufacturer guidelines (Veterinary Medical Research and Development, Pullman, WA, USA). All calves had serum IgG < 0.73 g·dL^{-1}, with mean IgG concentrations for treatments ranging from 0.52 to 0.64 g·dL^{-1}, thereby indicating failure of passive transfer [2, 3].

Calves were housed in individual wooden hutches and offered 440 g (DM) of nonmedicated milk replacer containing 20% fat and 20% CP from spray-dried animal plasma (American Protein Corporation, IA, USA). The milk replacer was reconstituted in 3.8 L of warm water to achieve a solution with 11.6% DM. Milk replacer was offered into two daily feedings of 1.9 L at 7:00 and 16:00 h during the first 42 d of study.

All calves were fed the same mixture of grains (Tab. I) to meet or exceed the nutrient requirements for a pre- and early weaned Holstein calf to achieve adequate weight gain as suggested by the NRC [14] and others [3]. Grain contained the ionophore monensin to control coccidia. Because ionophores such as monensin only interfere with gram positive bacteria and coccidia by affecting ion transport and cellular energy metabolism, it is unlikely that monensin would influence response to feeding live yeast or affect patterns of antibiotic resistance in gut bacteria. Grain was fed once a day before weaning, in the afternoon, and twice a day after weaning, in the morning and afternoon, for ad libitum intake and orts from each individual calf were weighed daily to calculate DM intakes. Amounts of grain offered were adjusted daily to allow for at least 5% orts.

The grain and orts from each treatment were sampled weekly, dried at 55 °C for 48 h to determine the DM content. Dried samples were processed and composited samples were analyzed for OM and CP [15], ADF and NDF [16], and minerals using an ICP plasma emission spectrometer (Thermo Garrell Ash, Franklin, MA, USA).

Table I. Ingredient composition and nutrient content of the calf grain.

	DM basis
Ingredient, %	
Steam-rolled barley	28.0
Steam-rolled corn	27.0
Soybean hulls	11.0
Rapeseed meal, solvent extracted	11.0
Soybean meal, solvent extracted	11.0
Cane molasses	8.0
Beef tallow	2.0
Minerals and vitamins[1]	2.0
Nutrient	
OM, %	93.4
CP, %	19.5
ME[2], Mcal·kg^{-1}	3.1
NDF, %	22.2
Fat, %	5.0
Ca, %	0.87
P, %	0.59
K, %	1.28
Mg, %	0.40
Na, %	0.23
Cl, %	0.52
S, %	0.43
Zn, mg·kg^{-1}	113
Cu, mg·kg^{-1}	16
Mn, mg·kg^{-1}	92
Monensin, mg·kg^{-1}	22.0

[1] Zinpro 4-plex, 4.3%; calcium carbonate, 67.5%; magnesium oxide, 11.3%; salt, 16.8%; and (per kg) 33 mg of I, 14 mg of Se, 165 000 IU of vitamin A, 22 000 IU of vitamin D, and 11 000 IU of vitamin E.
[2] ME = metabolizable energy according to NRC (2001).

2.2. Treatments and body weight measurements

Treatments consisted of no added live yeast (control), live yeast as an active dry yeast product added to the grain (SC; *S. cerevisiae*; Levucell SC20 containing the Pasteur Institute CNCM I-1077 strain of *S. cerevisiae*, Lallemand Animal Nutrition,

Lallemand Biochem International, Milwaukee, WI, USA), live yeast as an active dry yeast product added to the milk replacer (SB; *S. cerevisiae,* spp. *boulardii*; Levucell SB20 containing the Pasteur Institute CNCM I-1079 strain of *S. cerevisiae boulardii*, Lallemand Animal Nutrition, Lallemand Biochem International, Milwaukee, WI, USA), and the respective live yeasts added to the grain and to the milk replacer (SCSB). Treatments were top-dressed onto the grain (*S. cerevisiae* in treatments SC and SCSB) once daily with 500 mg of the product containing a total of 10 billion CFU and calves were observed to assure yeast consumption. The live yeast added to the milk replacer in treatments SB and SCSB was incorporated with a total of 500 mg of the product containing a total of 10 billion CFU, but divided into two daily feedings with half of that amount per feeding. The study was conducted from October to December of 2002 and lasted 84 d.

