University of São Paulo
"Luiz de Queiroz" College of Agriculture

Effects of feeding different probiotic types on metabolic, performance, and carcass responses of *Bos indicus* feedlot cattle offered a high-concentrate diet

Bruno Garcia de Carvalho Dias

Dissertation presented to obtain the degree of Master in Science. Area: Animal Science and Pastures

Piracicaba
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versão revisada de acordo com a Resolução CoPGr 6018 de 2011

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CONTENTS

RESUMO

Efeito de diferentes tipos probióticos no metabolismo, desempenho, e carcaça de bovinos *Bos indicus* confinados com dieta de alto concentrado

Aditivos alimentares, tais como probióticos (DFM), são incluídos nas dietas para melhorar a fermentação ruminal, a saúde do trato gastrointestinal e o desempenho animal. Entre os probióticos mais estudados estão as leveduras, as bactérias produtoras de ácido lático e os bacillus. Assim, nós hipotetizamos que a suplementação com diferentes probióticos poderia melhorar a digestibilidade dos nutrientes, a fermentação ruminal e o desempenho de bovinos *Bos indicus* em confinamento recebendo dieta de terminação. No Exp. 1, 30 novilhos Nelore canulados no rúmen foram alocados em 3 blocos (10 baias por bloco) com base no peso corporal inicial (PI; 350 ± 35.0 kg) e os seguintes tratamentos foram sorteados dentro de cada bloco: (1) ***Controle***: dieta sem DFM, (2) ***EFSC***: CONT + 1 gram/animal por dia de uma mistura de *Enterococcus faecium* e *Saccharomyces cerevisiae* (Probios® Precise, Chr. Hansen A/S, Horsholm, Denmark), e (3) ***BLBS***: CONT + 2 grams/animal por dia de uma mistura de *Bacillus licheniformis* e *Bacillus subtilis* (Bovacillus^{TM}, Chr. Hansen A/S). O período experimental durou 35 dias, entre os dias 30 e 34, foi calculada a produção total de fezes e nos dias 34 e 35, fezes e fluido ruminal foram coletados às 0, 3, 6, 9, 12, 15, 18 e 21 h após a alimentação. No Exp. 2, 240 touros Nelore foram blocados pelo peso inicial (PI; 374± 35.3 kg) em 1 das 60 baias (4 animais/baia) e as baias foram aleatoriamente distribuídas para receber os mesmos tratamentos do Exp. 1. Durante os 115 dias do período experimental foram ofertadas 3 dietas de adaptação e então a dieta de terminação (20% silagem de milho, 33% milho Flint moído seco, 45% de fibra seca de destilaria mais solúveis e 2% da mistura de minerais e vitaminas, com os respectivos aditivos. No dia 113, foi realizada a avaliação por ultrassonografia de carcaça e o rendimento de carcaça foi obtido no dia do abate dos animais (dia 117). No Exp. 1 a inclusão ou tipo de DFM não afetou a digestibilidade dos nutrientes ou os parâmetros de fermentação ruminal ($P \geq 0.40$). No entanto, a suplementação com DFM, independentemente do tipo, produziu menor média de N-amoniacal (14.7 vs. 15.7 mg/L; $P = 0.05$). Além disso, os níveis de N-amoniacal foram menores para EFSC às 3 e 6 h após a alimentação vs. CONT ($P \leq 0.04$), e menor para BSBL vs. CONT às 6 h ($P < 0.01$). No Exp. 2 nenhum efeito foi observado no CMS diário ($P \geq 0.18$) ou peso final ($P \geq 0.12$). Apesar disso, a suplementação com DFM, independentemente do tipo, tendeu a melhorar o GMD (1.57 vs. 1.50 kg; $P = 0.10$) e a EA (145 vs. 140 g/kg; $P = 0.07$). Nenhum efeito foi observado para os dados de carcaça na ultrassonografia ou abate ($P \geq 0.22$). Em resumo, a suplementação com DFM, independentemente do tipo, tendeu a melhorar o desempenho dos touros *Bos indicus.*

Palavras-chave: *Bacillus*, Zebuínos, Confinamento, *Enterococcus faecium*, Metabolismo, *Saccharomyces cerevisiae*

ABSTRACT

Effects of feeding different probiotic types on metabolic, performance, and carcass responses of *Bos indicus* feedlot cattle offered a high-concentrate diet

Feed additives, such as direct-fed microbials (**DFM**), are included into feedlot diets to improve rumen fermentation, gastrointestinal tract health, and overall animal performance. Among the strains of interest in DFM products, yeast, lactic acid and bacilli are highlighted. Hence, we hypothesized that the supplementation of different DFM would improve nutrient digestibility, rumen fermentation and performance of feedlot *Bos indicus* cattle receiving a finishing diet. In Exp. 1, 30 rumen-cannulated Nellore steers were blocked based on initial body weight (**BW**; 350 ± 35.0 kg) in 1 of 30 pens and, within each block, animals were randomly assigned to: (1) ***Control***: corn-based diet without DFM , (2) ***EFSC***: CONT + 1 gram/head per day of a DFM based on *Enterococcus faecium* and *Saccharomyces cerevisiae* (Probios® Precise, Chr. Hansen A/S, Horsholm, Denmark), and (3) ***BLBS***: CONT + 2 grams/head per day of a DFM based on *Bacillus licheniformis* and *Bacillus subtilis* (Bovacillus^{TM}, Chr. Hansen A/S). The experimental period lasted 35 days, while between days 30 to 34, total fecal collection was performed and on days 34 and 35, feces and rumen fluid were collected at 0, 3, 6, 9, 12, 15, 18, and 21 h post-feeding. In Exp. 2, 240 Nellore bulls were blocked based on initial body weight (**BW**; 374 ± 35.3 kg) into 1 of 60 feedlot pens (4 bulls/pen) and pens within blocks were randomly assigned to receive the same treatments as Exp. 1. There were 3 adapting diets and the finishing diet (20% corn silage, 33% ground flint corn, 45% distiller's bran plus solubles and 2% minerals and vitamins mixture) that was offered throughout the experimental period (115 days). On day 113, carcass ultrasound evaluations were performed and carcass traits were also obtained upon slaughter on day 117. In Exp.1the inclusion or type of DFM did not affect either nutrient digestibility or ruminal fermentation parameters ($P \geq 0.40$). In contrast, DFM supplementation, regardless of type, yielded a lower mean ammonia concentration (14.7 vs. 15.7 mg/L; $P = 0.05$). Moreover, ammonia levels were lower in EFSC at 3 and 6 h post-feeding vs. CONT ($P \leq 0.04$), but also lower for BSBL vs. CONT at 6 h ($P < 0.01$). In summary, DFM supplementation, regardless of type, reduced proteolysis, with no effect on other parameters of rumen fermentation. In Exp. 2 no effects were observed on daily DMI ($P \geq 0.18$) or final BW ($P \geq 0.12$). Nonetheless, DFM supplementation, regardless of type, tended to improve ADG (1.57 vs. 1.50 kg; $P = 0.10$) and FE (145 vs. 140 g/kg; $P = 0.07$). No further effects were observed on carcass traits measured via ultrasound or at slaughter ($P \geq 0.22$). In summary, DFM supplementation, regardless of type, tended to benefit feedlot performance of *Bos indicus* bulls.

