

Using ruminally protected and nonprotected active dried yeast as alternatives to antibiotics in finishing beef steers: growth performance, carcass traits, blood metabolites, and fecal *Escherichia coli*¹

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ABSTRACT: The objective of this study was to evaluate the effects of supplementing the diet of finishing beef steers with active dried yeast (ADY) in ruminally protected and nonprotected forms on growth performance, carcass traits, and immune response. Seventy-five individually-fed Angus steers (initial body weight (BW) \pm SD, 448 \pm 8.4 kg) were assigned to a randomized complete design with 5 treatments: 1) control (no monensin, tylosin, or ADY), 2) antibiotics (ANT, 330 mg monensin + 110 mg tylosin·steer⁻¹d⁻¹), 3) ADY (1.5 g·steer⁻¹d⁻¹), 4) encapsulated ADY (EDY; 3 g·steer⁻¹d⁻¹), and 5) a mixture of ADY and EDY (MDY; 1.5 g ADY + 3 g EDY·steer⁻¹d⁻¹). Active dried yeast with 1.7×10^{10} cfu/g was encapsulated in equal amounts of ADY and capsule materials (stearic acid and palm oil). Steers were fed a total mixed ration containing 10% barley silage and 90% barley-based concentrate mix (dry matter [DM] basis). The ANT, ADY, and EDY were top-dressed daily to the diet at feeding. Intake of DM, final BW, averaged daily gain (ADG), and gain-to-feed ratio (G:F) were unaffected by ADY or EDY. Carcass traits including hot carcass weight (HCW), dressing percentage, marbling score and quality grade did not differ among treatments, although fewer severely abscessed livers were observed ($P < 0.05$) with ADY and MDY compared

with the other treatments. Plasma urea N tended ($P < 0.10$) to be greater in steers fed ANT, ADY, or EDY on day 56 and 112, while glucose remained stable in all treatments except greater ($P < 0.02$) plasma glucose occurred in steers fed MDY on day 112. Serum nonesterified fatty acid (NEFA) was unaffected by ADY or EDY, but it was greater ($P < 0.03$) in steers fed ANT compared with control. Plasma haptoglobin (Hp) and serum amyloid A (SAA) were affected by yeast supplementation on day 112, with greater ($P < 0.01$) Hp in steers fed ADY, EDY, or MDY and lesser ($P < 0.01$) SAA in steers fed EDY and MDY than control. Lipopolysaccharide binding protein concentrations were greater ($P < 0.01$) in steers fed EDY and MDY on day 56. Supplementing with ADY (protected or nonprotected) or ANT had no effect on fecal IgA contents on day 56 and 112. Steers fed yeast (EDY or MDY) tended ($P < 0.10$) to have fewer fecal *Escherichia coli* counts than the control and ANT on day 56 and 112. These results indicate that feeding ADY to feedlot cattle may exhibit antipathogenic activity that conferred health and food safety beneficial effects including reduced liver abscess and potentially pathogen excretion, thus yeast may be an alternative to in-feed antibiotics in natural beef cattle production systems.

Key words: active dried yeast, antibiotics, beef steers, growth performance, immune response

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INTRODUCTION

Antibiotics are widely used in North American feedlot operations to promote feed efficiency, growth performance, and animal health (Ashima et al., 2016). However, the use of antibiotics as growth promotants is under increased scrutiny because of potential antibiotic residue accumulation in animal products and increasing risk of antimicrobial resistant pathogens (WHO, 2006). Probiotics have been studied as alternatives to antibiotics, and some have been demonstrated to improve feed digestibility, enhance feed efficiency, reduce risk of intestinal infections, and restore gut microflora in some studies (Liong, 2007; Abd El-Tawab et al., 2016).

Active dried yeast (ADY) has been the main probiotic studied in ruminants and it has been shown to have a range of beneficial effects on rumen fermentation and nutrient digestibility (Denev et al., 2007; McAllister et al., 2011). Additionally, ADY demonstrates activity against zoonotic pathogens and thus may improve gut health, intestinal microbial balance (Broadway et al., 2015), and post-ruminal digestion in ruminants if it is metabolically active in the lower digestive tract (Jiao et al., 2017). However, few studies have been conducted to examine the post-ruminal effects and on immune responses to ADY in ruminants. Recently, Jiao et al. (2017) reported that ruminally protected ADY fed to heifers was released in the small intestine and increased the intestinal digestibility of nutrients. We hypothesize that feeding ruminally protected ADY would improve feed efficiency and lower gut immune response in finishing beef steers. Thus, the objective of this study was to determine the effects of supplementing diets with ruminally protected and nonprotected ADY on growth performance, carcass traits, animal health, and immune response in finishing beef steers. In addition, changes in blood metabolite concentrations and immune response with increasing days on-feed were also examined.

MATERIALS AND METHODS

The study was conducted in the Individual Feeding Facility at Agriculture and Agri-Food Canada's Research Centre in Lethbridge, Alberta. The study received approval of the institutional Animal Care Committee and was conducted in accordance with the guidelines of the Canadian Council on Animal Care (2009).

Encapsulation of Yeast

Active dried yeast (*Saccharomyces cerevisiae*, Biomate) was provided by AB Vista (Marlborough, Wiltshire, UK). The number of yeast colonies detected on potato dextrose agar medium was 1.7×10^{10} cfu/g (Petran et al., 2001). Ruminally protected ADY was prepared as reported by Wang et al. (2011) using stearic acid and palm oil-based capsule material (King Techina Feed Co., Ltd, Hangzhou, China). Encapsulated ADY (EDY) consisted of equal amounts of ADY and capsule material. The stability of EDY in the rumen, abomasum and its release in the intestine were determined under simulated gastrointestinal conditions as described by Jiao et al. (2017). The nonencapsulated ADY lost activity after incubation in simulated rumen and abomasum fluids, while EDY was stable after incubation. The yeast was released from the capsule material after incubated in simulated intestinal fluid containing lecithin, bile salts, bile acid, and trypsin with over 90% survival rate (Shen et al., 2017).