Calves were weighed on the first day of treatment, and every 14 d thereafter. At the end of the study, all calves were weighed on two consecutive days and final weight was averaged for those days. Calves were weighed always at the same time, immediately prior to afternoon feeding.

2.3. Blood sample collection and analyses

Blood samples (8 mL) were collected weekly from every calf 30 min after the morning feeding by puncture of the jugular vein into evacuated tubes containing sodium EDTA (Vacutainer®; Becton Dickinson, Franklin Lakes, NJ, USA). The initial sample was collected the day before initiation of treatments and used for covariate adjustment of data. Samples collected during the treatment period coincided when calves were 14 d old and thereafter. Samples were immediately placed on ice and tubes were centrifuged at 2000 × *g* for 15 min in a refrigerated centrifuge at 8 °C for plasma separation. Plasma was frozen at –25 °C and later analyzed for glucose and β-hydroxybutyrate (BHBA). Plasma glucose concentration was determined by direct measurement using the YSI Model 2700 SELECT Biochemistry Analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH, USA). Measurement of plasma BHBA [17] was performed by using a Wako kit (Autokit 3-HB; Wako Pure Chemical Industries Ltd., Richmond, VA, USA).

2.4. Fecal consistency scoring and health events

Fecal consistency was scored daily as an indicator of presence and severity of diarrhea. Feces were scored according to the following scale: (1) feces of firm consistency; (2) feces of moderate consistency; and (3) watery feces indicating profuse diarrhea. At the same time, health of each individual calf was evaluated and recorded. Duration of illnesses, as well as days of the respective treatments was evaluated. Calves with temperature above 39.5 °C and requiring antibiotic treatment received 1.0 mg of flunixin meglumine·kg^{-1} of body weight per day, for 1 to 3 d (Banamine®; Schering-Plough Animal Health Corp., Union, NJ, USA) and 2.0 mg of ceftiofur sodium·kg^{-1} of body weight per day, for 3 d (Naxcel®; Pfizer Animal Health, Kalamazoo, MI, USA). Calves with diarrhea that required treatment received 2 to 4 L of oral fluids containing electrolytes between milk feedings with additional 60 mL of a suspension containing 1.75% bismuth subsalicylate (Bismusal suspension®, Agripharm, USA) given orally as needed. All calves received a single dose of a modified live vaccine containing bovine rhinotracheitis virus, bovine viral diarrhea virus, bovine parainfluenza-3 virus, and bovine respiratory syncytial virus (Bovishield® 4, Pfizer Animal Health, New York, NY, USA) at 60 d of age. Calves that died during the course of the study were subjected to a complete post-mortem examination performed at the California Veterinary Diagnostic Laboratory in Tulare, CA, to determine the cause of death.

2.5. Fecal culture and identification of *E. coli*

A fecal sample was collected from individual calves on cotton-tipped swabs every 2 weeks and streaked for isolation of *E. coli* directly on McConkey agar and incubated for 24 h at 37 °C. Two lactose-positive colonies of different colony morphology were picked and re-streaked onto McConkey agar and incubated for 24 h at 37 °C. Biochemical confirmation of the strains was performed on all isolates. The biochemical tests used for typing were triple sugar iron, sulfide indole motility, urea, Simmon's citrate and oxidase. *Escherichia coli* was defined as oxidase negative, indole positive, Simmons citrate negative, urease negative and hydrogen sulfide negative.