Keywords: *Bacillus*, Zebu, Feedlot, *Enterococcus faecium*, Metabolism, *Saccharomyces cerevisiae*

1 INTRODUCTION

Probiotics are promising, as they meet the demands of niche markets where the use of ionophore antibiotics is not allowed and because they have the potential to improve animal health and performance. The European Union has banned the feeding of antibiotics as growth promoters to animals (EU Regulation No. 1831/2003 of the European Parliament and of the Council of 22 September 2003). Furthermore, the Food and Drug Administration has gone on to define direct-fed microbials as "a source of live, natural microorganisms" (FDA, 2003).

The first objective in feeding DFM to cattle was based on the idea of health responses, that included establishing a desirable microflora and preventing the establishment of pathogenic organisms in intestine (Krehbiel et al., 2003; McAllister et al., 2011). However, economic reasons for feeding DFM cattle include: increased performance in young calves, improved average daily gains and better feed efficiency, improved receiving period health, increased immunity, prevention of ruminal acidosis, increased concentrations of propionate and changes in the rumen microflora (Nocek et al., 2002; Krehbiel et al., 2003; Beauchemin et al., 2003b; Sun et al., 2011; Colombo et al., 2021).

Cattle arrive at feedlot and undergo changes in the ruminal microbiota, which was adapted to digest forage and will gradually be introduced to high-grain diets. The DFMs can alter the species that make up the microbial population to be safer and more efficient in degrading finishing diets producing a higher proportion of propionate (Wilson and Krehbiel, 2012). These changes increase the energy use of the diet and can lead to greater weight gain and feed efficiency (Krehbiel et al., 2003).

Enterococcus faecium is a lactic acid-producing bacterium responsible for creating a stable concentration of lactate in the rumen, thus providing constant stimulation for lactate-consuming bacteria to prevent lactate accumulation and reduce the risk of acidosis (Nocek et al., 2002). Mixing different DFM such as bacteria and yeast cultures prevented pH drop and acidosis (Nocek et al., 2002). The mode of action commonly attributed to *Saccharomyces cerevisiae* is the creation of a more anaerobic and stable environment, which stimulates cellulolytic and lactate-using bacteria (Newbold et al., 1996).

The *Bacillus* spp. are gram-positive, aerobic and facultative anaerobic bacteria, which produce cellulases, amylases and expansin-like protein (Pech-Cervantes et al., 2019; Luise et al., 2022) and can survive for at least 24 h in the rumen fluid *in vitro* (Dong et al., 2011). Sun et al. (2012) when supplementing dairy cattle with *Bacillus subtilis*, observed a decrease in the acetate:propionate ratio and improved milk production. Studies with supplementation of

this strain of DFM for feedlot beef cattle are rare. However, Smock et al., (2020) suggest that feeding *Bacillus subtilis* during the period of receiving improved health and performance.

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2 EFFECTS OF FEEDING DIFFERENT PROBIOTIC TYPES ON METABOLIC, PERFORMANCE, AND CARCASS RESPONSES OF *Bos indicus* FEEDLOT CATTLE OFFERED A HIGH-CONCENTRATE DIET[1]

Abstract

Two experiments were designed to evaluate the effects of different probiotic combinations on rumen fermentation characteristics, performance, and carcass characteristics of feedlot *Bos indicus* beef bulls offered a high-concentrate diet. In Exp. 1, 30 rumen-fistulated Nellore steers were blocked by initial body weight (BW; 350 ± 35.0 kg) and within blocks (n = 10), animals were randomly assigned to receive: 1) high-concentrate diet without probiotic supplementation (n = 10; **CONT**), 2) CONT plus 1 g/head of a probiotic mixture containing three strains of *E. faecium* and one strain of *S. cerevisiae* (3.5×10^9 CFU/g; n = 10; **EFSC**), and 3) CONT plus 2 g/head of a probiotic mixture containing *Bacillus licheniformis* and *B. subtilis* (3.2×10^9 CFU/g; n = 10; **BLBS**). The experimental period lasted 35 d, being 29 d of adaptation and 6 d of sampling. From d 34 to 35 of the experimental period, ruminal fluid and fecal samples were collected every 3-h, starting immediately before feeding (0 h) for rumen fermentation characteristics and apparent nutrient digestibility analysis, respectively. In Exp. 2, 240 Nellore bulls were ranked by initial shrunk BW (374 ± 35,3 kg), assigned to pens (n = 4 bulls/pen), and pens randomly assigned to receive the same treatments as in Exp. 1 (n = 20 pens/treatment). Regardless of treatment, all bulls received the same step-up and finishing diets throughout the experimental period, which lasted 115 d. In both Exp., data were analyzed as orthogonal contrasts to partition specific treatment effects: 1) Probiotic effect: CONT vs. PROB, 2) Probiotic type: EFSC vs. BLBS (SAS Software Inc.). In Exp. 1, no contrast effects were observed on any of the nutrient intake, digestibility, and rumen fermentation analyses ($P \geq 0.13$). Nonetheless, supplementation of probiotics, regardless of type (P = 0.59), reduced mean acetate:propionate ratio and rumen ammonia-N concentration vs. CONT ($P \leq 0.05$). In Exp. 2, no significant effects were observed for final BW and DMI ($P \geq 0.12$), but ADG and FE tended to improve ($P \leq 0.10$) when probiotics were offered to the animals. Probiotic supplementation or type of probiotic did not affect carcass traits ($P \geq 0.22$). In summary, supplementation of probiotics containing a mixture of *E. faecium* and *S. cerevisiae* or a mixture of *B. licheniformis* and *B. subtilis* reduced rumen acetate:propionate ratio and rumen ammonia-N levels and tended to improve performance of feedlot cattle offered a high-concentrate diet.

Key words: *Bacillus*, zebu, feedlot, *Enterococcus faecium*, metabolism, *Saccharomyces cerevisiae*

2.1 Introduction

Probiotics are classified as live microorganisms that, when administered in adequate amounts, confer health benefits to the host (FAO/WHO, 2001). In cattle, most published

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studies have attempted to evaluate how probiotics may favor nutrient digestion in the rumen through modulation of rumen fermentation characteristics, promoting the establishment of beneficial rumen microflora, and/or enhancing fiber and overall nutrient digestibility (Krehbiel et al., 2003). Nonetheless, as its main premise, probiotics improve gastrointestinal tract (**GIT**) health, including the rumen and lower GIT.

Finishing feedlot cattle are often fed high-concentrate, high-starch diets for an extended period (> 100 d; Samuelson et al., 2016; Silvestre and Millen, 2021), so that the rationale that the maintenance of rumen health is imperative to meet a desirable performance during this feeding period. Furthermore, flint corn cultivars in Brazil have a high proportion of vitreous endosperm (Correa et al., 2002) and when fed as whole or ground dry corn the resulting flow of starch to the large intestine of feedlot cattle (Marques et al., 2016; Gouvêa et al., 2016; de Melo et al., 2019) may challenge its health (Gressley et al., 2011). Different probiotic strains, such as *Enterococcus faecium*, *Bacillus* spp., and *Saccharomyces cerevisiae*, can support rumen and lower GIT metabolism through different mechanisms (McAllister et al., 2011; Chiquette et al., 2015; Luise et al., 2022). However, to the best of our knowledge, no other research evaluated the effects of different probiotics on rumen and intestinal metabolism, performance, and carcass traits of *Bos indicus* beef bulls offered a high-concentrate diet. Hence, we hypothesized that combining different probiotics would improve rumen and intestinal metabolism and performance of feedlot *B. indicus* beef bulls fed a high-concentrate diet. Therefore, our objective was to evaluate different probiotic combinations on rumen fermentation characteristics and total tract nutrient digestibility (Exp. 1), performance, and carcass characteristics (Exp. 2) of feedlot *B. indicus* beef bulls fed a high-concentrate diet.