Animals, Design, and Treatments

Seventy-five Angus steers were housed in individual feedlot pens (4.9 × 1.8 m) bedded with sawdust in a sheltered barn. The steers were given 4 wk to adapt to the new environment and the high-grain finishing diet (Table 1) before starting the experiment. During the adaptation period, the steers were ear tagged and vaccinated following standard operating procedures of the beef facility. The experiment was a randomized complete design. The steers were initially weighed (448 ± 8.4 kg) on 2 consecutive days, blocked by body weight (BW) and randomly allocated into 1 of 5 treatments. The treatments were: 1) control (no monensin, tylosin, or ADY), 2) antibiotics (ANT; 330 mg monensin + 110 mg tylosin·steer⁻¹d⁻¹), 3) ADY (1.5 g·steer⁻¹d⁻¹), 4) EDY (3 g·steer⁻¹d⁻¹), and 5) a mixture of ADY and EDY (MDY; 1.5 g ADY + 3 g EDY·steer⁻¹d⁻¹). The daily amount of antibiotics or yeast was mixed with 50 g of dry-rolled barley and delivered to each animal once daily by top-dressing onto the feed at feeding time. The control animals received a top-dress of 50 g of dry-rolled barley without antibiotics or yeast.

Table 1. Ingredients and chemical composition of the experimental diet (DM basis)

Item	
Ingredient, % of DM	
Barley silage ¹	10
Barley grain ² , dry-rolled	87
Barley, ground	0.92
Canola meal	0.83
Calcium carbonate	1.04
Molasses	0.04
Salt	0.09
Feedlot premix ³	0.02
Urea	0.06
Vitamin E (500,000 IU/kg)	0.002
Canola oil	0.02
Chemical composition, % of DM	
DM, %	74.9
OM	90.6
CP	12.5
NDF	17.7
ADF	5.3
Starch	52.3
NEm ⁴ , Mcal/kg	2.67
NEg ⁴ , Mcal/kg	1.90

ADF, acid detergent fiber; CP, crude protein; NDF, neutral detergent fiber

¹Composition (DM basis): 31.8% DM, 48.2% NDF, 25.9% ADF, 16.4% starch, and 12.1% CP based on 4 samples composited by period.

²Composition (DM basis): 90.2% DM, 97.0% OM, 14.6% NDF, 3.7% ADF, 55.9% starch and 14.5% CP based on 4 samples composited by period.

³Supplied per kilogram of dietary DM: 15 mg Cu, 65 mg Zn, 28 mg Mn, 0.7 mg I, 0.2 mg Co, 0.3 mg Se, 6,000 IU vitamin A, 600 IU vitamin D, and 47 IU vitamin E.

⁴NEm and NEg were estimated from [NRC \(2000\)](#).

Diet and Feeding

Steers were fed a total mixed ration (TMR) containing 10% barley silage, 87% dry-rolled barley grain and 3% vitamin and minerals supplement (dry matter [DM] basis) to meet the nutrient requirements of finishing beef cattle ([NASEM, 2016](#)). Diet composition and nutrient ingredients are given in [Table 1](#). The diets were prepared daily using a feed mixer (Data Ranger, American Calan Inc., Northwood, NH) and the steers were fed ad libitum once daily at 0900 a.m. such that refusals in each feed bunk did not exceed approximately 5% of the daily amount fed. The steers had free access to water.

Feed Sampling and Measurements

Feed offered was recorded daily for each steer, and refusals were weighed weekly. Samples of dietary ingredients, diets, and refusals were collected

weekly and subsampled for determination of DM content. The daily feed intake was calculated for each steer as the daily DM offered minus the weekly DM refused divided by 7. Weekly samples (ingredients, TMR, and refusals) were pooled over 4-wk periods, mixed, subsampled, and dried in an oven at 55 °C for 48 h. The dried samples were then ground through a 1-mm screen and stored for chemical analysis. Steers were weighed at the beginning and the end of the experiment and at 4-wk intervals during the experiment. The averaged daily gain (ADG) was determined for each 4-wk period and for the overall experiment by subtracting initial BW from BW at the end of the period or experiment and dividing by the number of days on-feed. Feed efficiency was determined as the ratio of ADG to dry matter intake (DMI). All feed and ingredient chemical analyses including DM, ash, organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), starch, and crude protein (CP) were carried out as described by [Yang et al. \(2010\)](#) with each sample performed in duplicate. If the coefficient of variation (CV) for the replicate analysis was > 5%, the analysis was repeated.

Blood Sampling and Analysis

Ten of the 15 steers from each treatment were randomly selected and blood samples were collected before feeding on day 0, 56, and 112 via jugular venipuncture. Two 10-mL vacuum tubes containing Na heparin, and one 10-mL vacuum tube without additive were obtained per animal at each sampling time (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Plasma samples were centrifuged at 3,000 × g for 20 min at 4 °C, and serum samples were centrifuged at 2,000 × g for 15 min at 4 °C. Plasma and serum were frozen at -20 °C until analyzed. Blood glucose, blood urea N (BUN), and nonesterified fatty acid (NEFA) were determined as described by [Yang et al. \(2010\)](#). In brief, a subsample (1 mL) of the plasma was centrifuged at 16,000 × g for 2 min at 4 °C to remove fibrinogen, and the supernatant was analyzed for glucose and urea N using a dry chemistry analyzer (VetTest analyzer, model 8008, IDEXX Lab, Westbrook, ME), while concentrations of serum NEFA were determined using a commercially available enzymatic colorimetric procedure (NEFA-HR 2, Wako Chemicals Inc., Richmond, VA). Concentrations of serum amyloid A (SAA), plasma haptoglobin (Hp), and lipopolysaccharide binding protein (LBP) were determined using bovine ELISA kits (Cloud-Clone Corp., Katy, TX; Product No.: SEA885Bo, SEA817Bo

and SEB406Bo) according to the manufacturer's instructions. The minimum detectable dose of SAA, Hp, and LBP is typically less than 0.067 ng/mL, 5.9 ng/mL, and 0.287 ng/mL, respectively.