2.6. Determination of *E. coli* antibiotic susceptibility

Two *E. coli* isolates per fecal sample randomly selected from the MacConkey agar plates were used for antibiotic susceptibility determination using the Kirby-Bauer disc diffusion method [18]. This isolate selection process was intended to identify the dominant *E. coli* clones in the fecal sample and within the study populations. Antimicrobial disk susceptibility tests were performed as recommended by the Clinical and Laboratory Standards Institute using *E. coli* ATCC 25922 and *Salmonella* Typhimurium (VMTRC bovine clinical isolate) as quality control standards. Biochemically confirmed *E. coli* isolates were transferred from McConkey agar plates to trypticase soy broth. The cells were allowed to grow at 37 °C for 4 to 6 h. Three mL of 0.85% NaCl sterile solution was inoculated with the trypticase soy broth suspension to achieve an optic density corresponding to 0.5 McFarland units. The amount of TSB inoculum added to the saline solution varied by isolate. Within 15 min of adjusting the turbidity suspension, a sterile nontoxic swab was dipped into the adjusted suspension and streaked onto Mueller-Hinton agar plates (150 × 15 mm) to form a uniform lawn of bacterial growth. Drug-impregnated disks were placed on the surface of the agar using a disk dispenser. Twelve antibiotics from the antibiotic monitoring panel used by the National Antimicrobial Resistance Monitoring System [19] were selected for the tests. The following 12 antibiotic discs were used: amikacin 30 μg, amoxicillin/clavulanic acid 20.10 μg^{-1}, ampicillin 10 μg, cephalotin 30 μg, ceftiofur 30 μg, chloramphenicol 30 μg, gentamicin 10 μg, nalidixic acid 30 μg, streptomycin 10 μg, sulfisoxazole 250 μg, tetracycline 30 μg, sulfamethoxazole/trimethoprim 23.75/1.25 μg^{-1} (Becton Dickinson and Co., Cockeysville, MD, USA). Detailed explanation of this methodology has been described previously [20].

2.7. Experimental design and statistical analyses

The experimental design was a randomized complete block design [21]. Calves were blocked according to body weight and serum total protein concentration, and within each block randomly assigned to one of the four treatments. Continuous data collected over time were analyzed by ANOVA for repeated measures [22] by the MIXED procedure of SAS [23]. Calf nested within treatment was used as the random effect in the model, and the covariance structure for the data was tested and the structure that best fitted the data chosen [22]. Data was analyzed for the entire experimental period, or divided into two phases, prior to weaning and after weaning. For the analyses of blood metabolites, results from the first blood sample collected immediately prior to initiation of treatment were used as covariate.

Number of days with diarrhea was analyzed by the GENMOD procedure of SAS [23] using a Poisson distribution and log transformation function [22]. Treatment costs and average daily fecal scores were analyzed by ANOVA [22] using the GLM procedure of SAS [23].

The statistical unit for the analysis of antibiotic resistance was the *E. coli* isolate. Cluster analysis was performed to group the isolates with similar resistance patterns together. The clusters were obtained using the squared Euclidean distance as a dissimilarity measure and Wards minimum variance method. For each cluster, the sum of the mean zone sizes to the 12 antibiotics was calculated and the susceptibility clusters ranked in decreasing order of the mean zone size sums. Less resistant clusters had large sums while multiple resistant clusters had relatively small sums [20]. Cumulative multinomial logistic regression was thereafter used to determine the exposure factors (treatment, age of calves, and interaction between treatment and age of calves) associated with changes in antibiotic resistance as determined by belonging to a more resistant cluster. The predictors sampling date and treatments were entered as categorical predictors.

Treatment differences with $P \leq 0.05$ were considered significant and $0.05 < P \leq 0.10$ a tendency. When treatment effect was observed ($P < 0.10$) for the ANOVA, individual comparisons were then performed using the PDIFF statement in SAS [23].

3. RESULTS

Four calves died during the first week in the study, one from each treatment, two as result of enteritis and colitis caused by C*ryptosporidium parvum* and *Coronavirus* sp., and the other two from pneumonia and septic shock caused by *Streptococcus* sp. and *Salmonella dublin*. Because there was no time to determine any treatment effect on these calves, their data were not included in the statistical analyses for the study. Therefore, data from 48 calves were used in the statistical analyses.

3.1. Performance of calves

The effects of live yeast added to grain or milk replacer on performance of Holstein calves are shown on Table II. As expected, serum total IgG and protein concentrations were similar among treatments and they were low, which was indicative of failure of passive transfer [2, 3].

Intake of DM grain prior to weaning was greater ($P < 0.05$) for SC than control calves, and calves fed SB tended to consume more grain ($P < 0.10$) than controls, but no differences between SCSB and other treatments were observed for grain intake prior to weaning. After weaning, grain intake tended to be greater ($P < 0.10$) for calves fed SC than controls, but did not differ among other treatments. When overall DM intake (grain and milk replacer) measured as % of body weight was evaluated, calves fed SC had greater ($P < 0.05$) and calves fed SB tended to have greater ($P < 0.10$) DM intake than control calves. When the entire study period was evaluated (Fig. 1) it was observed that calves in the control group had consistently less intake of grain than calves fed SC.