2.2 Materials and Methods

2.2.1 Experiment 1: Metabolism trial

This experiment was conducted at the metabolism barn facility located at the University of São Paulo (USP), Escola Superior de Agricultura Luiz de Queiroz (**ESALQ**), located in Piracicaba, São Paulo, Brazil (22°43′31″ S, 47°38′51″ W, and elevation of 546 m) from April to May 2021. Minimum and maximum temperature during the experimental period was 7.8 and 29.1°C, respectively, whereas total rainfall was 31 mm. All animals utilized herein were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the ESALQ/USP Institutional Animal Care and Use Committee (# 6538141220).

2.2.1.1 Animals, housing, and diets

Thirty rumen-fistulated Nellore (*Bos taurus indicus*) steers were enrolled in the present experiment [initial body weight (**BW**) 350 ± 35.0 kg]. Throughout the experiment, all steers were housed in individual pens with concrete floor, in the same covered barn, and with *ad libitum* access to water.

Steers were assigned to treatments in a randomized complete block design, using initial BW as the blocking factor. Within blocks (n = 10), animals were randomly assigned to receive 1 of 3 treatments: 1) high-concentrate diet without probiotic supplementation (n = 10; **CONT**), 2) CONT plus daily top-dressing supplementation of 1 g/head of a probiotic mixture containing three strains of *E. faecium* and one strain of *S. cerevisiae* (3.5×10^9 CFU/g; Probios® Precise; Chr. Hansen A/S, Valinhos, SP, Brazil; n = 10; **EFSC**), and 3) CONT plus daily top-dressing supplementation of 2 g/head of a probiotic mixture containing *Bacillus licheniformis* and *B. subtilis* (3.2×10^9 CFU/g; Bovacillus™; Chr. Hansen A/S; n = 10; **BLBS**). The complete composition and nutritional profile of the diets are reported in Table 2. Flint Corn was processed through a hammer mill (Indústria e Comercial Lucato, Limeira, SP, Brazil) to achieve a mean particle size of 1.84 mm (Table 2), according to procedures described by Yu et al. (1998), using sieves with 6.0, 3.5, 2.0, and 1.25-mm square pores (Produtest T model; Telastem Peneiras para Análises Ltda., São Paulo, SP, Brazil). Diets were mixed using a feed wagon (Rotormix-40; Casale Equipamentos, São Carlos, SP, Brazil). The experimental period lasted 35 d, being 29 d of adaptation and 6 d of sampling, and all steers were fed once a day (1200 h) from d 0 to 35.

Table 1. Corn grain particle size distribution for Exp. 1 and 2.

Pores in the sieve	***% of total***
> 6.0 mm	0.0
≤ 6.00 and > 3.5 mm	0.7
≤ 3.50 and > 2.0 mm	30.8
≤ 2.00 and > 1.25 mm	51.0
≤ 1.25 mm	17.6
Mean particle size of corn, mm[1]	1.84

[1]Corn retained on the 6 mm screen was determined in 20 randomly particles using a digital caliper. The residue retained in the bottom was assumed to have a mean particle size of 0.625 mm. Based on Yu et al. (1998).

Table 2. Nutritional profile of the diets offered during Exp. 1 and 2.

Item	Diets[1]			
	ADAP-1	**ADAP-2**	**ADAP-3**	**FIN**
Inclusion,[2] % DM				
Corn silage	50.0	40.0	30.0	20.0
Ground corn	15.0	25.0	30.0	33.0
Dried distiller bran plus solubles	33.0	33.0	38.0	45.0
Mineral-vitamin mix[3]	2.0	2.0	2.0	2.0
Nutritional profile				
DM	50.5	54.7	63.0	69.3
Crude protein, % DM	13.8	13.8	14.9	16.3
Ether extract, % DM	4.5	4.8	5.2	5.7
Neutral detergente fiber, % DM	51.0	46.3	43.6	41.8
Starch, % DM	29.0	33.0	33.6	33.0
Total digestible nutrientes,[4] % DM	72.0	74.9	77.8	80.8
Metabolizable energy,[4] Mcal/kg	2.60	2.71	2.81	2.92
Net energy for maintenance,[4] Mcal/kg	1.69	1.78	1.87	1.97
Net energy for gain,[4] Mcal/kg	1.08	1.16	1.24	1.32

[1]**ADAP-1:** step-up diet fed from d 0 to 5; **ADAP-2:** step-up diet fed from d 6 to 10; **ADAP-3:** step-up diet fed from d 10 to 15; **FIN:** finishing diet.
[2]**DM** = dry matter.
[3]Composition: 275 g/kg Ca, 20 g/kg Mg, 15 g/kg Na, 550 ppm Cu, 1,400 ppm Mn, 2,500 ppm Zn, 15 ppm Co, 25 ppm I, 5 ppm Se, 65,000 IU Vit. A, 14,000 IU Vit. D3, and 500 IU Vit. E.
[4]Estimated with the equations proposed by NASEM (2016) and the tabular TDN values of dent corn and dry distiller's grain plus soluble, respectively for experimental flint corn and DDBS. The corn silage TDN value was obtained according to equation proposed by Weiss et al. (1992) using its chemical composition.

2.2.1.2 Sampling

At the beginning (d 0) of the experimental period, individual shrunk BW was recorded after 16 h of feed and water withdrawal to determine animal initial BW and to perform the randomization of the animals into blocks and treatments. Throughout the experimental period (d 0 to 35), total dry matter (**DMI**) and nutrient intake was recorded daily by collecting and weighing feed refusals approximately 24-h apart. Moreover, total digestible nutrient (**TDN**) intake was calculated according to equations proposed by Weiss et al. (1992). Samples of the offered and non-consumed diet were collected daily from each pen and dried for 48 h at 50 ± 5°C in forced air ovens for dry matter (**DM**) and, consequently, DMI calculation.

From d 34 to 35 of the experimental period, ruminal fluid samples were collected (approximately 100 mL) every 3-h, starting immediately before diet feeding (0 h), by squeezing the ruminal contents into 4 layers of cheesecloth and the ruminal fluid pH was immediately determined (Digimed-M20; Digimed Instrumentação Analítica; São Paulo, SP, Brazil). Approximately 50 mL of the ruminal fluid were collected and stored (-20°C) for subsequent analysis of rumen ammonia and molar proportions of individual volatile fatty

acids (**VFA**; acetate, propionate, butyrate), acetate:propionate (**Ac:Pr**) ratio, and total VFA. Frozen ruminal samples were prepared for analysis by thawing, centrifuging (15,000 × *g*) for 10 min at room temperature and analyzed for VFA and rumen ammonia-N according to procedures described by Ferreira et al. (2016) and Broderick and Kang (1980), respectively.

2.2.1.3 Total tract apparent nutrient digestibility

From d 30 to 34, total fecal material was collected from the individual pens, weighed, sampled (approximately 10% of wet weight), and frozen at -20ºC for DM analysis and determination of total fecal excretion. From d 34 to 35, approximately 50 g of fecal samples were directly collected from the rectum of each animal, every 3-h, starting immediately before feeding (0 h), and stored at -20°C for nutrient analysis and subsequent apparent nutrient digestibility calculations. The samples collected from the rectum on d 34 were weighed and summed at total fecal material collected from individual pens.