Fecal Sampling and Analysis

At the same time of day that blood samples were collected, fresh feces (approximately 400 g wet) were collected via rectum from the same 10 steers per treatment used for blood sampling. The pH of the samples was measured immediately after sampling (Benchtop Meter, VWR International, Radnor, PA), and then the samples were subdivided into 2 portions and retained for determination of generic *Escherichia coli* counts and fecal IgA concentration. For total generic *E. coli* enumeration, 1 g of feces was weighed into a Hungate culture tube containing 9 mL of 0.1% peptone dilution water and vortexed vigorously. Serial dilutions to 10^{-6} were prepared in peptone water. One milliliter of 10^{-4} , 10^{-5} and 10^{-6} dilution was dispensed onto an *E. coli* Petrifilm plate (3M Canada, ON, Canada) and incubated at 35 °C for 24 h. After incubation, plates were counted and all blue colonies associated with gas bubbles were reported as *E. coli* (10^7 cells/g feces). For IgA determination, 1 g of feces was weighed and placed immediately in ultra-purified water at a concentration of 10% (wt/vol) by adding 9 mL of water in a 15 mL centrifuge tube. The tubes were vortexed and incubated overnight before centrifuging at $2,000 \times g$ for 15 min at 4 °C. The supernatants were collected and analyzed for total IgA (Bovine IgA ELISA Quantitation Set, Bethyl Laboratories, Montgomery, TX).

Carcass Traits

At the end of the experiment, the steers were shipped to a commercial abattoir for slaughter. Hot carcass weights (with kidneys removed), dressing percentage, 12th-rib back fat thickness (BFT), longissimus muscle (LM) area, marbling score, quality grade, saleable meat yield, and abscessed livers were recorded for each carcass. Dressing percentage was calculated as hot carcass weight (HCW) divided by final BW $\times 100$. Marbling score was estimated according to pictorial standards from 1 (devoid) to 10 (abundant marbling; USDA, 1989). Quality grade was carried out as Canada AAA standards: Canada grade A is approximately equivalent to USDA Standard; AA is approximately equivalent to USDA Select; and AAA is approximately equivalent to USDA Choice. Saleable meat yield was

estimated with consideration for the length, width, and fat cover of the rib eye muscle between the 11th and 12th rib, as estimated lean yield = $57.96 - 0.027 \text{ HCW} + 0.202 \text{ LM area} - 0.703 \text{ BFT}$. Liver abscess scores were determined based on the ranking scale used by the Canadian Beef Grading Agency. Severe liver abscesses were defined as liver with at least four small abscesses or at least one abscess with diameter larger than 2.5 cm.

Statistical Analysis

The data for BW, DMI, ADG, and gain-to-feed ratio (G:F) were analyzed using the Mixed procedure of SAS (version 16.0.0, SAS Inst. Inc., Cary, NC) as a completely randomized design to account for time (days on feed) effects. The model included treatment, period (weigh day), and their interaction as fixed effects and steers within treatment as random effect. For repeated measures, various covariance structures were tested and AR(1) was selected based on the lowest value for Akaike's information criteria. Data for carcass characteristics, blood metabolites, acute phase proteins, fecal pH, IgA, and *E. coli* counts were analyzed using the Mixed procedure of SAS with treatment as fixed effects and steers within treatment as random effects. To test the effects of increasing days on-feed on blood and fecal variables, orthogonal contrasts were conducted to determine linear and quadratic responses. Meat quality grade and liver abscess data were analyzed using the GLIMMIX procedure (SAS Inst. Inc., Cary, NC). Least square means were compared using the Tukey correction for multiple comparisons, and treatment effects were declared significant at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Growth Performance and Carcass Traits

Treatment \times period effects occurred ($P < 0.05$) for most variables measured, thus, the data are presented by treatment within period in the tables. Final BW was not affected by treatments (Table 2). During the first 28 d, there were no differences in DMI of steers fed yeast (ADY, EDY, or MDY) compared with control, but ANT had lesser ($P < 0.01$) DMI than the other treatment. The DMI did not differ among treatments for the remaining periods, but overall the steers fed yeast (ADY, EDY, or MDY) had greater ($P < 0.05$) overall DMI than the steers fed ANT, while the overall DMI did not

Table 2. Growth performance of finishing steers fed a diet supplemented with antibiotics (ANT), active dried yeast (ADY), encapsulated ADY (EDY), or mixture of ADY and EDY (MDY)

Item	Treatments ¹					SEM	<i>P</i> <
	Control	ANT	ADY	EDY	MDY		
BW, kg							
Initial	448	448	448	448	448	8.4	1.00
Final	627	623	628	625	631	10.9	0.99
DMI, kg/d							
Day 1–28	9.97 ^a	8.82 ^b	10.11 ^a	9.81 ^a	10.35 ^a	0.32	0.01
Day 29–56	11.32	10.76	11.73	11.62	11.60	0.35	0.32
Day 57–84	11.35	11.18	11.95	12.17	12.05	0.40	0.31
Day 85–112	11.42	11.39	11.70	11.75	11.85	0.38	0.88
Overall	11.0 ^{a,b}	10.5 ^b	11.4 ^a	11.3 ^a	11.5 ^a	0.20	0.05
ADG, kg/d							
Day 1 to 28	1.84 ^b	1.57 ^c	1.78 ^b	1.85 ^b	2.13 ^a	0.12	0.04
Day 29 to 56	1.74 ^{a,b}	1.92 ^a	1.80 ^a	1.51 ^b	1.49 ^b	0.11	0.02
Day 57 to 84	1.40	1.31	1.50	1.65	1.64	0.11	0.13
Day 85 to 112	1.40	1.46	1.36	1.27	1.27	0.09	0.50
Overall	1.59	1.57	1.61	1.57	1.63	0.06	0.94
G:F, g/kg							
Day 1 to 28	186.0	176.9	174.9	188.5	205.6	10.8	0.29
Day 29 to 56	155.4 ^b	178.6 ^a	154.2 ^b	130.6 ^c	128.4 ^c	8.4	0.01
Day 57 to 84	122.2	114.1	125.6	135.7	136.4	8.1	0.26
Day 85 to 112	123.1	130.6	115.1	110.0	106.5	7.7	0.18
Overall	146.7	150.1	142.5	141.2	144.2	4.7	0.58

^{a,b,c}Least square means within a row with different superscripts differ ($P < 0.05$).