Gain of body weight in the first 42 days of the study followed similar patterns as grain intake. Calves in the SC had greater ($P < 0.02$) and those in the SB tended to have greater ($P < 0.10$) body weight gains prior to weaning than calves in the control group. This positive effect was caused by the greater grain intakes for calves fed SC and SB, but not because of improved efficiency of feed conversion. When the entire study period was evaluated, there was an interaction between treatment and age of calves on body weight gain ($P = 0.03$), which demonstrated that the positive effects of SC and SB on weight gain were observed early in life, but not after weaning. However, body weight of calves was always lower for controls compared to calves fed SC ($P < 0.03$). Efficiency of feed conversion was low and even negative in the first 2 weeks in the study (Fig. 2), but after 32 d of age calves were consuming an average of 2.2 kg of DM for every 1.0 kg of body weight gain.

Table II. Effect of live yeast products added to the grain or milk replacer on performance of Holstein dairy calves.

Item[2]	Treatment[1]				SEM
	Control	SC	SB	SCSB	
Serum TP at 5 d of age, g·dL^{-1}	5.15	5.08	4.91	5.04	0.13
Serum IgG at 5 d of age, g·dL^{-1}	0.57	0.62	0.52	0.64	0.06
Grain DM intake, g·d^{-1}					
Prior to weaning	438.3^{b}	682.3^{a}	611.4^{ab}	500.0^{ab}	73.8
After weaning	2193.9^{c}	2575.5^{d}	2379.2^{cd}	2400.0^{cd}	150.0
DM intake, % body weight					
Prior to weaning	1.53^{b}	1.72^{a}	1.69^{ab}	1.58^{ab}	0.07
After weaning	2.58	2.66	2.60	2.70	0.09
Body weight gain, g·d^{-1}					
Prior to weaning	298.0^{a}	464.7^{b}	416.7^{ab}	379.3^{ab}	52.7
After weaning	907.1	1037.2	975.1	975.9	68.7
Mean body weight, kg	64.0^{a}	73.2^{b}	67.4^{ab}	69.4^{ab}	3.06
Feed efficiency					
Prior to weaning	0.276^{c}	0.401^{d}	0.331^{cd}	0.331^{cd}	0.050
After weaning	0.431	0.429	0.425	0.417	0.014
Plasma glucose, mg·dL^{-1}					
Prior to weaning	74.0^{b}	78.1^{a}	74.5^{ab}	77.3^{ab}	1.49
After weaning	74.5^{b}	83.4^{a}	79.7^{ab}	80.0^{ab}	2.44
Plasma BHBA, μM·L^{-1}					
Prior to weaning	77.5	91.9	79.3	86.4	8.0
After weaning	272.8	275.7	269.8	251.7	15.0

[a,b] Different superscripts in the same row differ ($P \leq 0.05$).
[c,d] Different superscripts in the same row tend to differ ($P \leq 0.10$).
[1] Control = no live yeast; SC = live yeast as *S. cerevisiae* added to the grain; SB = live yeast as *S. cerevisiae boulardii* added to the milk replacer; and SCSB = the respective live yeasts added to the grain and milk replacer.
[2] TP = total protein; IgG = immunoglobulin G; DM = dry matter; Feed efficiency = daily body weight gain/daily DM intake; BHBA = β-hydroxybutyrate.

3.2. Plasma metabolites

Concentrations of glucose in plasma of calves were affected by treatment (P = 0.05; Fig. 3) and were consistently greater for calves receiving SC than controls pre- and post-weaning (Tab. II). Concentrations of glucose increased with age ($P < 0.0001$), but no interaction between treatment and age was observed (P = 0.21). Changes in plasma glucose concentrations followed changes in DM intake.

Plasma BHBA concentrations were not affected (P = 0.75) by treatment (Fig. 4), either before or after weaning (Tab. II). However, concentrations of BHBA in plasma increased ($P < 0.0001$) with increasing age of calves.

