scientific reports

Check for updates

OPEN

Feeding biotreated rumen digesta affects nutrient digestion, ruminal fermentation, and blood parameters in calves

Kian Sadeghi[1,6], Farhad Parnian-Khajehdizaj[2,6]✉, Mahdi Ganjkhanlou[1], Samad Zamanzad-Ghavidel[3], Reza Faraji[1], Valiollah Palangi[4] & Rasoul Kowsar[5]✉

The objective of this research was to examine the impact of feeding biologically treated rumen digesta (BTRD) to Holstein steer calves at levels of 0, 10, 20, and 30% (DM-based) on feed consumption, nutrient digestion, growth performance, rumen fermentation, and plasma metabolites. Sixteen Holstein steer calves with an initial BW of 113 ± 8 kg were randomly allocated in a randomized complete design. Dietary inclusion of BTRD in calves diet did not altered ($P < 0.05$) total intakes of DM, OM, and CP, when compared to those on the control diet over the experiment, but improved ($P < 0.01$) NDF and ADF consumption. Dietary inclusion of BTRD by 20% increased significantly DM and OM digestibility, average daily gain and feed efficiency (FE) ($P < 0.05$) and decreased feed conversion ratio (FCR) during 60 to 120 days. Plasma glucose, triglyceride, total protein, creatinine, or urea-N concentrations between treatments were not affected, but uric acid was reduced linearly ($P = 0.02$) in steers given BTRD supplements. Supplemented diets containing BTRD had the highest levels of plasma cholesterol and HDL, which increased with increasing BTRD inclusion levels. BTRD supplements were found to lower MDA levels in steers than control diets. This study found that dietary BTRD supplementation improved performance and health status of Holstein steer calves as well as their oxidative parameters.

Keywords Holstein calves, Rumen digesta, Growth performance, Blood metabolites

With the rise in the world's population, global demand for livestock products is growing at a significant rate, so food supply has been raised as one of the most important issues in the world today. FAO projections indicate that in order to feed 9.1 billion individuals by 2050, a 70% rise in overall food production will be necessary[1]. In other words, animals cannot convert more feed into meat, as cattle, pigs, and broilers require 8, 4, and 1 kg of cereals per animal to produce 1 kg meat[2]. FAO projects that cereal production in 2050 will nearly hit one billion tons, while meat production is expected to rise by over 200 million tons to 470 million tons[1]. However, increasing the area under cultivation is a logical response to increasing food production, but in the past few decades, about a third of the arable land has been threatened by erosion, seawater, and pollutants that destroy soil health and biological productivity. It is essential to utilize agricultural waste and by-products efficiently as alternative animal feed in order to address these issues and enhance production. To meet the global demand for meat, the number of slaughterhouses is growing, as well as the volume of raw organic materials produced as by-products of slaughterhouses[3]. Wastewater from slaughterhouses contains large amounts of nitrogen and phosphorus, which contribute to pollution of the environment and groundwater. Although the high moisture content of rumen digesta and its bad smell are considered to be the main obstacles, the use of rumen digesta with proper processing can provide a valuable source of nutrients if used as a supplement in the diet of different livestock species[4]. Rumen digesta from slaughterhouses have previously been used as animal feed for cattle[5,6] and small ruminants[7–9]. Furthermore, it encompasses microbial proteins, microbial fermentation byproducts

[1]Department of Animal Science, Campus of Agriculture and Natural Resources, University of Tehran, Karaj 31587-77871, Iran. [2]Animal Science Research Department, East Azarbaijan Agricultural and Natural Resources Research and Education Center, AREEO, Tabriz, Iran. [3]Livestock Production Improvement Deputy, East Azarbaijan Agriculture Jihad Organization, Tabriz, Iran. [4]Visiting Researcher at Department of Life Sciences, Western Caspian University, Baku, Azerbaijan. [5]Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan, Iran. [6]These authors contributed equally as the first author to this work. ✉email: farhad.parnian@yahoo.com; rasoul_kowsarzar@yahoo.com

like volatile fatty acids (VFA), amino acids, and non-protein nitrogen (NPN) devoid of any anti-physiological components[3,10]. This composition exerts a beneficial impact on both rumen efficiency and functionality. Several studies have previously demonstrated the nutritional potential of dried rumen digesta[4,11,12]. Prior research has explored different processing methods, including sun drying[4,7,13], mixing with barley grain[12], or corn flour[14] before sun drying, and forced drying in the oven[15] to enhance palatability and address challenges associated with this feed source. The present investigation was carried out in order to examine the impact of incorporating biologically treated rumen digesta into the diet of steer calves on feed consumption, nutrient digestibility, growth performance, rumen fermentation, and plasma metabolites. Treating rumen digesta with beneficial and effective microorganisms including including two types of lactic acid bacteria and yeast during ensiling is a highly effective method for preventing spoilage and unpleasant odors, while also preserving or enhancing its nutritional quality.

Materials and methods

All experimental protocols for using the experimental animals were approved by the Animal Ethics and Environment Committee of the University of Tehran (6/6/30854). All methods were carried out in accordance with relevant guidelines and regulations. The study was reviewed and approved by the Animal Ethics and Environment Committee of the University of Tehran, according to the ARRIVE guidelines.

Preparation of biologically treated rumen digesta

Rumen digesta was collected from a slaughterhouse located in the Tabriz Province that slaughters several species of ruminants. Figure 1 shows the preparation of biologically treated rumen digesta (BTRD). However, the fresh rumen digesta was dehydrated by mechanical pressure using air cylinders to separate the extra water and then the dry matter was increased from 15% to 40–50% by mechanical pressure. The dehydrated and compressed rumen digesta was spread on the ground to spray the effective microbial solution on rumen digesta (EM Silage, AGRITON Group, 1:10 w/w). EM Silage contains a mix of Effective Micro-organisms (EM), including two types of lactic acid bacteria and yeast as *Lactobacillus plantarum* NBRC 3070, Lactobacillus casei NBRC 3425, and *Saccharomyces cerevisiae* NBRC 0203, carefully selected to enhance the fermentation process of forage crops. During the ensiling process, EM not only accelerate pH decline but also produce a number of bio-active substances. The Lactic Acid bacteria converts cellulose and glucose into volatile fatty acids which increase nutrition values as well as improving aerobic stability. The added yeast produces propylene glycol in the clamp and is then available itself in the rumen. The sprayed materials were ensiled in closed buckets for 30–35 days in order for bioconversion. At the end of ensiling, the silage was removed and dried at the range of 120–150 °C for 90 min in the automatic drum dryer. The dried silage was then chopped and pelleted with a 6.0 mm die diameter and used in the experiment. The end BTRD contained 13.78% CP, 2.53 Mcal/kg ME, 6.69% Ash, and 3% ether extract.