¹Control = no yeast and no antibiotics; ANT (330 mg monensin + 110 mg tylosin·steer⁻¹d⁻¹); ADY (1.5 g ADY·steer⁻¹d⁻¹); EDY (3 g·steer⁻¹d⁻¹); EDY consisted of 1.5 g ADY and 1.5 g capsule material); and MDY (1.5 g ADY + 3.0 g EDY·steer⁻¹d⁻¹); Treatment × days on-feed were significant at $P < 0.05$ for DMI, ADG, and G:F.

differ between the steers fed yeast and the control steers.

Average daily gain differed ($P < 0.04$) among treatments in the first 2 periods. During day 1 to 28 ADG was greatest ($P < 0.04$) for MDY, intermediate for ADY, EDY, and control, and least for ANT. However, from day 29 to 56, the ADG of steers supplemented with EDY and MDY was lesser ($P < 0.02$) than that of those supplemented with ADY, ANT, and control. No treatment effects on ADG were observed during day 57 to 84, day 85 to 112, and overall. Feed efficiency measured as G:F was not affected by treatments except during day 29 to 56, where G:F was greatest ($P < 0.01$) for ANT, least for EDY and MDY, and intermediate for ADY and control.

Carcass traits including HCW, dressing percentage, BFT, LM area, marbling score, and saleable meat yield were not affected by treatments (Table 3). However, steers supplemented with ANT had ($P < 0.01$) lower quality grade when compared with the yeast supplementation and control groups. Proportion of abscessed livers did not differ, whereas, the proportion of severely abscessed livers was reduced ($P < 0.05$) by supplementing

with ADY and MDY compared with the other treatments.

Blood Metabolites and Immune Status

Concentration of BUN did not differ among treatments on d 0, but it tended ($P < 0.10$) to be greater on day 56 by supplementing ANT, ADY, or EDY, and on day 112 by supplementing ANT, ADY, EDY, or MDY compared with control (Table 4). Plasma glucose concentration was not different among treatments on day 0 and 56, but on day 112 it was greater ($P < 0.02$) for steers supplemented with MDY compared with control, ANT, or ADY, with EDY being intermediate and not different from the other treatments. Serum NEFA concentration on day 0 was similar for all treatments, but by day 56 it tended ($P < 0.10$) to differ with greater concentrations for ANT than for the other treatments. By day 112, concentrations were greater ($P < 0.03$) for ANT compared with control and yeast treatments, which were similar.

There were interactions for plasma Hp, SAA, and LBP concentration between treatment and days on feed (Table 5). There were no differences

Table 3. Carcass characteristics of finishing steers fed a diet supplemented with antibiotics (ANT), active dried yeast (ADY), encapsulated ADY (EDY), or mixture of ADY and EDY (MDY)

Item	Treatments ¹					SEM	P <
	Control	ANT	ADY	EDY	MDY		
HCW, kg	369	366	373	368	369	6.62	0.96
Dressing, %	58.9	58.7	59.4	59.1	58.9	0.33	0.66
BFT, mm	19.2	20.1	18.9	18.2	19.0	1.23	0.87
LM area, cm ²	86.1	82.7	88.9	87.3	86.7	1.88	0.20
Marbling score ²	6.13	6.08	6.13	6.07	5.71	0.20	0.52
Saleable meat yield ³ , %	51.9	50.7	52.7	52.9	52.1	1.09	0.63
Quality grade							
AAA ⁴ , %	100 ^a	86.7 ^b	100 ^a	100 ^a	100 ^a	...	0.01
Liver score, %							
Abscessed livers ⁵ , %	46.7	60.0	60.0	46.7	53.3	...	0.75
Severely abscessed ⁶ , %	26.7 ^a	26.7 ^a	6.7 ^b	20.0 ^a	6.7 ^b	...	0.05

BFT, back fat thickness

^{a,b}Least square means within a row with different superscripts differ ($P < 0.05$).

¹Control = no yeast and no antibiotics; ANT (330 mg monensin + 110 mg tylosin·steer⁻¹d⁻¹); ADY (1.5 g ADY·steer⁻¹d⁻¹); EDY (3 g·steer⁻¹d⁻¹); EDY consisted of 1.5 g ADY and 1.5 g capsule material); and MDY (1.5 g ADY + 3.0 g EDY·steer⁻¹d⁻¹).

²According to pictorial standards (from 1 = devoid to 10 = abundant marbling; USDA, 1989);

³Estimated lean yield = 57.96 – 0.027 HCW + 0.202 LM area – 0.703 BFT; BFT = 12th-rib back fat thickness.

⁴Canada grade AAA = equivalent to USDA choice.

⁵The percentage of liver with at least 1 abscess.