3.3. Fecal scores and diarrhea

Adding a live yeast product to the diet of young calves had no effects ($P > 0.10$) on fecal scores either prior to or after weaning and fecal scores were generally low (Tab. III). However, calves receiving SC and SB had fewer ($P < 0.05$) days with diarrhea prior to weaning than control calves. Similarly,

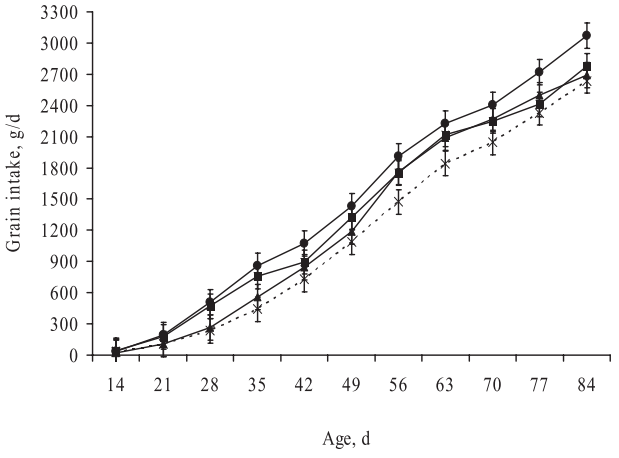

Figure 1. Temporal changes in grain dry matter (DM) intake in control calves (---x---), in calves fed live yeast in the grain (SC; —●—), in the milk replacer (SB; —■—), and in the grain and milk replacer (SCSB; —▲—).

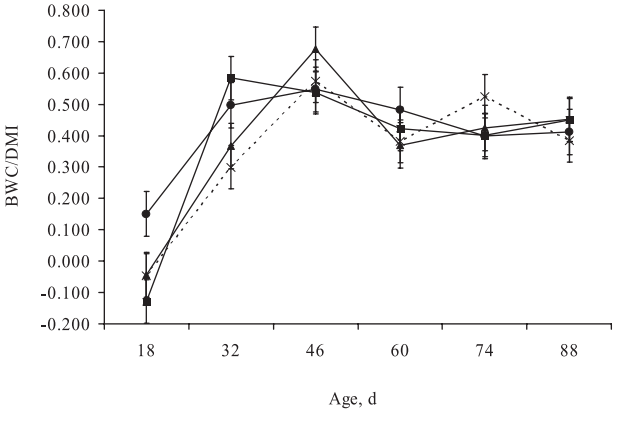

Figure 2. Patterns of feed efficiency (body weight change/dry matter intake: BWC/DMI) in control calves (---x---), in calves fed live yeast in the grain (SC; —●—), in the milk replacer (SB; —■—), and in the grain and milk replacer (SCSB; —▲—).

calves fed SC, and SCSB had fewer ($P < 0.05$) days with diarrhea after weaning than control calves. Although calves fed live yeast had reduced days with diarrhea, the total cost associated with treatment of diarrhea was not affected by treatment ($P = 0.59$).

3.4. Antibiotic susceptibility

A total of 12 clusters described the susceptibility variability for the 445 isolates that were obtained from the calves. Table IV depicts the *E. coli* distribution within the susceptibility clusters ranked from 1–12 according to increasing levels of antibiotic resistance. The data are stratified by treatment group and calf age. The majority of the 13 d isolates were found in the high resistance clusters (clusters 9, 10, 11, and 12). In contrast, isolates from 25, 38, 59, 74 d calves were predominantly in the more susceptible clusters (clusters 1, 2, 3, and 4). The multinomial logistic regression indicated that the age of calves was the most important predictor of level of resistance (Tab. V). Prevalence of multi-resistant *E. coli* decreased 4.5-fold in calves older than 13 d of age. No

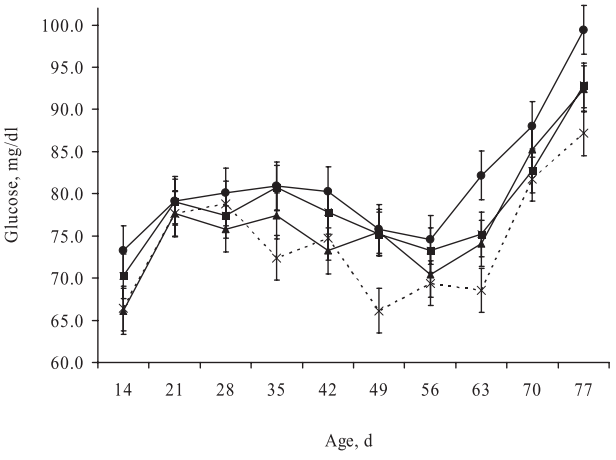

Figure 3. Temporal changes in plasma glucose in control calves (---x---), in calves fed live yeast in the grain (SC; —●—), in the milk replacer (SB; —■—), and in the grain and milk replacer (SCSB; —▲—).