Animals and experimental diets

Sixteen three-month old Holstein steer calves with an initial BW of 113 ± 8 kg were obtained from Khoram Dareh's Cultivation and Agricultural Industry Company located in Zanjan Province, Iran. Before beginning the experimental period, animals were dewormed (5 ml of 1% w/v ivermectin), and provided with vitamins A, D, and E. The calves were randomly allocated in a randomized complete design for 201 days including 21 days as adaptation and 180 days for the experiment. Every calf was kept in its own housing and given experimental diets (Table 1) while having individual access to water. There were four treatment diets: control, a diet without supplemented biologically treated rumen digesta, BTRD-10, a diet supplemented with 10% biologically treated rumen digesta, BTRD-20, a diet supplemented with 20% biologically treated rumen digesta, and BTRD-30, a diet supplemented with 30% biologically treated rumen digesta. Nutritional requirements were met or exceeded using CNCPS (Version 5.0.40) to make diets isonitrogenous and isoenergetic. The provision of diets as a total mixed ration (TMR) occurred thrice daily (0800, 1300, and 1800 h) to allow for ad libitum intake, targeting 5% daily refusals. The experiment was divided into three periods as 1–60 d, 61–120 d, and 121–180 d, respectively. To determine the average daily gain (ADG) of the animals, their weights were measured at the beginning and end of each period. Prior to morning feeding, the offered feed quantity and orts were recorded and sampled daily in the last weeks of each period. The samples were then combined per animal for the determination of dry matter intake (DMI) by drying in a forced-air oven at 60 ˚C for 48 h. Two feces sub-samples were taken per calf per day at 10:00 and 16:00 for four days at the end of each period. According to the research conducted by Ireland-Perry and Stallings[16], as well as the study by Woolsoncroft et al.[17], it has been found that a higher score on the fecal consistency scale indicates looser feces. In this scale, a score of 1 represents a cow on dry hay, while a score of 5 represents water. Sub-samples of feces were collected while calves defecated or when they were grabbed. Subsequently, they were preserved by freezing at -20 °C for analysis in the laboratory at a later time.

Rumen and blood sampling and analysis

At the end of the each period, the rumen fluid of the calves were obtained via stomach vacuum pumps four hours after their morning meal. The next step involved filtering the rumen fluids through four layers of cheesecloth, followed by an immediate measurement of the pH of the filtered fluids. To analyze VFAs, a 10 mL portion of rumen filtrate was frozen in 15 mL polyethylene tubes with 2 mL of 25% metaphosphoric acid (wt/vol). Additionally, 2 mL of a 5% solution (V/V) of sulfuric acid was immediately mixed with another 10 mL of strained rumen fluid. The resulting solution was subsequently stored at a temperature of − 20 °C until analysis for ammonia nitrogen (NH_3-N). In order to collect blood samples from the coccygeal vein, a K_3EDTA Vacuette tube (Greiner Bio-one, Kremsmunster, Austria) was utilized. The collected samples were then subjected to centrifugation at $3,000 \times g$ for 15 min at a temperature of 4 °C. This centrifugation process effectively separated the plasma, which was subsequently stored at − 20 °C.

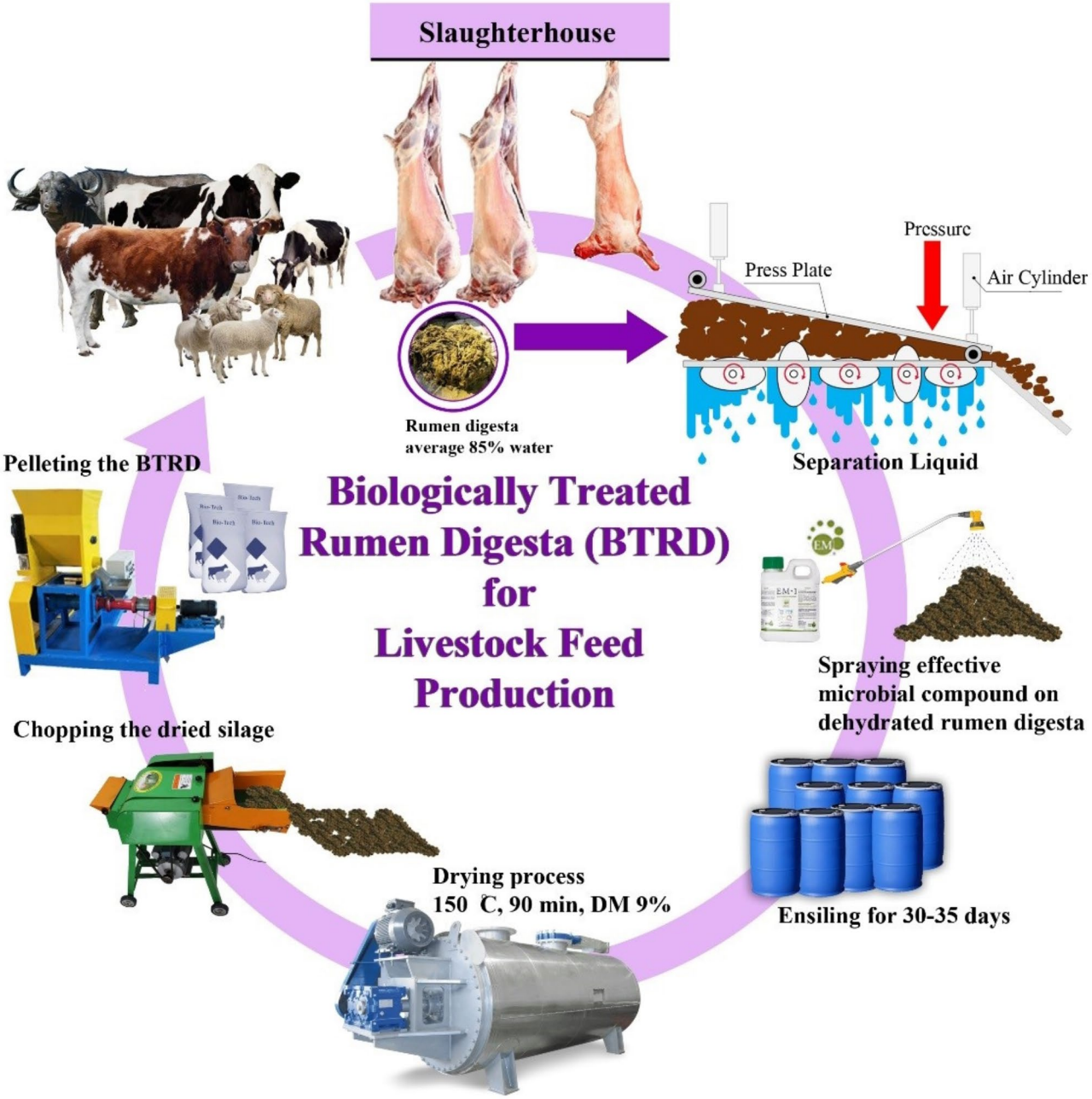

Fig. 1. The process of biologically treatment of rumen digesta to produce animal feed.