Frozen samples were thawed and dried in a forced air-oven at 55°C for 72 h. Diet (offer and orts) and fecal samples were ground into a 1-mm screen using a Willey mill (Marconi Equipamentos Laboratoriais, Piracicaba, SP, Brazil). Dry matter content was determined by drying the samples in an oven at 105°C for 24 h and ash content was determined by burning the samples in a muffle furnace at 550°C for 4 h (method 930.15; AOAC, 1986). Total nitrogen (**N**) determination was performed using a Leco FP-528 (Leco Corporation; Saint Joseph, MI), according to the methodology proposed by AOAC (1997), whereas ether extract followed the method 920.85 (AOAC, 1986), and ash-corrected neutral detergent fiber (**aNDF**) content was analyzed according to procedures described by Van Soest et al. (1991), using a sodium sulfite for all samples and heat-stable alpha-amylase for corn samples. Following NDF determination, acid detergent fiber (**ADF**) was evaluated according to procedures described by Goering and Van Soest (1970) in an Ankom-200 (Ankom Tech. Corp.). To indicate total starch, the Total Starch Assay AA/AMG Kit (Megazyme, Chicago IL, USA; method 996.11; AOAC, 1986 and method 76-13.01) was used. Apparent digestibility was calculated according to the formula: TTAD (%) = (((DMI × NCDM) – (FDM × NCFM)) × 100) / (DMI × NCDM), where TTAD = total tract apparent digestibility, DMI = dry matter intake, NCDM = nutrient content of the DMI (%), FDM = fecal dry matter, and NCFM = nutrient content of the fecal DM (%).

The calculations of observed net energy for maintenance ($\mathbf{NE_m}$) and gain ($\mathbf{NE_g}$) were performed from the TTAD calculations. The equations used for calculations included NE_m =

1.37 × ME – 0.138 × ME^2 + 0.0105 × ME^3 – 1.12 and for NE_g = 1.42 × ME - 0.174 × ME^2 + 0.0122 × ME^3 – 1.65 (NASEM, 2016), in which metabolizable energy (**ME**) = 0.82 × digestible energy (**DE**; NRC, 1984). The DE was obtained from the assumption that DE = TDN × 4.409 (NASEM, 2016), and the TDN was calculated according to the equation: TDN = DCHO + DCP + DEE × 2.25 (NRC, 2001). To predict expected dietary NE_m and NE_g the respective NASEM (2016) equations were used and the tabular values from NASEM (2016) for dry dent corn and DDGS (dry distiller's grain plus soluble) were assumed for the experimental ground flint corn and dry distiller's bran with solubles (**DDBS**) respectively, while the corn silage TDN was calculated according to the equation proposed by Weiss et al. (1992) using its chemical composition. Then, the observed to expected NE_m and NE_g ratios were calculated.

2.2.2 Experiment 2: Performance trial

This experiment was conducted at the experimental feedlot located at the USP, ESALQ, located in Piracicaba, São Paulo, Brazil (22°43′31″ S, 47°38′51″ W, and elevation of 546 m) from May to August 2021. Average temperature within each month from the experimental period (from May to August) was 19.8, 18.5, 17, and 20.9°C, respectively, whereas total rainfall was 22.9, 12.8, 23.3, and 12.4 mm, respectively. All animals utilized herein were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the ESALQ/USP Institutional Animal Care and Use Committee (# 6538141220).

2.2.2.1 Animals, housing, and diets.

From d -3 to -1 of the study, all animals were housed in group-pens with *ad libitum* access to water and a diet containing (DM basis) 50% corn silage, 13% ground flint corn, 33% DDBS, and 2% mineral-vitamin mix, in order to acclimate animals to the facilities prior to the beginning of the experiment.

On the morning of d 0, 240 Nellore (*B. taurus indicus*) bulls were ranked by initial shrunk BW (after 16 hours of feed and water restriction; 372 ± 34.9 kg; initial age = 24 ± 2 mo) and randomly assigned to treatments in a randomized complete block design. Within blocks (n = 10), animals were randomly assigned into pens (n = 4 bulls/pen) and pens were randomly assigned to receive 1 of 3 treatments (as reported in Exp. 1): 1) CONT (n = 20), 2) EFSC (4.0 × 10^9 CFU/g; Probios® Precise; Chr. Hansen A/S; n = 20), and 3) BLBS (3.2 × 10^9

CFU/g; Bovacillus™; Chr. Hansen A/S; *n* = 20). Conversely to what has been described for Exp. 1, probiotics were added and mixed with the other ingredients of the diet. Regardless of treatment, all bulls received the same step-up and finishing diets throughout the experimental period, which lasted 115 d. The adaptation diet was offered for 15 d and consisted of 3 step-up diets (5 d each) ranging from 50:50 to 30:70 roughage:concentrate ratio in step-up diets 1 and 3, respectively, whereas the finishing diet had a 20:80 roughage:concentrate ratio. The CONT diet was the same as aforementioned (Exp. 1) and was formulated using NASEM (2016) to provide an average daily gain (**ADG**) of 1.5 kg during the experimental period. DDBS is a co-product resulting from the fiber separation process before fermentation by the corn ethanol industry (Lima et al., 2022). The DDBS used in the experiment had 27.2% of CP, 3.15 of Ash, 7.99 of EE and 55.3 of NDF. Corn silage TDN was 59.03%, according to Weis et al., (1992) using feed composition.

On d 0, all bulls were individually identified with ear tags, vaccinated against clostridial (Covexin-9; MSD, São Paulo, Brazil) pathogens and dewormed with 1 mL/50 kg BW of an antiparasitic (Evol; Ouro Fino Saúde Animal, Cravinhos, SP, Brazil). Throughout the experimental period, diets were supplied once daily as a total mixed ration using a feed wagon (Rotormix-40; Casale Equipamentos, São Carlos, SP, Brazil) with an electronic scale (ez3400VL; Digi Star, Fort Atkinson) and offered to ensure *ad libitum* intake and result in 3% orts. Between the feeding of EFSC and BSL, as well as following complete feeding of BLBS pens, the feed wagon was washed with a 4% chloride solution (Barbarex, Nova Odessa, SP, Brazil) to avoid any cross-contamination among treatments. Additionally, all animals had full access to water and were maintained into open-sided paved pens with a coverall in the feed bunk (4.0 to 5.0 m of linear feed bunk per pen).

2.2.2.2 Sampling and carcass measurements.

Individual shrunk BW of bulls was collected on d 0 and 115 after 16 h of feed and water withdrawal and used to calculate the BW change (final minus initial BW) and ADG during the experiment. Feed bunkers were visually evaluated each day and managed for a maximum of 3% orts. For dietary DM adjustment, samples of each ingredient were collected twice a week and dried at 105 ºC for 24 h. Twice week orts were removed, weighed, sampled and discarded. Samples of feed and orts were dried at 105 ºC for 24 h to determine DM and calculate the DMI. At the end of the experiment, total BW gain and total DMI were used for

feed efficiency (**G:F**) calculation, whereas mean BW was used for determination of DMI as a percentage of BW.