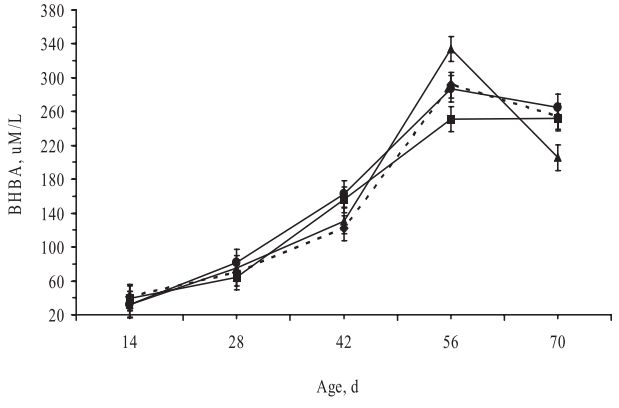

Figure 4. Temporal changes in plasma BHBA in control calves (---x---), in calves fed live yeast in the grain (SC; —●—), in the milk replacer (SB; —■—), and in the grain and milk replacer (SCSB; —▲—).

interaction (P = 0.11) between treatment and age of calves was observed for antibiotic resistance level. There was a treatment effect on antibiotic resistance, and calves fed SCSB had increased (P = 0.03) odds of multiple antibiotic resistance in isolated fecal *E. coli* than control calves.

4. DISCUSSION

Ranches raising dairy calves in the western US have become a large industry and it is not uncommon for bull calves arriving at these ranches to have inadequate passive transfer [24]. In spite of that, calves are successfully raised with survival in the first 4 weeks of life greater than 86% [24]. However, calves with failure of passive transfer experience greater morbidity and require additional treatments for health problems, which might impair performance and increase the use of antibiotics [3]. In the current study, all calves were characterized as having inadequate passive transfer because concentrations of IgG in serum were below recommended for adequate passive transfer (> 1.0 g·dL^{-1}) [2, 3]. In fact, calves with concentration of serum IgG < 1.0 g·dL^{-1} were less likely to survive than those with serum IgG greater than 1.0 g·dL^{-1} [3].

Table III. Effect of live yeast products added to the grain or milk replacer on fecal score, days with diarrhea, and costs associated with treatment of diarrhea in Holstein calves.

	Treatment[1]				
Item	Control	SC	SB	SCSB	SEM
Fecal score, 1–3					
Prior to wean	1.19	1.14	1.12	1.20	0.03
After wean	1.04	1.02	1.03	1.01	0.01
Days with diarrhea					
Prior to wean	5.83[a]	4.00[b]	4.00[b]	5.50[ab]	– –
After wean	2.08[a]	1.46[b]	2.83[a]	0.92[b]	– –
Treatment cost, US$/calf	3.03	1.49	1.43	1.96	0.92

[a,b] Different superscripts in the same row differ ($P \leq 0.05$).
[1] Control = no live yeast; SC = live yeast as *S. cerevisiae* added to the grain; SB = live yeast as *S. cerevisiae boulardii* added to the milk replacer; and SCSB = the respective live yeasts added to the grain and milk replacer.

Table IV. The distribution of *E. coli* isolates within antibiotic resistance clusters. The data are stratified by treatment group and calf age. The clusters are ranked from most susceptible (cluster 1) to most resistant (cluster 12) with the rankings based on the sum of the mean zone sizes for the 12 antibiotics tested in each cluster.