Laboratory analyses and calculations

To establish DM and carry out chemical analysis on fecal samples, the samples were thawed and dried at 60 °C for 72 h. Subsequently, we ground the dried samples of feed and feces in a Willey mill that had a 1-mm sieve. Feed and feces were measured according to AOAC procedures for DM, ash, EE, and CP[18]. Fibertec™ (Foss, Denmark) fiber analyzers were used to determine NDF, which incorporated sodium sulfite in addition to heat-stable amylase as described by Van Soest et al.[19]. An analysis of lignin content was conducted according to Van Soest[20]. Daily DMI was calculated by subtraction of DM offered from DM refused. Feed conversion ratio (FCR) was calculated as DMI/ADG. In order to calculate nutrient digestibility coefficients, Acid-insoluble ash (AIA) were calculated according to Van Keulen and Young[21]. Following thawing, frozen ruminal samples were centrifuged at 10,000 × g for 10 min at 4 °C. According to Broderick and Kang[22], NH_3–N was measured using a phenol-hypochlorite assay at 630 nm. The concentration of ruminal VFA was determined through the use of a 15 m (0.53 mm i.d.) fused silica column (DBFFAP column; J & W Scientific, Folsom, CA) coupled with gas chromatography (Varian 3700; Varian Specialties Ltd., Brockville, Ontario, Canada). An automated analyzer (Abbott, model Alcyon 300, USA) was used to determine plasma metabolites (Pars Azmun Inc., Tehran, Iran).

	Treatments[1]			
		Supplemented with BTRD		
	Un-supplemented	BTRD -10	BTRD -20	BTRD-30
Diet ingredients, % DM				
Alfalfa hay	0.89	0.88	0.86	0.88
Corn silage	3.54	3.54	3.50	3.35
Wheat bran	13.55	20.04	14.84	17.33
Ground corn	27.10	17.68	16.02	8.03
Ground barley	31.23	26.82	24.18	21.28
Soybean meal	20.33	17.68	17.48	15.95
BTRD	0.00	10.02	19.88	30.02
Sodium monensin	0.03	0.03	0.03	0.03
Calcium carbonate	0.95	0.94	0.86	0.94
Sodium bicarbonate	0.59	0.59	0.59	0.56
Salt	0.59	0.59	0.59	0.47
Vitamin and mineral premix	1.19	1.18	1.16	1.15
Energy content and chemical composition				
NEm, Mcal/kg	1.61	1.52	1.55	1.50
NEg, Mcal/kg	1.02	0.93	0.95	0.91
Crude protein, % DM	17.40	17.30	17.30	17.40
Neutral detergent fiber, % DM	20.20	23.90	23.00	25.20
Acid detergent fiber, % DM	11.16	12.23	12.10	13.50
Lignin, % DM	1.31	1.59	1.57	1.77
Lignin, % NDF	6.49	6.65	6.83	7.00
Ether extract, % DM	2.90	3.00	2.90	2.90
Ash, % DM	5.60	5.50	5.20	5.00

Table 1. Ingredients and chemical components of the diets used in the experiment. [1]Control = diet with no supplemented BTRD, BTRD-10 = diet supplemented with 10% BTRD, BTRD-20 = diet supplemented with 20% BTRD, and BTRD-30 = diet supplemented with 30% BTRD.

Statistical analyses

The data was analyzed using SAS's GLM procedure (version 9.4, SAS) with a randomized complete design. The fixed effect of treatment were modeled as follows:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij},$$

where Y_{ij} = the response of animal j fed diet i, μ = the overall mean, T_i = a fixed effect of BTRD supplemented diet i, and ε_{ij} = residual error associated with each Y_{ij}. The least squares means with a significant F-test ($P < 0.05$) were compared using PDIFF of SAS. The responses of male calves to increasing addition levels of BTRD were examined using orthogonal linear and quadratic polynomial contrasts. Statistical significance was declared at $P \leq 0.05$ and trends at $P = 0.05$ to $P \leq 0.10$.

The experiment data spanning from 1 to 180 days were measured multiple times and analyzed using SAS MIXED procedures. The Compound Symmetry covariance structure [CS] was selected after evaluating several covariance structures based on the smallest Akaike's information criterion. The animal within treatments was considered the SUBJECT for repeated measures, with period included in the following model:

$$Y_{ijkl} = \mu + T_i + A\,(T)_{ij} + P_k + P * T_{ik} + \varepsilon_{ijkl},$$

where Y_{ijkl} = the response of animal j fed diet i at period k, μ = the overall mean, T_i = a fixed effect of BTRD supplemented diet i as treatment, $A(T)_{ij}$ = a random effect of animal j fed BTRD supplemented diet i, P_k = effect of period as time, P^*T_{ik} = intraction effect between time and treatment, and ε_{ijkl} = residual error associated with each Y_{ijkl}. In the analysis, the macro PDMIX800.sas[23] was used with a significance level set at $\alpha = 0.05$ to compare mean values using least squared means, resulting in grouped letter outcomes.

Results

Nutrient intake and digestibility

There was no problem with palatability when BTRD was added to the diet of steer calves. However, the addition of BTRD did not have a significant impact on the intake of dry matter and organic matter throughout the entire experiment (1–180 days). On the other hand, it did improve the intake of NDF and ADF ($P < 0.01$). When BTRD was included in the diet by 20%, there was a significant improvement in the digestibility of DM and OM for all periods ($P < 0.05$). Replacing 10% of the control diet with BTRD did not result in any significant changes in DM digestibility, but a 20% inclusion led to an improvement (Table 2). The urine pH of the calves remained similar at all levels of BTRD inclusion during the first 60 days, but increased with BTRD inclusion in the subsequent periods (Table 3). In comparison to the control group, the feces of the steers became wetter and looser as the supplementation levels increased ($P < 0.05$).