Samples of ingredients were collected weekly throughout the experimental period, pooled across weeks, and analyzed for nutrient concentration (ESALQ Lab; Piracicaba, SP, Brazil). All samples were analyzed in duplicates by wet chemistry procedures for concentrations of crude protein [**CP**; method 984.13; AOAC (2006)], NDF (Van Soest et al., 1991); modified for use in an Ankom-200 fiber analyzer; Ankom Technology Corp., Fairport, NY], and ADF (method 973.18 modified for use in an Ankom-200 fiber analyzer; Ankom Technology Corp.; AOAC, 2006). Moreover, TDN concentration was calculated as reported in Exp. 1.

The observed NE for each diet was calculated from the performance data using the equations reported by Zinn and Shen (1998) based on pen average values. Energy gain (**EG**) was calculated as $EG = (0.0557 \times BW^{0.75}) \times ADG^{1.097}$ (NRC, 1984), in which EG is daily energy deposited (Mcal/d) and BW is mean shrunk BW. The equation used to calculate maintenance energy expended (**MEx**; Mcal/d) was $MEx = 0.077 \times BW^{0.75}$ (NRC, 1996). From the calculated amounts of energy required for maintenance (NE_m) and gain ($NE_{g)}$, the NE_m of each diet was obtained by the quadratic equation $NE_m = [-b \pm (b^2 - 4ac)^{1/2}]/2a$, in which $a = -0.877 \times DMI$, $b = 0.877 \times MEx + 0.41 \times DMI + EG$, and $c = -0.42 \times MEx$ and the NE_g of each diet was obtained by the equation $NE_g = 0.877 \times NE_m - 0.41$ (Zinn and Shen, 1998). Expected dietary NE_m and NE_g were predicted as reported in Exp 1.

On d 113 of the experimental period, all bulls were submitted to ultrasound evaluations (Aloka SSD-500V with a 17.2 cm/3.50 MHz convex probe; Hitachi Healthcare Americas, Twinsburg, OH), performed by the same trained technician (DGT Brasil, Presidente Prudente, SP, Brazil). Evaluations were conducted according to procedures described by the Ultrasound Guidelines Council (UGC, 2014) and measurements of the ribeye area (**REA**), marbling, and backfat thickness (**BFT**) were collected on the *Longissimus thoracis* muscle between the 12th and 13th ribs.

All animals were slaughtered on the morning of d 117 following a waiting period of approximately 16 hours, in a commercial packing plant (Frigorífico Zanqueta, Bauru, SP, Brazil). Hot carcasses were separated into two symmetrical sections, weighed to obtain hot carcass weight (**HCW**), and individually identified. Dressing percent (**DP**) was calculated by dividing the HCW and final BW of each animal.

2.2.3 Statistical analysis

For both experiments, all data were analyzed using the PROC MIXED procedure of SAS (Version 9.4; SAS Inst. Inc.; Cary, NC) and the Satterthwaite approximation to determine the denominator df for the test of fixed effects and block as random variable. All results are reported as least square means, separated using the PDIFF structure, and adjusted with the TUKEY option of SAS (for orthogonal contrast analysis only; SAS Inst. Inc.). For all the data, significance was set at $P \leq 0.05$ and tendencies were denoted if $P > 0.05$ and $P \leq 0.10$. Moreover, specifically for DM and nutrient intake, results are reported according to the main effects if no interactions were significant or according to the highest-order interaction detected.

Exp. 1. Animal was considered the experimental unit for all analyses performed herein. All data were analyzed as orthogonal contrasts to partition specific treatment effects: 1) Probiotic effect: CONT vs. PROB, 2) Probiotic type: EFSC vs. BLBS. Moreover, rumen VFA (mmol/L and proportion), pH, and ammonia-N data were analyzed using the REPEATED statement of SAS, using the fixed effects of treatment, hour, and the resulting interaction. Data were analyzed using animal as the random variable, whereas the specified term for the repeated statement was hour, the subject was animal(treatment), and the covariance structure was first-order autoregressive, which provided the best fit for these analyses according to the smallest Akaike Information Criterion (**AIC**).

Exp. 2. The model statement used for all performance and carcass data contained the fixed effects of treatment. All data were analyzed using block and pen(treatment) as random variables, whereas animal(pen) was also included in the random statement for BW, ADG, and carcass ultrasound data. Orthogonal contrasts were used to partition specific treatment comparisons: 1) Probiotic effect: CONT vs. PROB, 2) Probiotic type: EFSC vs. BLBS. Moreover, for daily DM, NE_m, and NE_g intakes, values were averaged within each wk and analyzed as repeated measures. The specified term for the repeated statement was wk, the subject was pen(treatment), and the covariance structure was autoregressive 1, which provided the best fit for these analyses according to the smallest AIC.

2.3 Results

2.3.1 Experiment 1

No contrast effects were observed on any of the nutrient intake and digestibility analyses reported herein ($P \geq 0.18$; Table 3). Similarly, total VFA, rumen pH, and individual

proportion of the main VFA were not impacted by DFM supplementation or DFM type ($P \geq 0.13$; Table 4). Nonetheless, acetate:propionate ratio and mean rumen ammonia-N concentration was lower for DFM-supplemented steers vs. CON ($P \leq 0.05$; Table 4).

Table 3. Nutrient intake and digestibility in beef *B. indicus* steers receiving a high-concentrate diet (**CONT**; $n = 10$) with the addition of a probiotic containing *Enterococcus faecium* and *Saccharomyces cerevisiae* (1 g/head per d; **EFSC**; $n = 10$) or a mixture of *Bacillus licheniformis* and *B. subtilis* (2 g/head per d; **BLBS**; $n = 10$) in Exp. 1[1]

Item	***Treatments***			***SEM***	***Contrasts***[2]	
	CONT	***EFSC***	***BLBS***		***1***	***2***
Nutrient intake, kg/d[3]						
DM	7.76	7.57	7.72	0.303	0.76	0.72
CP	0.80	0.82	0.80	0.038	0.87	0.79
EE	0.38	0.38	0.38	0.014	0.91	0.93
Carbohydrate	3.82	3.89	3.78	0.168	0.92	0.65
TDN	5.48	5.44	5.56	0.288	0.94	0.72
DE, Mcal/d[4]	24.1	24.5	24.0	1.03	0.94	0.73
ME, Mcal/d[4]	19.8	20.1	19.7	0.84	0.94	0.73
NE_g, Mcal/d[4]	8.1	8.5	8.0	0.42	0.73	0.45
Observed NE_m, Mcal/kg	1.66	1.74	1.64	0.044	0.55	0.14
Observed NE_g, Mcal/kg	1.05	1.12	1.03	0.039	0.55	0.13
Observed:Expected NE_m	0.92	0.96	0.91	0.024	0.55	0.13
Observed:Expected NE_g	0.89	0.94	0.87	0.033	0.28	0.14
Digestibility, %[5]						
DM	67.0	69.3	66.2	1.53	0.70	0.17
OM	69.4	72.1	68.8	1.49	0.59	0.14
CP	60.8	63.1	60.7	1.85	0.64	0.38
NDF	63.6	65.8	61.8	2.23	0.95	0.22
EE	90.3	91.3	90.1	0.63	0.61	0.21
Carbohydrate						
Total	69.9	72.7	69.1	1.54	0.61	0.13
Non-fiber	81.4	85.5	82.5	1.55	0.18	0.19
Starch	94.0	93.5	93.7	0.48	0.52	0.66
Ash	33.9	31.3	30.8	4.25	0.58	0.94
TDN	70.9	73.4	70.3	1.95	0.58	0.13

[1]**CONT** = high-concentrate diet without the addition of probiotics; **EFSC** = 1 g/head per d of a probiotic containing three strains of *E. faecium* and one strain of *S. cerevisiae* (3.5×10^9 CFU/g; Probios® Precise, Chr. Hansen A/S, Valinhos, SP, Brazil); **BLBS** = 2 g/head per d of a probiotic containing *B. licheniformis* and *B. subtilis* (3.2×10^9 CFU/g; Bovacillus™, Chr. Hansen A/S).
[2]Contrast analysis: 1) CONT vs. PROB and 2) EFSC vs. BLBS.
[3]**DM** = dry matter; **CP** = crude protein; **EE** = ether extract; **TDN** = total digestible nutrient.
[4]**DE** = digestible energy; **ME** = metabolizable energy; **NE_g** = net energy for gain.
[5]**OM** = organic matter; **NDF** = neutral detergent fiber.