⁶The percentage of liver with at least 4 small abscesses or at least 1 abscess with a diameter greater than 2.5 cm.

in plasma Hp concentration among treatments on day 0 and 56, but it was greater ($P < 0.01$) for yeast treatments (ADY, EDY, and MDY) than control and ANT on day 112. Similarly, no differences in plasma SAA concentration were observed among treatments on day 0 and 56, whereas the plasma SAA concentration was reduced ($P < 0.01$) on day 112 by supplementing ANT, EDY, or MDY, compared with control. No differences in plasma LBP concentration were observed among treatments on day 0 and 112, whereas on day 56 steers supplemented with EDY or MDY had greater ($P < 0.01$) plasma LBP concentration than steers fed control, ANT, or ADY. The LBP concentration increased ($P < 0.01$) considerably from day 0 to 56 without further change to day 112 in steers supplemented with EDY or MDY, whereas the LBP concentration did not change from day 0 to 56 but doubled from day 56 to 112 in control steers.

Fecal IgA and *E. coli* Counts

Fecal pH was not affected by treatments throughout the entire experimental period, whereas it linearly ($P < 0.01$) increased with increasing days on-feed except for the control and MDY steers (Table 6). Fecal IgA concentration in steers fed ANT was greater ($P < 0.02$) than the other treatments on day 0. However, on day 56 and 112, there were

no treatment effects on fecal IgA concentration. The fecal IgA concentration consistently declined ($P < 0.01$) with increasing days on feed with all treatments. The fecal *E. coli* counts tended ($P < 0.07$) to be different among treatments on day 0 and 56, with greater counts in the control and ANT steers on day 0 and greater counts in the ANT and EDY steers on day 56. There was no difference in fecal *E. coli* counts between control and ANT, whereas steers supplemented with ADY, EDY, or MDY tended ($P < 0.07$) to have lesser *E. coli* counts than control steers on day 112. Although the *E. coli* counts lowered from day 0 to 56 for all treatments, only counts for steers supplemented with EDY or MDY further declined ($P < 0.01$) from day 56 to 112.

DISCUSSION

Survival of EDY Under Simulated Gastrointestinal Conditions

Yeast cell viability can be reduced during production, storage, and delivery as well as by the ruminal environment such as low pH when cattle are fed high-grain diets. Thus responses to ADY have been variable when it was used in animal studies (Sullivan and Bradford, 2011; Sartori et al., 2017). Fratianni et al. (2014) found dramatic loss of yeast cell viability after 4 wk of storage at 4 °C,

Table 4. Blood metabolites of finishing steers fed a diet supplemented with antibiotics (ANT), active dried yeast (ADY), encapsulated ADY (EDY), or mixture of ADY and EDY (MDY)

Item	Treatments ¹					SEM	P <
	Control	ANT	ADY	EDY	MDY		
Animal# ²	10	10	10	10	10		
BUN ³ , mg/dL							
Day 0	6.71	6.44	7.09	7.07	6.87	0.45	0.85
Day 56	12.81	14.62	14.21	15.29	13.12	0.79	0.10
Day 112	10.29	12.25	10.81	12.21	12.07	0.69	0.10
Linear	0.01	0.01	0.01	0.01	0.01		
Quadratic	0.01	0.01	0.01	0.01	0.01		
Glucose, mg/dL							
Day 0	99.30	99.23	95.08	85.63	91.50	6.22	0.45
Day 56	81.42	75.63	75.86	80.46	75.85	4.55	0.82
Day 112	85.42 ^b	82.65 ^b	79.39 ^b	87.80 ^{a,b}	95.23 ^a	3.35	0.02
Linear	0.02	0.01	0.01	0.80	0.53		
Quadratic	0.03	0.01	0.03	0.26	0.01		
NEFA, mM							
Day 0	0.20	0.17	0.24	0.25	0.26	0.04	0.43
Day 56	0.11	0.18	0.15	0.13	0.14	0.02	0.10
Day 112	0.13 ^b	0.20 ^a	0.14 ^b	0.15 ^b	0.15 ^b	0.02	0.03
Linear	0.04	0.29	0.01	0.01	0.01		
Quadratic	0.06	0.85	0.16	0.02	0.03		

^{a,b}Least square means within a row with different superscripts differ ($P < 0.05$).

¹Control = no yeast and no antibiotics; ANT (330 mg monensin + 110 mg tylosin·steer⁻¹d⁻¹); ADY (1.5 g ADY·steer⁻¹d⁻¹); EDY (3 g·steer⁻¹d⁻¹); EDY consisted of 1.5 g ADY and 1.5 g capsule material; and MDY (1.5 g ADY + 3.0 g EDY·steer⁻¹d⁻¹); Treatment × days on-feed was significant at $P < 0.05$ for blood urea N and blood glucose concentrations and at $P < 0.08$ for blood NEFA.

²Ten steers from each treatment group were randomly selected for blood and fecal sample collection (Tables 4, 5 and 6).

³BUN = blood urea N

and a total loss of viability after simulated gastrointestinal digestion. Several reports stated that encapsulation may increase viability of probiotic bacteria against harsh environmental conditions of the gastrointestinal tract of animals (Chavarri et al., 2010; Ghorbani-Choboghlo et al., 2015). Our previous study (Jiao et al., 2017) showed that encapsulation of ADY resulted in improved yeast survival under simulated gastrointestinal conditions compared with unprotected ADY, indicating that ADY may enter the intestine without significant loss of viability. These results indicate that the capsule material protected the ADY from digestion in the rumen and abomasum, but the ADY was released from the capsule material in the intestine when subjected to pancreatic juice and bile. Therefore, encapsulation technologies offer promise for maintaining the viability of yeast cells after passing through the rumen and abomasum, to be released in the intestine.