		Treatments[1]				Age of calves, days				
Cluster	Freq[2]	CON	SC	SB	SCSB	13	25	38	59	74
1	68	21	19	14	14	14	6	26	14	8
2	26	13	2	3	8	1	9	12	2	2
3	13	1	6	2	4	1	2	9	0	1
4	96	21	23	33	19	7	28	12	23	26
5	8	7	0	1	0	0	0	2	3	3
6	88	8	23	32	25	0	3	14	33	38
7	21	7	6	2	6	4	5	2	3	7
8	25	10	5	10	0	9	9	2	0	5
9	17	4	3	7	3	9	7	2	0	0
10	29	7	9	4	9	26	0	0	2	1
11	22	7	2	1	12	7	5	4	4	2
12	32	8	5	5	14	14	4	7	4	3
Sum[3]	445	114	103	114	114	92	78	92	88	96

[1] Control = no live yeast; SC = live yeast as *S. cerevisiae* added to the grain; SB = live yeast as *S. cerevisiae boulardii* added to the milk replacer; and SCSB = the respective live yeasts added to the grain and milk replacer.
[2] Freq = Number of *Escherichia coli* isolates that fall in each cluster category.
[3] Sum = Sum of the number of *E. coli* isolates for the 12 clusters in each column.

Few studies have evaluated the effects of adding live yeast to the grain or milk replacer on performance of young calves. Preliminary reports [12, 13] indicated that feeding *S. cerevisiae* to young calves increased DM intake and weight gain. When newborn calves were fed 0, 1, or 2% of the grain as a culture of *S. cerevisiae* [9], grain intake in the first 6 weeks of life increased with the 2% yeast culture treatment. However, Quigley et al. [25] did not find any effect of yeast culture on DM intake and weight gain in

Table V. Results of cumulative multinomial logistic regression predicting the odds of isolating increasing multi-drug resistant commensal *Escherichia coli* from experimental calves.

Comparisons	Odds ratio	95% Wald's CI	$P <$
Treatments[1]			
Control	1.00		
SC	0.92	0.57, 1.48	0.74
SB	1.05	0.66, 1.66	0.83
SCSB	1.65	1.05, 2.62	0.03
Age of calves, days			
13	1.00		
25	0.21	0.12, 0.37	0.0001
38	0.09	0.05, 0.15	0.0001
59	0.18	0.11, 0.31	0.0001
74	0.22	0.13, 0.38	0.0001

[1] Control = no live yeast; SC = live yeast as *S. cerevisiae* added to the grain; SB = live yeast as *S. cerevisiae boulardii* added to the milk replacer; and SCSB = the respective live yeasts added to the grain and milk replacer.

young Jersey calves in the first 12 weeks of life, and suggested that the numerous health problems experienced by those calves might have masked response to treatments.

Chaucheyras-Durand and Fonty [8] observed that *S. cerevisiae* increased activity of fibrolytic enzymes, decreased rumen ammonia and increased VFA concentrations in the rumen of gnotobiotic lambs. Their data suggested that daily consumption of live yeast influenced microbial colonization of the rumen, which could potentially influence digestive processes. The same group observed that *S. cerevisiae* influenced establishment of ciliate protozoa in the rumen [11]. Furthermore, incubating a strain of *S. cerevisiae* with *Streptococcus bovis* and *Megasphaera elsdenii* reduced availability of glucose for lactate synthesis by *S. bovis* and enhanced utilization of L-lactate by *M. elsdenii* [26], which suggest that live *S. cerevisiae* can potentially minimize fluctuation in rumen pH and reduce the risk for acidosis. In fact, rumen pH was increased when live *S. cerevisiae* was incubated in vitro with mixed ruminal microorganisms [27].

It is possible that inclusion of *S. cerevisiae* in the grain for calves in SC might have enhanced colonization of the rumen and enzymatic activities responsible for digestion of carbohydrates, which would stimulate DM intake. Furthermore, a more stable ruminal environment when yeast is fed would also favor performance of calves [1]. However, most of these mechanisms have been demonstrated in vitro or in lambs with controlled rumen flora, and limited data are available to determine mechanistic aspects of the effects of live yeast and yeast culture on young calves. Intriguing was lack of positive effects on performance of calves when fed SCSB, which was not expected.

Early after birth, calves usually maintain or even lose weight in spite of consumption of milk, which can result in negative efficiency of feed utilization [3]. We observed a period of low and even negative feed efficiency in the first 2 weeks of the study, which was associated with the low weight gain in calves. However, efficiency of feed conversion into body weight increased from 0.4 to 0.6 after 32 d in the study. This is a typical pattern for calves in the first weeks of life [3, 9]. In spite of the changes in feed efficiency over time, yeast only had minor effects on efficiency of feed conversion into body weight. These are similar to results obtained with yeast culture by others [9, 25].