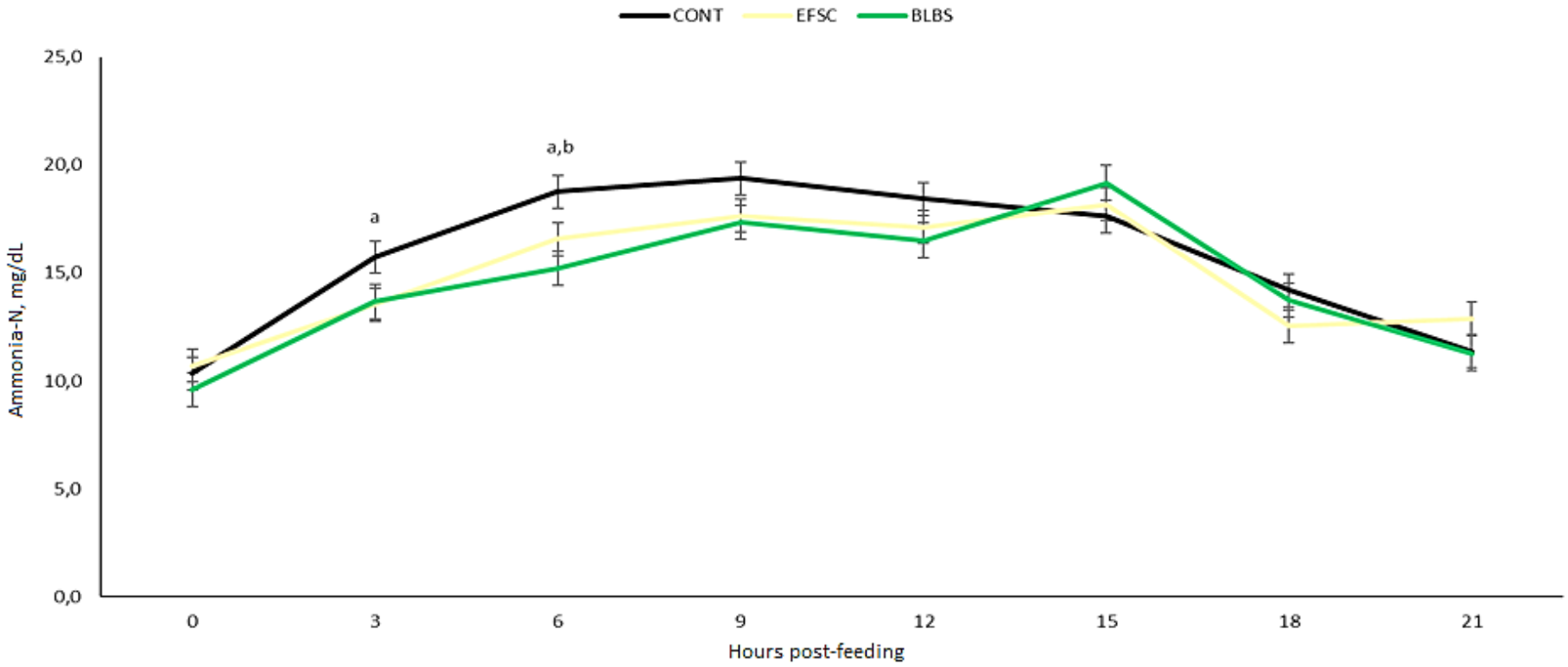
When rumen fermentation traits were analyzed as repeated measures, no treatment × hour interactions were observed for individual and total rumen VFA (concentration and proportion), Ac:Pr ratio, and pH ($P \geq 0.41$; data not shown). On the other hand, the same interaction tended to be observed ($P = 0.08$) for rumen ammonia-N. Steers fed EFSC had lower rumen ammonia-N vs. CONT at 3 and 6 h post-feeding ($P = 0.04$), whereas the same results tended to be observed at 9 h post-feeding ($P = 0.10$). Similarly, supplementation with BLBS reduced ammonia-N at 6 h ($P < 0.01$) and tended to reduce the concentration of this metabolite at 3, 9, and 12 h post-feeding ($P \leq 0.08$; Figure 1). Conversely, no differences on rumen ammonia-N were observed between EFSC and BLBS at any timepoint of the sampling period ($P \geq 0.14$; Figure 1).

Table 4. Rumen volatile fatty acids (**VFA**; mmol and proportion), pH, and ammonia concentration of beef *B. indicus* steers receiving a high-concentrate diet (**CONT**; $n = 10$) with the addition of a probiotic containing *Enterococcus faecium* and *Saccharomyces cerevisiae* (1 g/head per d; **EFSC**; $n = 10$) or a mixture of *Bacillus licheniformis* and *B. subtilis* (2 g/head per d; **BLBS**; $n = 10$) in Exp. 1[1]

Item	***Treatments***			***SEM***	***Contrasts***[2]	
	CONT	***EFSC***	***BLBS***		***1***	***2***
VFA, mmol/100 mol						
Acetate	46.6	46.2	44.8	1.35	0.49	0.45
Propionate	20.2	23.1	24.7	1.56	0.12	0.39
Butyrate	24.0	21.8	23.2	1.64	0.61	0.14
Isobutyrate	2.3	2.2	1.7	0.17	0.15	0.04
Valerate	2.1	2.0	1.9	0.22	0.55	0.68
Isovalerate	4.9	4.6	3.6	0.38	0.10	0.09
Total VFA	85.3	84.2	96.8	7.32	0.56	0.25
Acetate:propionate ratio	2.71	2.38	2.10	0.164	0.03	0.25
pH	6.40	6.41	6.28	0.087	0.59	0.31
Ammonia-N, mg/L	15.7	14.9	14.6	0.43	0.05	0.59

[1]**CONT** = high-concentrate diet without the addition of probiotics; **EFSC** = 1 g/head per d of a probiotic containing three strains of *E. faecium* and one strain of *S. cerevisiae* (3.5×10^9 CFU/g; Probios® Precise, Chr. Hansen A/S, Valinhos, SP, Brazil); **BLBS** = 2 g/head per d of a probiotic containing *B. licheniformis* and *B. subtilis* (3.2×10^9 CFU/g; Bovacillus™, Chr. Hansen A/S).
[2]Contrast analysis: 1) CONT vs. PROB and 2) EFSC vs. BLBS.