Animal growth performance

During the first 60 days of the experiment, the average daily gains of steer calves were not affected, but increased from 61 to 120 days of the experiment (Table 3, Fig. 2). Holstein steers fed BTRD did not suffer any adverse effects in terms of final weight or daily gain. There was an improvement in final weight and feed efficiency (ADG/DMI) in calves fed 20% BTRD for all periods. Moreover, this level of inclusion resulted in a decreased ($P = 0.01$) feed conversion ratio during 61 to 120 days.

Rumen fermentation characteristics

Elevating dietary BTRD levels resulted in an increase in rumen pH across all time periods. Increasing dietary BTRD levels led to an increase in rumen pH across all time periods. The ruminal NH_3-N concentration experienced a slight decrease with the increase in BTRD levels during the 61–120 or 121–180 periods. Throughout this research, the total VFA production, as well as the proportions of acetate, propionate, butyrate, and valerate, and the acetate: propionate ratio in the rumen fluid of steer calves fed BTRD supplemented diets, did not show any significant difference ($P > 0.05$) when compared to those on a control diet (Table 4).

Plasma metabolites

The addition of BTRD did not have a significant impact on plasma glucose levels (Table 5). Triglyceride levels were consistent across all treatment groups. However, plasma cholesterol and HDL levels were higher in diets supplemented with BTRD, with an increase observed as the amount of BTRD included in the diet increased. Plasma total protein, creatinine, and urea-N concentrations remained unaffected by the supplementation. Interestingly, the concentration of uric acid decreased in a linear fashion ($P = 0.02$) in steers that were fed BTRD-included diets. Steers that received BTRD supplements exhibited lower malondialdehyde (MDA) concentrations compared to those on a control diet. The total antioxidant capacity was similar for diets with 10% and 20% BTRD inclusion when compared to the control, but it decreased linearly ($P < 0.05$) with a 30% inclusion of BTRD.

Discussion

Nutrient intake and digestibility

No health issues, such as diarrhea or other negative health impacts linked to the supplementary diets, were observed in any of the calves during the experiment. The same result was noted in cows by Cherdthong et al.[5] and Seankamsorn et al.[6], and in sheep by Olafadehan et al.[8], and Osman and Abass[9] when incorporating dried rumen digesta into the basal diet. The lack of substantial influence from dietary supplementation of BTRD on DM and OM intake over the experiment corresponds with the conclusions of multiple studies that reported no significant difference in DM and OM digestibility when varying levels of DRD were administered to sheep[8,12,24]. The process of pelleting diets based on plant leaf meal has been shown to enhance the digestibility of various nutrients and positively influence rumen ecology in ruminants[25,26].

Additionally, a study conducted by Mondal et al.[24] revealed that Bengal goats, when provided with a diet containing 10% DRD supplementation, did not exhibit any noteworthy variations in their dry matter intake. In a previous investigation by Wanapat et al.[27], it was observed that the inclusion of 50, 100, and 150 g of dried rumen pellets per day in the diet of swamp buffalo resulted in significant disparities in the overall feed intake across different treatments. Notably, the highest intake was recorded at the 150 g inclusion level. The digestibility of DM was significantly depressed (73.4 vs. 74.4%) when 30% of the diet was supplied by BTRD ($P < 0.05$). Similarly, dietary crude protein intake was similar for treatments when it was calculated for all over (1–180 d) the experiment, while CP digestibility was decreased by 30% inclusion of BTRD. Utomo et al.[28] investigated replacing Napier grass in the diet of Ongole crossbred cattle with rumen digesta silage at 0, 33, 67, and 100% and reported that feed intake was not influenced by 33 and 67% but depressed by 100% replacement level. Similar to our result, in a study conducted by Patra and Ghosh[29], replacing para grass in growing kids diet with dried rumen digesta (DRD) at 0, 25, 50 and 100% revealed that DM digestibilities did not differ by the inclusion of 25 and 50% DRD but it was depressed significantly by 100% replacement level. The increased ADF and lignin content in the diet likely contributed to this outcome. However, the increased NDF intake at the present study was inconsistence with Seankamsorn et al.[6], who evaluated the inclusion of 0, 50, 100, and 150 g DM/d dried rumen digesta in buffalo and reported that NDF and ADF intake were not influenced as inclusion level of dried rumen digesta increased. Nevertheless, investigations conducted on 16S ribosomal DNA sequences and real-time PCR to assess the abundance of cellulolytic bacteria have demonstrated a rise in *R. flavefaciens* and the overall population of bacteria in the rumen of beef cattle four hours after feeding, as soybean meal is gradually replaced with increasing levels of DRD. Additionally, the incorporation of rumen digesta barley meal, constituting 25%