Effect of Yeast Supplementation on Growth Performance and Carcass Traits

Feed intake responses of beef cattle to live yeast (LY) supplementation have been variable;

some studies reported increased DMI (Robinson, 2010; Habeeb, 2017), whereas, other studies stated a reduction of DMI (Rodrigues et al., 2013; Sartori et al., 2017). Furthermore, there appears to be more studies that report no impact of LY supplementation on DMI than studies that report positive or negative effects (Beauchemin, 2012). These discrepancies among studies may be due to differences in yeast strains used (Newbold et al., 1995), the amount of yeast fed, type of animals, or the age of animals. In the current study, the lack of differences in overall DMI between control and yeast supplementation (ADY, EDY, and MDY) is in accordance with our previous study using beef heifers supplemented with protected or nonprotected ADY (Jiao et al., 2017). Vyas et al. (2014) also observed no difference in DMI by beef heifers fed ADY versus control diets. It is well established that DMI can be affected by ruminal digestibility and rate of passage of feeds out of the rumen. Jiao et al. (2017) reported that the ruminal fermentation characteristics and ruminal digestibility was not affected by supplementing ADY, EDY, or MDY; thus, the lack of yeast impact on DMI in the current study was as expected. Ruminal VFA concentrations exert some

Table 5. Plasma acute phase protein concentration in finishing steers fed a diet supplemented with antibiotics (ANT), active dried yeast (ADY), encapsulated ADY (EDY), or mixture of ADY and EDY (MDY)

Item ¹	Treatments ²					SEM	P <
	Control	ANT	ADY	EDY	MDY		
Animal# ³	10	10	10	10	10		
Hp, µg/mL							
Day 0	338	304	304	403	381	44.3	0.42
Day 56	197	213	188	177	236	20.9	0.32
Day 112	202 ^b	164 ^b	356 ^a	359 ^a	372 ^a	22.0	0.01
Linear	0.01	0.01	0.23	0.25	0.81		
Quadratic	0.05	0.53	0.01	0.01	0.01		
SAA, µg/mL							
Day 0	5.82	4.53	3.97	4.43	5.98	0.69	0.18
Day 56	5.54	5.92	3.54	5.24	5.03	0.63	0.14
Day 112	6.66 ^a	4.36 ^{b,c}	5.60 ^{a,b}	4.06 ^c	4.03 ^c	0.47	0.01
Linear	0.33	0.88	0.04	0.65	0.02		
Quadratic	0.35	0.04	0.09	0.15	0.97		
LBP, µg/mL							
Day 0	17.96	15.31	13.93	14.95	18.71	1.66	0.21
Day 56	17.13 ^b	25.43 ^b	22.90 ^b	33.78 ^a	32.99 ^a	2.79	0.01
Day 112	39.73	38.12	40.71	31.42	31.62	4.13	0.33
Linear	0.01	0.01	0.01	0.01	0.01		
Quadratic	0.01	0.72	0.23	0.01	0.03		

^{a,b,c}Least square means within a row with different superscripts differ ($P < 0.05$).

¹Hp = haptoglobin; SAA = serum amyloid A; LBP = lipopolysaccharide binding protein.

²Control = no yeast and no antibiotics; ANT (330 mg monensin + 110 mg tylosin·steer⁻¹d⁻¹); ADY (1.5 g ADY·steer⁻¹d⁻¹); EDY (3 g·steer⁻¹d⁻¹); EDY consisted of 1.5 g ADY and 1.5 g capsule material); and MDY (1.5 g ADY + 3.0 g EDY·steer⁻¹d⁻¹); Treatment × days on-feed was significant at $P < 0.01$ for Hp, SAA and LBP.

³Ten steers from each treatment group were randomly selected for blood and fecal sample collection (Tables 4–6).

control of DMI with propionate evoking a greater decrease in feed intake than acetate and butyrate (Allen, 2000). Total VFA concentration and molar proportion of acetate and propionate did not differ between heifers fed control or ADY in the study of Jiao et al. (2017). However, the numerically greater DMI (+3.8%, $P < 0.11$) on day 1 to 28 of steers supplemented with MDY compared with control steers in the current study is of interest because it suggests that MDY supplementation might have partially alleviated the negative impact of stress or rumen acidosis at the start of the study, as discussed previously (Yang et al., 2010). It has been suggested that feeding LY to receiving cattle can minimize the negative effects of adaptation on feed intake by altering ruminal fermentation patterns and improving cattle health (Adams et al., 1981; Finck et al., 2014). Mohammed et al. (2017) also reported that ADY supplementation reduced subacute rumen acidosis in beef heifers by reducing the duration of pH < 5.8 (indicative of subacute ruminal acidosis) when compared with control heifers when the cattle were exposed to an acidosis challenge.

The overall ADG and feed efficiency were not affected by LY supplementation in the present study.

Similarly, Mir and Mir (1994) reported that supplementation of LY in steer diets did not improve feed efficiency. However, in the current study, the improved ADG of steers fed MDY during day 1 to 28 indicates that feeding LY promoted growth performance in the beginning of the finishing period when LY was delivered in both rumen (ADY) and intestine (EDY). The steers might have been more stressed during the first few weeks of the study even though they were adapted to the high-grain diet during the transition period (Broadway et al., 2015). Supplementation of LY in both rumen and intestine (i.e., MDY) to steers appeared to mitigate adverse effects of the stress leading to numerically greater DMI and possibly improved intestinal function, and consequently improved ADG. However, the impact of LY supplementation may be diminished with increasing days on-feed as reported by Beauchemin et al. (2003). Improved performance of steers early in the study due to MDY supplementation may have been due to delivery of LY to both the rumen and intestine rather than only one site, or it might have been due to the greater total dose of LY for MDY (3 g ADY) compared with ADY (1.5 g/d) or EDY (1.5 g/d) alone. However,

Table 6. Fecal pH, IgA, and *Escherichia coli* counts in finishing steers fed a diet supplemented with antibiotics (ANT), active dried yeast (ADY), encapsulated ADY (EDY), or mixture of ADY and EDY (MDY)