Figure 1. Rumen ammonia-N concentration (mg/L) of beef *B. indicus* seers receiving a high-concentrate diet (**CONT**; *n* = 10) with the addition of a probiotic containing *Enterococcus faecium* and *Saccharomyces cerevisiae* (1 g/head per d; **EFSC**; *n* = 10) or a mixture of *Bacillus licheniformis* and *B. subtilis* (2 g/head per d; **BLBS**; *n* = 10) in Exp. 1. A treatment × hour interaction tended to be observed (*P* = 0.08). Within hour, different letters denote differences at *P* < 0.05. a = CONT vs. EFSC (*P* = 0.04); b = CONT vs. BLBS (*P* < 0.01).



2.3.2 Experiment 2

At the beginning of the feedlot performance trial, BW did not differ among treatments ($P \geq 0.59$; Table 5). Similarly, contrasts were not significant for final BW ($P \geq 0.12$), DMI reported as kg/d ($P \geq 0.21$) or % of BW ($P \geq 0.18$). Nonetheless, ADG and G:F tended to improve ($P \leq 0.10$) when probiotics were offered to the animals, with no further differences between the type of probiotic being offered to the feedlot cattle herd ($P \geq 0.22$; Table 5). When DM, NE_m, and NE_g intake data were analyzed as repeated measures, no treatment × wk interactions were observed ($P \geq 0.74$; data not shown). Probiotic supplementation or type of probiotic did not affect any of the carcass traits measured in the present experiment ($P \geq 0.22$; Table 5).

Table 5. Feedlot performance of beef *B. indicus* bulls receiving a high-concentrate diet (**CONT**; *n* = 20) with the addition of a probiotic containing *Enterococcus faecium* and *Saccharomyces cerevisiae* (1 g/head per d; **EFSC**; *n* = 20) or a mixture of *Bacillus licheniformis* and *B. subtilis* (2 g/head per d; **BLBS**; *n* = 20) in Exp. 2[1]

Item	***Treatments***			***SEM***	***Contrasts[2]***	
	CONT	***EFSC***	***BLBS***		***1***	***2***
Performance data						
Body weight, kg						
Initial	374.4	374.1	373.9	11.77	0.59	0.79
Final	546.7	554.3	554.1	14.00	0.12	0.98
Average daily gain, kg	1.50	1.57	1.57	0.038	0.10	0.98
Dry matter intake						
kg/d	10.7	11.0	10.7	0.34	0.67	0.21
% Body weight	2.32	2.37	2.31	0.300	0.97	0.18
Feed efficiency, g/kg	140	143	147	3.5	0.07	0.22
Carcass data						
Hot carcass weight, kg	311.1	315.0	313.9	8.41	0.22	0.71
Dressing percent, %	56.8	56.8	56.7	0.21	0.96	0.60
Ribeye area, cm^2	77.8	78.4	78.4	1.17	0.84	0.66
Backfat thickness, mm	5.12	5.01	5.13	0.210	0.59	0.79

[1]**CONT** = high-concentrate diet without the addition of probiotics; **EFSC** = 1 g/head per d of a probiotic containing three strains of *E. faecium* and one strain of *S. cerevisiae* (3.5×10^9 CFU/g; Probios® Precise, Chr. Hansen A/S, Valinhos, SP, Brazil); **BLBS** = 2 g/head per d of a probiotic containing *B. licheniformis* and *B. subtilis* (3.2×10^9 CFU/g; Bovacillus™, Chr. Hansen A/S).
[2]Contrast analysis: 1) CONT vs. PROB and 2) EFSC vs. BLBS.

Regardless of type ($P \geq 0.28$), probiotic supplementation tended to increase diet observed NE_m and NE_g and observed to expected ratios of NE_m and NE_g ($P \leq 0.10$; Table 6).

Table 6. Energy intake of beef *B. indicus* bulls receiving a high-concentrate diet (**CONT**; $n = 20$) with the addition of a probiotic containing *Enterococcus faecium* and *Saccharomyces cerevisiae* (1 g/head per d; **EFSC**; $n = 20$) or a mixture of *Bacillus licheniformis* and *B. subtilis* (2 g/head per d; **BLBS**; $n = 20$) in Exp. 2[1]

Item	***Treatments***			***SEM***	***Contrasts[2]***	
	CONT	***EFSC***	***BLBS***		***1***	***2***
NE_g intake, Mcal/d	13.7	14.3	14.2	0.27	0.13	0.84
Expected[3]						
NE_m	1.97	1.97	1.97	--	--	--
NE_g	1.32	1.32	1.32	--	--	--
Observed[4]						
NE_m	1.93	1.95	1.99	0.022	0.10	0.28
NE_g	1.28	1.30	1.33	0.019	0.10	0.28
Observed:Expected						
NE_m	0.98	0.99	1.01	0.011	0.10	0.32
NE_g	0.97	0.99	1.01	0.015	0.09	0.30

[1]**CONT** = high-concentrate diet without the addition of probiotics; **EFSC** = 1 g/head per d of a probiotic containing three strains of *E. faecium* and one strain of *S. cerevisiae* (3.5×10^9 CFU/g; Probios® Precise, Chr. Hansen A/S, Valinhos, SP, Brazil); **BLBS** = 2 g/head per d of a probiotic containing *B. licheniformis* and *B. subtilis* (3.2×10^9 CFU/g; Bovacillus™, Chr. Hansen A/S).
[2]Contrast analysis: 1) CONT vs. PROB and 2) EFSC vs. BLBS.
3Estimated with the equations proposed by NASEM (2016) and the tabular TDN values of dent corn and dry distiller's grain plus soluble, respectively for experimental flint corn and DDBS. The corn silage TDN value was obtained according to the equation proposed by Weiss et al., (1992) using its chemical composition.
[4]Calculated according to Zinn and Shen (1998).

2.4 Discussion

The main goal of the present manuscript was to evaluate whether (1) probiotic supplementation and (2) supplementation of different probiotic strains would impact rumen fermentation characteristics, total tract nutrient digestibility, performance, and carcass traits of *Bos indicus* animals offered a high-concentrate diet. The probiotics evaluated in both experiments present a different composition of strains (*E. faecium* + *S. cerevisiae* for EFSC and *B. licheniformis* + *B. subtilis* for BLBS) with different modes of action, precluding potential differences on rumen and intestinal metabolism, as well as performance of feedlot beef cattle. To the best of our knowledge, a limited number of

research evaluated the probiotics described here for beef cattle and, therefore, data will be discussed on single-strain or different combinations of the same strains mostly for lactating dairy cows and/or calves.