	Treatment[1]				SEM	*P-value*		
		Supplemented with BTRD						
	Un-supplemented	BTRD-10	BTRD-20	BTRD-30		Linear	Quadratic	Cubic
Dry matter								
Intake, kg/d								
1–60 d	3.10	3.11	3.04	3.14	0.036	0.77	0.23	0.12
61–120 d	3.94^{a}	3.82^{b}	3.75^{c}	3.93^{a}	0.010	< 0.01	< 0.0001	< 0.001
121–180 d	5.42^{a}	5.46^{a}	5.33^{b}	5.46^{a}	0.015	0.99	< 0.01	< 0.001
1–180 d	4.15	4.13	4.04	4.17	0.202	0.97	0.69	0.75
Digestibility, %								
1–60 d	75.70^{ab}	74.07^{b}	76.73^{a}	70.54^{c}	0.603	0.63	0.04	< 0.001
61–120 d	74.04^{b}	73.48^{b}	76.20^{a}	68.84^{c}	0.634	0.05	0.06	< 0.001
121–180 d	73.49^{b}	72.58^{b}	74.41^{a}	67.83^{c}	0.654	0.10	0.04	< 0.0001
1–180 d	74.41^{b}	73.38^{b}	76.11^{a}	69.07^{c}	0.389	0.02	< 0.01	< 0.0001
Organic matter								
Intake, kg/d								
1–60 d	2.92^{ab}	2.98^{a}	2.88^{b}	2.94^{ab}	0.034	0.48	0.22	0.13
61–120 d	3.72^{a}	3.73^{a}	3.56^{c}	3.61^{b}	0.010	0.40	< 0.0001	< 0.001
121–180 d	5.11^{b}	5.19^{a}	5.05^{c}	5.16^{a}	0.014	0.09	< 0.01	< 0.0001
1–180 d	3.92	3.97	3.83	3.90	0.191	0.94	0.69	0.76
Digestibility, %								
1–60 d	76.92^{b}	78.79^{b}	83.63^{a}	76.54^{b}	0.927	< 0.01	0.02	< 0.0001
61–120 d	77.30^{b}	78.70^{b}	83.65^{a}	76.84^{b}	0.924	0.01	0.02	< 0.0001
121–180 d	77.79^{b}	79.28^{b}	83.95^{a}	77.42^{b}	0.902	0.01	0.02	< 0.01
1–180 d	77.34^{c}	78.93^{c}	83.74^{a}	76.93^{c}	0.509	< 0.0001	< 0.0001	< 0.0001
Crude protein								
Intake, kg/d								
1–60 d	0.54^{ab}	0.55^{a}	0.53^{b}	0.54^{ab}	0.006	0.77	0.10	0.12
61–120 d	0.69^{a}	0.68^{a}	0.65^{c}	0.66^{b}	0.001	< 0.01	< 0.0001	< 0.001
121–180 d	0.94^{a}	0.95^{a}	0.92^{b}	0.94^{a}	0.003	0.99	< 0.0001	< 0.0001
1–180 d	0.72	0.73	0.70	0.71	0.035	0.97	0.61	0.75
Digestibility, %								
1–60 d	72.73	70.80	72.57	72.06	1.123	0.30	0.63	0.49
61–120 d	74.78^{a}	71.69^{b}	74.90^{a}	75.25^{a}	1.028	0.04	0.08	0.66
121–180 d	77.64^{a}	73.87^{b}	75.44^{ab}	76.09^{ab}	0.993	0.01	0.99	0.68
1–180 d	75.05^{a}	72.12^{b}	74.30^{a}	74.46^{a}	0.668	< 0.01	0.23	0.41
Neutral detergent fiber								
Intake, kg/d								
1–60 d	0.63^{d}	0.79^{a}	0.70^{c}	0.74^{b}	0.008	< 0.0001	0.14	< 0.0001
61–120 d	0.80^{d}	0.99^{a}	0.86^{c}	0.91^{b}	0.002	< 0.0001	0.08	< 0.0001
121–180 d	1.09^{d}	1.38^{a}	1.23^{c}	1.30^{b}	0.004	< 0.0001	< 0.0001	< 0.0001
1–180 d	0.84^{b}	1.05^{a}	0.93^{ab}	0.99^{a}	0.047	< 0.01	0.79	0.07
Digestibility, %								
1–60 d	59.42^{a}	55.35^{bc}	53.12^{c}	56.64^{b}	0.857	< 0.001	< 0.01	0.10
61–120 d	58.27^{a}	53.85^{bc}	53.80^{c}	56.19^{ab}	0.874	< 0.0001	0.08	0.15
121–180 d	56.28^{a}	55.65^{a}	51.97^{b}	52.76^{b}	0.910	0.51	< 0.0001	0.67
1–180 d	57.99^{a}	54.95^{b}	52.63^{c}	55.20^{b}	0.544	< 0.0001	< 0.0001	0.06
Acid detergent fiber								
Intake, kg/d								
1–60 d	0.35^{d}	0.38^{b}	0.37^{c}	0.42^{a}	0.004	< 0.0001	0.02	< 0.0001
61–120 d	0.44^{d}	0.47^{b}	0.45^{c}	0.53^{a}	0.002	< 0.0001	< 0.0001	< 0.0001
Continued								

	Treatment[1]							
		Supplemented with BTRD				*P-value*		
	Un-supplemented	BTRD-10	BTRD-20	BTRD-30	SEM	Linear	Quadratic	Cubic
121–180 d	0.60[d]	0.67[b]	0.64[c]	0.74[a]	0.001	< 0.0001	< 0.0001	< 0.0001
1–180 d	0.46[d]	0.51[b]	0.49[c]	0.56[a]	0.003	< 0.0001	< 0.0001	< 0.0001
Digestibility, %								
1–60 d	51.70[a]	50.36[a]	47.25[b]	47.92[b]	0.695	< 0.0001	0.16	0.08
61–120 d	43.24[a]	43.74[a]	40.15[b]	35.99[c]	0.809	< 0.0001	0.01	0.34
121–180 d	40.81[b]	48.92[a]	40.15[bc]	38.01[c]	0.764	< 0.0001	< 0.0001	< 0.0001
1–180 d	45.25[b]	47.68[a]	42.52[c]	40.67[c]	1.069	< 0.0001	0.01	0.01
Urine pH								
1–60 d	6.22	6.25	6.21	6.16	0.049	0.51	0.34	0.62
61–120 d	5.97[b]	6.51[a]	6.49[a]	6.53[a]	0.127	< 0.01	0.04	0.26
121–180 d	6.43[c]	6.62b[c]	7.02[a]	6.71[b]	0.097	0.05	< 0.01	0.10
1–180 d	6.20[b]	6.46[a]	6.57[a]	6.47[a]	0.072	< 0.01	0.01	0.84
Fecal score, 1–5								
1–60 d	1.85[b]	1.96[a]	1.91[ab]	1.97[a]	0.034	0.07	0.33	0.06
61–120 d	1.94[c]	2.04[ab]	2.12[a]	2.00[bc]	0.029	< 0.01	0.02	0.06
121–180 d	2.03[c]	2.43[a]	2.26[ab]	2.22[b]	0.063	< 0.01	< 0.01	0.19
1–180 d	1.94[b]	2.06[a]	2.15[a]	2.06[a]	0.039	< 0.01	0.02	0.49

Table 2. Influence of dietary supplementation of biologically treated rumen digesta (BTRD) on intake, apparent total-tract digestibility of nutrient, and fecal characteristic in Holstein steer calves during 180 days of the experiment, presented as least square mean with standard error of the mean (SEM) and P-value. [1]Control = diet with no supplemented BTRD, BTRD-10 = diet supplemented with 10% BTRD, BTRD-20 = diet supplemented with 20% BTRD, and BTRD-30 = diet supplemented with 30% BTRD. [ab] Values within a row with different superscripts differ significantly at $P \leq 0.05$.

DM, into a concentrate diet has the potential to enhance the bacterial community in the rumen and increase the digestible CP in growing lambs, as suggested by Abouhief et al.[4].