Item	Treatments ¹					SEM	P <
	Control	ANT	ADY	EDY	MDY		
Animal# ²	10	10	10	10	10		
pH							
Day 0	6.24	6.17	6.00	6.17	6.31	0.16	0.74
Day 56	6.52	6.53	6.59	6.45	6.52	0.11	0.81
Day 112	6.42	6.76	6.73	6.76	6.47	0.15	0.32
Linear	0.29	0.01	0.01	0.01	0.18		
Quadratic	0.18	0.24	0.12	0.97	0.47		
IgA, µg/g							
Day 0	0.80 ^b	1.66 ^a	0.43 ^b	0.45 ^b	0.57 ^b	0.280	0.02
Day 56	0.24	0.22	0.26	0.28	0.44	0.084	0.33
Day 112	0.17	0.16	0.17	0.14	0.18	0.072	0.99
Linear	0.01	0.01	0.01	0.01	0.01		
Quadratic	0.23	0.01	0.84	0.95	0.73		
<i>E. coli</i> , ×10 ⁷							
Day 0	17.34	14.75	6.35	8.24	11.56	2.101	0.07
Day 56	5.80	7.92	4.04	7.21	5.83	1.806	0.07
Day 112 ³	8.47	10.08	3.99	3.27	2.47	2.185	0.06
Linear	0.01	0.01	0.39	0.01	0.01		
Quadratic	0.19	0.63	0.84	0.57	0.21		

^{ab}Least square means within a row with different superscripts differ ($P < 0.05$).

¹Control = no yeast and no antibiotics; ANT (330 mg monensin + 110 mg tylosin·steer⁻¹d⁻¹); ADY (1.5 g ADY·steer⁻¹d⁻¹); EDY (3 g·steer⁻¹d⁻¹); EDY consisted of 1.5 g ADY and 1.5 g capsule material); and MDY (1.5 g ADY + 3.0 g EDY·steer⁻¹d⁻¹); Treatment × days on-feed was significant at $P < 0.01$ for IgA.

²Ten steers from each treatment group were randomly selected for blood and fecal sample collection (Tables 4–6).

³ANT differed ($P < 0.05$) from ADY, EDY, and MDY.

the subsequently reduced ADG and G:F of steers fed EDY and MDY compared with control from day 29 to 56 suggests that the early benefits of feeding LY diminished with time on feed. Nevertheless, the reduced ADG of steers fed EDY or MDY from day 29 to 56 was compensated from day 57 to 84, indicating fluctuation of growth performance throughout the finishing period. There are limited studies evaluating the effect of feeding LY on beef cattle growth performance in comparison with the considerable research that has been conducted with lactating dairy cows (Sartori et al., 2017). Lascano and Heinrichs (2007) concluded in a review that although results were highly variable, most studies reported limited influence of LY on DMI and ADG.

Feeding monensin to ruminants is known to reduce feed intake and improve feed efficiency (Tedeschi et al., 2003). In contrast, in the current study, overall DMI and G:F of steers supplemented with ANT did not differ from control steers. Comparisons of LY with monensin within the same study for effects on beef cattle growth performance are scarce. Erasmus et al. (2009) conducted a meta-analysis on the effect of LY addition fed with and without monensin to feedlot cattle fed

high-grain diets. The ADG and G:F of cattle supplemented with either LY (1.54 kg/d and 0.156) or monensin (1.54 kg/d and 0.158) were not different. The absence of differences in ADG and G:F between steers fed LY and ANT in the present study appears to be consistent with our previous findings in beef heifers receiving the same dietary treatments (Jiao et al., 2017) as in the present study. In that study, ruminal total VFA concentration was not different among treatments, but ratio of acetate to propionate was less for ANT than yeast supplementation. Those results indicate that ANT may promote propiogenesis, which is energetically more favorable for growth and improved feed efficiency, whereas LY may promote acetogenesis through enhanced fiber digestion. In the previous study of Jiao et al. (2017) digestibility of OM in the total digestive tract was greater with yeast (ADY, EDY, and MDY) than ANT. Results suggest similar effects of either LY or monensin on growth performance of feedlot cattle although mechanisms differ.

The lack of treatment effects on growth performance likely account for the similar carcass characteristics among treatments. Very few studies have examined the effects of feeding LY on carcass traits.

Liver abscesses are often associated with undesirable carcass traits (Brown and Lawrence, 2010; Rezac et al., 2014). Brown and Lawrence (2010) evaluated the association between liver abnormalities and carcass characteristics for 76,191 animals and found that mildly and severely abscessed livers were associated with reduced HCW, LM area, and 12th-rib subcutaneous fat. In the present study, proportion of severely abscessed livers was reduced by supplementing the diet with ruminally available ADY, although the proportion of total abscessed livers was not different among treatments. The lack of effect of ADY on HCW, LM area, 12th-rib subcutaneous fat, and marbling score in the present study suggests that the previously reported association between liver abnormalities and carcass traits by Brown and Lawrence (2010) may have been mediated by reduced DMI or ADG, which was not observed in our study. The reduction in severely abscessed livers with ruminally available ADY suggests that ADY may have alleviated rumen acidosis. Rapid starch degradation in the rumen of steers fed high-grain diet can lower ruminal pH and cause digestive disturbances such as acidosis and rumenitis, increasing the incidence of liver abscesses (McAllister et al., 1990). Supplementing ADY may prevent risk of ruminal acidosis by reducing lactate production. Meanwhile, the lack of effect of EDY on the incidence of total and severely abscessed livers indicates that encapsulated ADY bypassed the rumen. The lack of effect of ANT on carcass characteristics observed in the present study is consistent with previous studies that showed feeding monensin had no significant effect on carcass characteristics (Goodrich et al., 1984; Swyers et al., 2014). Antibiotics, particularly tylosin, are commonly used to control liver abscesses in feedlot cattle by minimizing occurrence of acidosis and subsequent rumenitis (Amachawadi and Nagaraja, 2016). However, in the present study, supplementation of tylosin had no effect on liver abscesses, which was not expected and difficult to explain.