Enterococcus faecium is a lactic-acid producing bacterium and together with oxygen-scavenging properties of *S. cerevisiae* may help to maintain an adequate rumen environment (Chiquette et al., 2012). Hence, the EFSC mixture used herein may have contributed to a constant and steady tonic level of lactic acid in the rumen, likely stimulating the growth and activity of fibrolytic and lactic acid-utilizing bacteria (Nisbet and Martin, 1991; Newbold et al., 1996; Chaucheyras-Durand et al., 2008), resulting in sustained low or undetectable concentrations of lactic acid in the rumen, which, in turn, would tend to increase the pH (Nocek et al., 2003). In fact, dairy cows supplemented with a mixture of *E. faecium* (2 out of 3 strains used herein) and *S. cerevisiae* had greater mean daily rumen pH, mean nadir pH, DM digestibility of forages and corn (Nocek et al., 2002a; Nocek et al., 2002b; Nocek and Kautz, 2006). In dairy cattle, pre- and post-partum supplementation of the same mixture of probiotics yielded greater milk production vs. non-supplemented cohorts over 70 days post-partum (Nocek et al., 2003; Nocek and Kautz, 2006). When evaluated during a sub-acute ruminal acidosis challenge in lactating dairy cows, *E. faecium* and *S. cerevisiae* supplementation alleviated the loss in milk yield, reduced the time that rumen pH was < 6.0, and increased maximum rumen pH (Chiquette et al., 2012: Chiquette et al., 2015), but did not impact total and proportion of individual VFA (Chiquette et al., 2012). On the other hand and in agreement to our results, others also did not report positive effects of *E. faecium* (1, 2, or 3 strains) and *S. cerevisiae* on rumen fermentation characteristics, such as rumen pH during a regular feeding regime, total, and proportion of individual VFA (Beauchemin et al., 2003; Chiquette, 2009; Chiquette et al., 2012; Chiquette et al., 2015), suggesting that effects related to diet composition and/or experimental period length and total dry matter intake, was not enough to cause ruminal challenges and, possibly, demonstrate positive effects of probiotic supplementation to beef animals. The inclusion of DDBS while reducing the amount of ground corn in the diet does not necessarily reduces its ruminal challenge. Garland et al. (2019) did not observe differences in ruminal pH of cattle fed either a high-corn diet or high-DDBS diet. In several studies, the partial replacement of corn by DDGS did not affect ruminal pH of feedlot cattle (Corrigan et al., 2009; Vander Pol et al., 2009). Cattle in the metabolism study consumed only 2.06% of BW during the sampling period, being lower than the values on performance

trial (Exp. 2), where mean DMI as %BW was roughly 2.33%. This lower DMI in the metabolism trial may not have challenged the ruminal environment to the experimental cattle. Nonetheless, acetate:propionate ratio was lower for DFM-supplemented animals, suggesting an improved energetic efficiency by feeding DFM.

On the other hand, *Bacillus* spp. are classified as gram-positive, catalase-positive, spore-forming, aerobic and facultative anaerobic bacteria (Luise et al., 2022). To the best of our knowledge, few experiments evaluated the effects of *Bacillus* spp. on lactate production. Other authors reported production of D- and L-lactate by *B. subtilis* in aerobic and anaerobic culture (Ohara and Yhata, 1996; Gao et al., 2012; Awasthi et al., 2018), but no such info has been reported in beef or dairy animals and/or rumen fluid with more acidic conditions (pH $\leq$ 6.5). In calves, supplementation of *B. subtilis* promoted rumen development mainly due to an altered rumen fermentation pattern (Sun et al., 2011). Molar proportions of propionate increased and NDF digestibility was lower when lactating dairy cows were fed *B. subtilis* in a 50% roughage diet (Sun et al., 2013). Altogether, these data suggest a positive effect of *B. subtilis* strains on rumen metabolism, but it is worth mentioning that the experiments above used higher dosages of *B. subtilis* (1×10^{10}, 5×10^{10}, and 1×10^{11} CFU/head per day; Sun et al., 2011; Sun et al., 2013) than the dose fed herein in combination with *B. licheniformis* (6.4×10^{9} CFU/head per day). As observed with EFSC feeding, cattle fed BLBS had lower acetate:propionate ratio, indicating an improved energetic efficiency of DFM-fed cattle and a likely reduction in methane emission (NASEM, 2016).

Probiotics are also known to improve post-ruminal metabolism, including alteration of gut microbial populations, improvement on nutrient digestibility, and improve immunity (Seo et al., 2010; McAllister et al., 2011). As an example, total-tract starch digestibility was improved when the same EFSC mixture was fed to dairy cows receiving an 18 and 22% starch diet pre- and post-partum, respectively, without further effects on fiber digestibility (AlZahal et al., 2014). On the other hand, *Bacillus* spp. are known to produce a different set of enzymes, including cellulases, expansin-like proteins, and amylases (Rojo et al., 2005; Pech-Cervantes et al., 2019; da Silva et al., 2021; Luise et al., 2022). Recently, Pan et al. (2022) reported overall improvement on *in vitro* DM, NDF, and starch digestibility of different forage sources (high- and low-quality) and high-starch substrates inoculated with BLBS. Therefore, it would be logical to speculate that the probiotics fed to feedlot beef steers would positively impact nutrient digestibility. However, no differences were observed when probiotics and type

of probiotics were fed in any nutrient digestibility evaluated in Exp. 1. Similarly, Souza et al. (2017) also did not report improvement on nutrient digestibility of lactating dairy cows offered a corn silage-based diet. One cannot disregard that those differences between grain types (flint vs. dent), processing, type and amount of the fiber included into the diet might also lead to differences on nutrient digestibility and rumen fermentation characteristics (Owens et al., 1997; Marques et al., 2016; Owens et al., 2016). On the other hand, despite the fact that both experimental DFMs did not contribute to decrease the load of dietary starch into the large intestine, they may have acted positively protecting the large intestinal epithelium in a challenging high-starch environment. Although probiotic supplementation has not impacted rumen ammonia-N in previous reports (Ghorbani et al., 2002; Chiquette et al., 2012), mean rumen ammonia-N concentration was lower in probiotic-fed steers, a decrease that was mainly observed in the initial 6 hours post-feeding, which is concomitant to the greater numerical rumen pH values observed in EFSC and BLBS (data not shown), likely suggesting a greater growth of cellulolytic bacteria in the rumen (Qadis et al., 2014).

Although no changes were observed on rumen individual proportion of VFA and nutrient digestibility, probiotic supplementation, regardless of type, tended to improve ADG and FE of *B. indicus* feedlot bulls. This potential improvement on performance could be explained by the reduced acetate:propionate ratio observed in Exp. 1, demonstrating that energetic efficiency was likely improved by DFM supplementation. Results of probiotic supplementation to feedlot beef cattle have been variable, ranging from no improvements (Beauchemin et al., 2003; Encinas et al., 2018; Lopes et al., 2021) to positive results (Swinney-Floyd et al., 1999; Rust et al., 2000; Hanford et al., 2011; Dick et al., 2013). The benefits of probiotic supplementation observed herein might be related to the impacts on rumen fermentation, gut health, pathogen inhibition associated with these strains, such as against *E. coli* and. *C. perfringens*, as well as potential benefits on leaky gut following a high-concentrate diet feeding (Copani et al., 2020; Segura et al., 2020).

Evaluating the effects of *B. subtilis* and yeast supplementation to receiving cattle, Colombo et al. (2021) did not report benefits on performance over a 45-day period. The number of articles evaluating BLBS is still limited, but Kritas et al. (2006) reported an improvement on milk production, milk fat, and protein in pregnant ewes receiving BLBS from 45 days pre- to 75 days post-lambing. In Holstein calves, ADG,

weaning BW, and starter intake was greater when BLBS was fed during the pre-weaning period (Kowalski et al., 2009).

2.5 Conclusions

In summary, supplementation of probiotics containing a mixture of *E. faecium* and *S. cerevisiae* or a mixture of *B. licheniformis* and *B. subtilis* reduced acetate:propionate ratio and rumen ammonia levels, and also tended to improve performance of feedlot cattle offered a high-concentrate diet. However, no further probiotic effects were observed on rumen fermentation characteristics, nutrient digestibility, and carcass traits. Nonetheless, additional Research is warranted to further understand potential effects of different probiotic strains on performance and metabolism of *B. indicus* feedlot cattle offered a high-concentrate diet.

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