Animal growth performance

Measuring growth performance in livestock is crucial for assessing economic traits such as average daily gain and feed conversion ratio. These traits are influenced by various factors including breed, diet, and management practices[30]. In the case of steers fed a 20% BTRD diet, their ADG was found to be higher due to the increased digestibility of DM and OM, resulting in improved FCR. The observed daily weight gains in these steers closely align with the range of 0.72–1.10 kg/d reported by Utomo et al.[28] in Ongole crossbred steers. Moreover, the research conducted by Cherdthong et al.[5] demonstrated that substituting soybean meal with dried rumen digesta at varying levels did not affect total feed intake in beef steers. Likewise, Abouhief et al.[4] observed no significant differences in daily dry matter intake, final body weight, and carcass weight in Najdi lambs when dried rumen digesta replaced barley mixture at different levels. The research conducted by Mondal et al.[24] and Osman and Elimam[31] demonstrated that the inclusion of increasing levels of DRD at 0, 10, and 20% did not have any negative impact on the final weight or daily weight gain of lambs. Similarly, Osman and Abass[9] observed a comparable trend in Sudan desert lambs that were fed concentrate diets containing DRD at the same levels. These consistent weight gains suggest that the BTRD-supplemented diets provided sufficient nutrition to meet the needs of the experimental steers. Consequently, although it is important to note that a careful approach is necessary when drawing conclusions from the results due to the limited number of observations in the trial, BTRD could serve as a valuable source of nutrients for ruminant livestock producers.

Rumen fermentation characteristics

The incorporation of dried rumen digesta instead of soybean meal at varying levels in Thai native beef steers did not have an impact on rumen fermentation characteristics, leading to comparable pH, NH_3-N, and total or individual VFA levels as reported by Cherdthong et al.[5]. During the initial phase, pH values fell within the range of 5.61 to 5.93, aligning with findings by Osman and Elimam[31] where ruminal pH in calves ranged from 5.94 to 5.97. The elevated ruminal pH with increasing dietary BTRD levels across all time periods was in consistency with observations of Dey et al.[32] who reported that ruminal pH was increased with inclusion of 10% dietary rumen content in kids. Allen[33] indicated a slight negative correlation between the concentration of volatile fatty acids in the rumen and the pH level of the rumen, noting that this relationship is influenced by dietary differences in the removal, buffering, and neutralization of acids present in the rumen. The incorporation of BTRD pellets into the diet seems to have resulted in changes to the NDF and likely the PeNDF content, which

	Treatment[1]							
		Supplemented with BTRD			SEM	*P-value*		
	Un-supplemented	BTRD-10	BTRD-20	BTRD-30		Linear	Quadratic	Cubic
Average daily gain, kg/d								
1–60 d	1.00	1.01	1.19	1.04	0.069	0.34	0.27	0.12
61–120 d	0.98^{ab}	0.96^{ab}	1.03^{a}	0.90^{b}	0.025	0.19	0.04	0.02
121–180 d	1.00	0.99	1.06	0.83	0.029	0.41	0.70	0.04
1–180 d	1.00^{ab}	0.99^{b}	1.10^{a}	0.93^{b}	0.038	0.45	0.01	0.01
ADG/DMI								
1–60 d	0.32^{b}	0.32^{b}	0.39^{a}	0.33^{ab}	0.021	0.32	0.18	0.05
61–120 d	0.24^{bc}	0.25^{b}	0.28^{a}	0.23^{c}	0.007	0.33	0.01	0.01
121–180 d	0.19^{ab}	0.18^{b}	0.20^{a}	0.15^{c}	0.005	0.01	0.01	0.01
1–180 d	0.25	0.25	0.29	0.24	0.021	0.96	0.25	0.20
Feed conversion ratio								
1–60 d	3.11	3.29	2.55	3.02	0.270	0.43	0.61	0.11
61–120 d	4.03^{ab}	3.99^{b}	3.64^{c}	4.35^{a}	0.105	0.22	0.01	0.01
121–180 d	5.40^{bc}	5.53^{b}	5.02	6.57	1.164	0.01	0.01	0.01
1–180 d	4.18	4.27	3.74	4.64	0.349	0.58	0.25	0.20

Table 3. Influence of dietary supplementation of biologically treated rumen digesta (BTRD) on growth performance in Holstein steer calves during 180 days of the experiment, presented as least square mean with standard error of the mean (SEM) and P-value. [1]Control = diet with no supplemented BTRD, BTRD-10 = diet supplemented with 10% BTRD, BTRD-20 = diet supplemented with 20% BTRD, and BTRD-30 = diet supplemented with 30% BTRD. [ab] Values within a row with different superscripts differ significantly at $P \leq 0.05$.