Effect of Yeast Supplementation on Blood Metabolites and Immune Status

Ruminal $\text{NH}_3\text{-N}$ concentration is a crude indicator of efficiency of dietary N conversion into microbial N (Firkins et al., 2007), while BUN concentration is useful as an indicator of protein status within a group of animals (Kohn et al., 2005). Studies reporting effects of LY on microbial N metabolism in the rumen are few and inconsistent (Chaucheyras-Durand et al., 2008). Our previous

study (Jiao et al., 2017) using rumen cannulated heifers receiving the same treatments as in the present study showed adding rumen protected ADY (i.e., EDY and MDY) increased protein digestibility in the total digestive tract without changing ruminal $\text{NH}_3\text{-N}$ concentration and ruminal degradability of protein. Thus, the trend of greater BUN with EDY and MDY versus control in the current study suggests increased intestinal absorption of amino acids. In addition, the trend of greater BUN with ANT than control was possibly because monensin inhibited ruminal protein degradation, and thus feed protein digestion in the intestine increased (Tedeschi et al., 2003). These results suggest that feeding ruminally protected LY or monensin may potentially improve protein efficiency although the mode of action is different between LY and monensin.

Blood glucose and NEFA are important indicators of energy status. Because the energy requirement of the steers was met in the current study (NASEM, 2016), differences in blood glucose and NEFA concentrations were not expected. Nevertheless, greater blood glucose concentration in the steers supplemented with MDY on day 112 may be attributed to greater intestinal digestibility of OM, as observed previously in heifers fed MDY (Jiao et al., 2017). More net glucose can be transferred from the small intestine to the liver if more starch is digested in the small intestine. Similar blood glucose concentration among the other treatments is consistent with several studies carried out with lambs fed diets containing LY (Antunovic et al., 2005; Ding et al., 2008). Similar blood NEFA concentrations for steers fed yeast and control was expected, but the greater serum NEFA concentration in steers supplemented with ANT may have been caused by lower DMI due to monensin addition. Increased serum NEFA was also observed in a previous study in which steers fed high-grain diets were supplemented with monensin (Yang et al., 2010), although DMI was not different between monensin and control steers in that study.

The release of acute phase proteins is commonly attributed to activation of the innate immune response to inflammation, tissue injury, and infection (Murata et al., 2004; Gruys et al., 2005). It is known that endotoxin stimulates release of proinflammatory cytokines by liver macrophages which in turn activates hepatocytic receptors and initiates synthesis of acute phase proteins (Gruys et al., 2005). The SAA and LBP directly participate in detoxification and removal of endotoxin during the acute phase response. The SAA can bind and neutralize

endotoxin and carry it to the liver to be excreted in bile, and intestinal epithelial cells can release SAA upon stimulation by the proinflammatory mediators (Vreugdenhil et al., 1999). The Hp is able to bind free hemoglobin, which is toxic and proinflammatory, in the plasma and reduce the oxidative damage associated with hemolysis (Murata et al., 2004). The LBP also participates in the detoxification and removal of endotoxin during an acute phase response (Schroedl et al., 2001). In the current study, the lowered plasma SAA concentrations on day 112 in steers supplemented with EDY and MDY suggests that adding protected ADY in high-grain diets may have reduced translocation of endotoxin through the hindgut into the bloodstream. Endotoxin can be released in both the rumen and hindgut (Li et al., 2016), but the site of translocation of endotoxin into blood (ruminal wall or hindgut epithelium) is not certain. The greater Hp of steers supplemented with EDY and MDY than that of control on day 112 was possibly caused by more free hemoglobin in the blood of steers supplemented with EDY and MDY. This effect is supported by 2.6 to 5.9% fewer red blood cells (Supplementary Table 1) in steers supplemented with EDY and MDY compared with control. Whether supplementing EDY and MDY to cattle fed high-grain diet can lead to hemolysis is not clear.

Effect of Yeast Supplementation on Fecal Microflora and Immune Status

The linear increase in fecal pH with increasing days on-feed indicates a reduction in carbohydrate fermentation in the hindgut over time. The differences in fecal IgA and *E. coli* counts among treatments on day 0 is consistent with the differences in blood lymphocyte and monocyte cells among treatments on day 0 (Supplementary Table 1), indicating that the animals were under different immune status at the start of the study. This initial difference led to the treatment \times days on feed interaction, because there was a substantial decrease in fecal IgA concentration for all treatments as days on-feed increased. The IgA secreted by the gut plays a crucial role in the mucosal defense and fecal IgA concentration can be used as an indicator of mucosal immunity (Suzuki et al., 2004). The decreased fecal IgA concentration with prolonged days on-feed suggests a reduction in the need for an immune response.

Fecal *E. coli* counts also tended to differ among treatments at the start of the study, with lower fecal *E. coli* counts for steers supplemented with LY (ADY, EDY, and MDY) than control. These differences in fecal *E. coli* counts remained until day 112.

It suggests beneficial effects of LY on the intestinal ecosystem. A reduction of fecal *E. coli* was also observed when dairy goats were offered 0.2 g/d of LY (Stella et al., 2007).

In conclusion, supplementation of a high-grain diet with ADY, either in encapsulated or nonencapsulated forms, as well as with ANT had overall no effects on DMI, final BW, ADG, feed efficiency, and most carcass characteristics when compared with control steers. Severely abscessed livers were reduced by supplementing the diet with ADY and MDY, indicating that ruminally available ADY may have alleviated rumen acidosis. The greater blood glucose concentrations with MDY suggest that more net glucose may have been transferred from the small intestine to the liver, although this potential improvement in energy efficiency did not enhance animal performance. The tendency for reduced fecal total *E. coli* counts with all three yeast treatments suggests possible antipathogenic activity of ADY in the lower gut. Supplementation with monensin and tylosin reduced DMI compared with LY treatments, but did not enhance feed efficiency. The study suggests that feeding ADY to feedlot cattle may exert potential beneficial health and food safety effects that reduce liver abscess and possibly pathogen excretion. Thus, yeast can be used as an alternative to in-feed antibiotics in natural beef cattle production systems.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Animal Science* online.

Conflict of interest statement. None declared.

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