The greater concentrations of glucose in calves receiving live yeast were probably the result of greater energy intake [28]. When Jersey calves were fed a culture of *S. cerevisiae* incorporated into the grain from birth to 12 weeks of age, plasma glucose concentrations remained unaffected by treatment when measured immediately prior to feeding and at 4 h postfeeding [25]. The lack of effect of yeast culture on plasma glucose in that study was probably associated with the lack of effects of treatment on DM intake.

Beta hydroxybutyrate originates from oxidation of butyrate by the rumen epithelium, or during hepatic ketogenesis in animals under negative energy balance. In young calves, plasma concentrations of BHBA more than doubled by 4 h after feeding [25] indicating that ruminal production of BHBA was the main source of ketones in calves with adequate energy intake. In fact, the mean concentrations of BHBA in the 42 d after weaning were 2.9 to 3.5 fold greater than the mean concentrations prior to weaning (Tab. II), indicating that BHBA increased as grain intake increased. Although calves in SC and SB treatments had greater DM intakes relative to body weight prior to weaning, which would likely increase rumen concentrations of VFA, these changes did not reflect in greater concentrations of plasma BHBA. Feeding a culture of *S. cerevisiae* did not alter concentrations of rumen VFA and plasma BHBA immediately after feeding, but it increased BHBA concentrations in plasma 4 h postfeeding [25]. Similar to our findings, feeding a culture of *S. cerevisiae* at 2% of the grain improved calf performance during the first 6 weeks of life, but had no impact on plasma BHBA [9].

We are not aware of any published data evaluating the effects of feeding a live yeast on diarrhea and health of newborn calves, but in feeder calves and lambs, feeding a culture of *S. cerevisiae* reduced morbidity, mortality, and number of treated sick days [29]. *Saccharomyces cerevisiae boulardii* is a nonpathogenic yeast used to treat antibiotic-associated diarrhea in humans [6], which results in clinically significant benefits [30]. Feeding *S. cerevisiae boulardii* resulted in similar performance to that observed in piglets receiving the antibiotics avilamycin and tylosine [31]. The calves fed live yeast had fewer days with diarrhea, and it is possible that both strains of *S. cerevisiae* reduced diarrhea by minimizing gut colonization by pathogenic microorganisms. *Saccharomyces cerevisiae* used in the grain has been shown to persist in the rumen as live yeast for approximately 30 h at a level close to the initial concentration after a single dose [32]. Interestingly, the authors [32] observed that 17 to 34% of yeast cells remained alive in the transit through the digestive tract, and suggested that their effects might extend beyond the rumen.

Because calves fed antimicrobials in milk have gut bacteria with increased antibiotic resistance [4], we attempted to use a non-antibiotic method to improve performance of calves with inadequate passive transfer and determine whether these live yeasts alter antibiotic resistance in fecal *E. coli*. An important finding in this study was that the levels of antibiotic resistance decreased with increasing age of calves. This trend has been noted in previous work [33] and may be associated with diet transition and development of an active rumen and changes in bacterial population in the gut. While *E. coli* isolated from calves fed SCSB tended to be more resistant to antimicrobials, this effect was not observed for the other groups. From these data, there was no consistent evidence that feeding either *S. cerevisiae* added to the grain or *S. cerevisiae boulardii* to the milk replacer resulted in shifts in the antibiotic resistance patterns of commensal fecal *E. coli* isolated from young calves.

5. CONCLUSIONS

Calves receiving SC treatment had improved performance, and most of the positive effects were observed prior to weaning. Calves fed SB tended to consume more grain and to have a greater DM intake relative to body weight than controls prior to weaning. In spite of the improved performance of calves fed SC, no additive effects were observed when yeast was incorporated into the grain and milk replacer. Calves receiving live yeast had fewer days with diarrhea, but costs associated with treatment of diarrhea were similar for all groups. Patterns of antibiotic resistance in *E. coli* were markedly affected by age of calves, but inconsistently affected by SC and SB. Results from the current study

suggest that addition of a live *S. cerevisiae* to the grain fed to calves with failure of passive transfer has the potential to improve performance prior to weaning because of increased grain intake and reduced days with diarrhea. However, in the current study, feeding of SB incorporated into the milk replacer was not beneficial to calf performance.

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