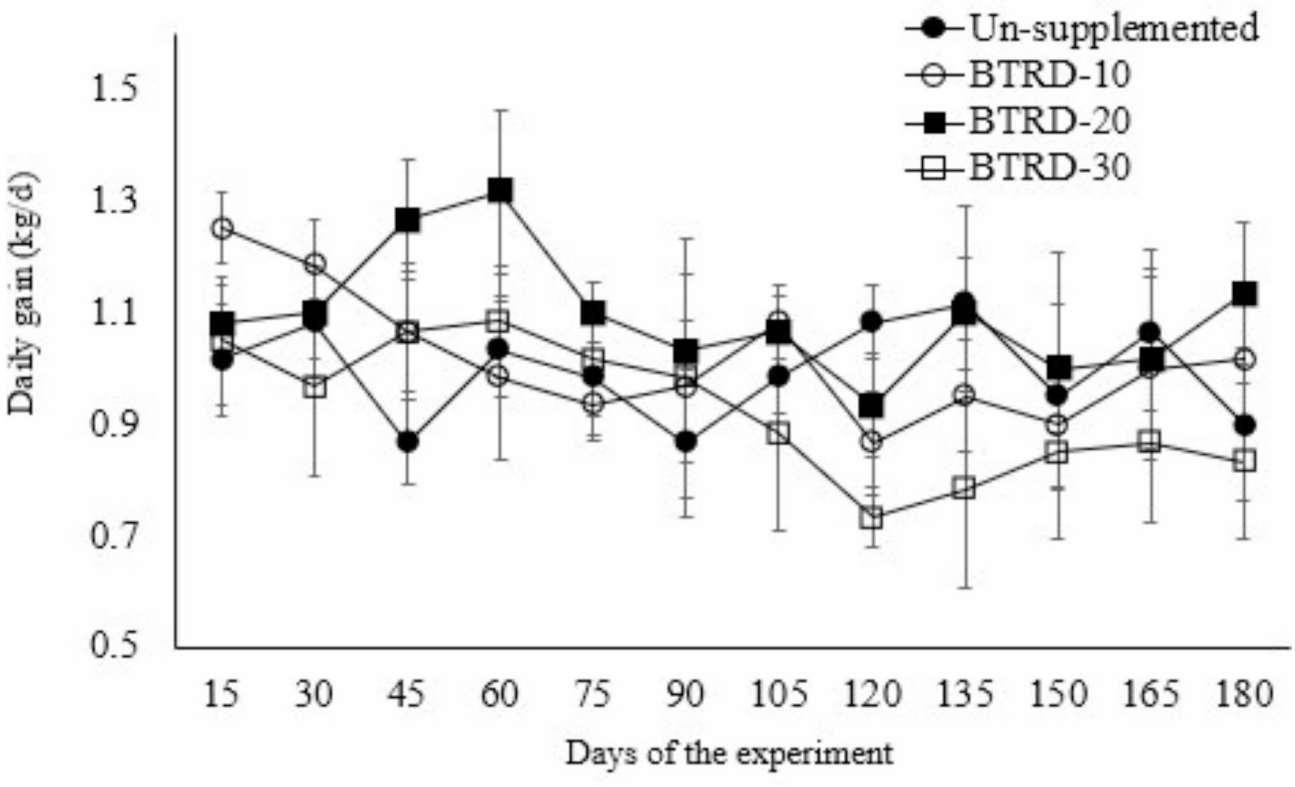

Fig. 2. Average daily gain (kg/d) by age in calves fed different dietary treatments (Control, BTRD-10, BTRD-20, and BTRD-30). Control = diet with no supplemented BTRD (●), BTRD-10 = diet supplemented with 10% BTRD(○), BTRD-20 = diet supplemented with 20% BTRD(▪), and BTRD-30 = diet supplemented with 30% BTRD(□).

	Treatment[1]							
		Supplemented with BTRD				*P-value*		
	Un-supplemented	BTRD-10	BTRD-20	BTRD-30	SEM	Linear	Quadratic	Cubic
Ruminal pH								
1–60 d	5.61^{c}	5.68^{bc}	5.93^{a}	5.84^{ab}	0.072	< 0.01	0.25	0.12
61–120 d	5.81^{c}	5.98^{b}	6.30^{a}	6.33^{a}	0.410	< 0.001	0.08	0.03
121–180 d	6.13^{b}	6.16^{b}	6.32^{ab}	6.43^{a}	0.067	< 0.01	0.56	0.51
1–180 d	5.85^{b}	5.94^{b}	6.18^{a}	6.20^{a}	0.056	< 0.001	0.49	0.13
NH_3–N, mg/dl								
1–60 d	13.10^{bc}	13.42^{ab}	13.69^{a}	12.82^{c}	0.152	0.41	< 0.001	0.12
61–120 d	11.65^{a}	10.74^{b}	10.37^{b}	10.39^{b}	0.352	0.04	0.20	0.14
121–180 d	9.94^{a}	8.33^{c}	7.44^{d}	8.87^{b}	0.068	< 0.001	< 0.001	< 0.001
1–180 d	11.14	10.83	10.50	11.11	0.429	0.83	0.28	0.62
Total VFA, m*M*	70.40	68.91	69.62	71.12	1.123	0.57	0.21	0.78
Individual VFA, mol/100 mol								
Acetate	52.80	53.68	53.15	53.08	1.184	0.95	0.69	0.73
Propionate	33.94	33.32	33.55	32.92	0.751	0.42	0.99	0.63
Butyrate	9.29	9.01	9.37	10.02	0.723	0.45	0.53	0.91
Isobutyrate	1.68	1.73	1.66	1.71	0.138	0.97	0.99	0.69
Isovalerate	1.48	1.43	1.42	1.45	0.190	0.12	0.83	0.72
Valerate	0.81	0.83	0.88	0.85	0.101	0.71	0.82	0.78
Acetate:Propionate	1.63	1.61	1.58	1.59	0.051	0.57	0.70	0.81

Table 4. Influence of dietary supplementation of biologically treated rumen digesta (BTRD) on rumen fermentation characteristics in Holstein steer calves during 180 days of the experiment, presented as least square mean with standard error of the mean (SEM) and P-value. [1]Control = diet with no supplemented BTRD, BTRD-10 = diet supplemented with 10% BTRD, BTRD-20 = diet supplemented with 20% BTRD, and BTRD-30 = diet supplemented with 30% BTRD. [ab] Values within a row with different superscripts differ significantly at $P \leq 0.05$.

could affect the ruminal retention rate and stratification of digesta in the rumen. This alteration may promote ruminating behavior and enhance salivary secretion, consequently decreasing the sensitivity of ruminal pH to changes in volatile fatty acid concentrations.

Studies by Südekum et al.[34] and Calabrò et al.[35] revealed that in the rumen fluid, NH_3–N is acquired through various processes such as feed intake, fermentation of feed protein, or hydrolysis of urea by ureolytic rumen bacteria. This NH_3–N is then absorbed through the rumen wall, passes out of the rumen, and is utilized by microbes. The optimal concentration of NH_3-N in the rumen for efficient fermentation and microbial growth is influenced by the type and level of fermented energy in the diet. Lower concentrations of ruminal NH_3-N indicate that rumen microbes are better equipped to utilize NH_3-N, as observed by Saxena et al.[36]. NH_3-N concentrations in rumen fluid were observed to vary between 7.44 and 13.69 mg/dl across different periods in our study. This result is in consistent with the findings of McDonald et al.[37], who proposed that NH_3-N levels exceeding 5 mg/dl are beneficial for improving rumen ecology, microbial protein production, nutrient absorption, and feed consumption. Notably, the optimal NH_3-N concentrations differed for grain and roughage digestion. Song and Kennelly[38] found that an increase in rumen NH_3-N levels on a silage diet resulted in higher mixed bacterial numbers and altered fermentation patterns in non-lactating Holstein cows. However, the extent of ruminal degradation was not affected by this change. VFAs, which are generated in the rumen through the fermentation of OM, can have a significant impact on ruminant livestock production and the composition of their products. This has been evidenced in research conducted by Weisbjerg and Børsting[39] and Liu et al.[40]. The relative proportions of VFAs in the rumen are influenced by several factors, including the availability and composition of the substrate, the rate at which depolymerization takes place, and the specific bacterial species that are present. Weisbjerg et al.[41] further investigated these factors. The present study revealed that the mean total VFA concentrations observed in all treatments ranged from 68.9 to 71.1 mM, which closely aligns with the findings reported by Cherdthong et al.[5]. These results provide clear evidence that the inclusion of BTRD in the diet of steer calves can effectively substitute both protein and energy sources, up to a maximum of 30%, without any statistically significant detrimental effects on VFA concentration. Consequently, the utilization of BTRD presents a promising approach to mitigate the environmental impact associated with waste incineration or landfill usage, by replacing imported commercial feedstuffs. Additionally, it offers the advantage of reducing energy consumption related to the transportation of imported feedstuffs.

Plasma metabolites

Blood metabolite concentrations serve as crucial indicators of nutrient supply in animals, reflecting their nutritional status. The consistent plasma glucose levels across treatments may be due to the unaffected dry

	Treatment[1]							
		Supplemented with BTRD				*P-value*		
	Un-supplemented	BTRD-10	BTRD-20	BTRD-30	SEM	Linear	Quadratic	Cubic
Glucose, mg/dl	94.00	94.50	90.25	91.25	4.119	0.51	0.95	0.59
Cholesterol, mg/dl	71.75^{b}	80.75^{ab}	98.75^{a}	90.00^{ab}	6.131	0.02	0.17	0.22
Triglyceride, mg/dl	14.75	18.25	14.75	17.00	2.171	0.74	0.77	0.21
HDL, mg/dl	35.00^{b}	40.50^{ab}	49.00^{a}	40.50^{ab}	3.599	0.14	0.07	0.23
Total protein, g/dl	7.10	6.98	7.35	6.85	0.229	0.72	0.43	0.21
Creatinine, mg/dl	0.16	0.20	0.12	0.10	0.054	0.30	0.58	0.53
Urea-N, mg/dl	19.25	16.75	20.75	18.50	1.763	0.83	0.94	0.13
Uric acid, mg/dl	1.63^{a}	1.33^{ab}	1.00^{ab}	0.75^{b}	0.261	0.02	0.92	0.93
BHBA, mmol/l	0.66	0.51	0.58	0.54	0.052	0.23	0.30	0.16
TAC, UI/l	0.82^{a}	0.75^{ab}	0.68^{ab}	0.56^{b}	0.079	0.03	0.77	0.85
MDA, nmol/ml	4.25^{a}	3.58^{ab}	2.63^{ab}	2.28^{b}	0.574	0.02	0.78	0.74
Enzymes								
AST, UI/l	91.00	94.25	90.25	74.50	11.63	0.32	0.43	0.93
ALT, UI/l	27.00	28.75	28.00	31.00	1.700	0.16	0.72	0.43
ALP, UI/l	437.47	349.75	353.00	395.00	86.357	0.75	0.47	0.89
GGT, UI/l	10.50	9.50	18.75	15.50	3.134	0.10	0.72	0.13
Ions								
Calcium, mg/dl	11.08	11.83	11.28	11.63	0.437	0.58	0.66	0.28
Phosphorus, mg/dl	7.50	7.70	7.45	7.28	5.164	0.52	0.56	0.72
Magnesium, mg/dl	2.07	1.88	2.07	2.35	0.155	0.17	0.15	0.67

Table 5. Influence of dietary supplementation of biologically treated rumen digesta (BTRD) on plasma metabolites in Holstein steer calves, presented as least square mean with standard error of the mean (SEM) and P-value. [1]Control = diet with no supplemented BTRD, BTRD-10 = diet supplemented with 10% BTRD, BTRD-20 = diet supplemented with 20% BTRD, and BTRD-30 = diet supplemented with 30% BTRD. TAC = total antioxidant capacity, AST = aspartate aminotransferase, ALT = alanine transaminase, ALP = alkaline phosphatase, GGT = gamma-glutamyl transferase. [ab] Values within a row with different superscripts differ significantly at $P \leq 0.05$.

matter intake (DMI) with dietary inclusion of BTRD, suggesting similar gluconeogenesis in steer calves through the incorporation of rumen digesta silage up to 30% of their diet. Rezai-Sarteshnizi et al.[42] observed decreased ruminal urea N and enhanced milk protein yield in Holstein dairy cows with 60 g dried rumen digesta. Urea nitrogen levels in plasma are indicative of protein degradability in the rumen and amino acid absorption post-ruminal. The concentration of ruminal NH_3-N decreased with increasing levels of BTRD during each period, but remained similar across all treatments when considering the overall period of 1–180 days. Interestingly, there were no significant differences in blood urea-N levels among the groups fed with BTRD. These findings align with a previous study conducted by Seankamsorn et al.[6], which investigated the inclusion of sun-dried rumen digesta in the diet of buffalo at varying levels. The study demonstrated that the reduction in rumen degradable protein resulting from the inclusion of soybean meal in the control diet was effectively compensated for by the dietary supplementation of BTRD, leading to a comparable nitrogen status in the serum across all treatments. According to a study conducted by Khattab et al.[43], there were no significant differences observed in the serum total protein levels of goats that were fed enzyme-treated and untreated dried rumen digesta. Similarly, the hematological indexes and serum metabolite values of lambs that were fed the DRD diet were found to be within normal ranges, as reported by Aruwayo et al.[44]. In another study by Oluwafemi and Iliyasu[45], it was discovered that rabbits fed DRD, with or without the addition of enzymes, did not experience any adverse effects on their serum total protein levels. The antioxidant capacity of a cell reflects the redox state of cells and the oxidation of biomolecules. Malondialdehyde, a biomarker of lipid peroxidation in tissues, serves as an indicator of oxidative stress and peroxidation of polyunsaturated fatty acids, as highlighted by Asghari et al.[46]. As a result of BTRD inclusion, cows had enhanced total antioxidant capacity due to decreased levels of oxidized MDA. To determine liver integrity, blood levels of liver enzymes such as ALT, AST, and GGT are measured. The similar liver enzymes indicate that BTRD up to 30% in the diet did not compromise liver function. In the present study, AST and ALT activity were comparable to that in the previous study on Holstein steers[47]. A higher level of these blood markers indicates that hepatocytes have been damaged and therefore liver function is impaired. Cebra et al.[48] report that AST and GGT activities are most frequently measured in dairy cows during early lactation when there is fatty liver syndrome, poor appetite, and the appearance of ketosis[49]. During oxidative stress, peroxidation damages the fatty acids in hepatocytes' cell walls[46]. Inclusion of BTRD led to an improvement in cows' overall antioxidant capacity by reducing oxidized MDA levels. Liver health was evaluated through the measurement of liver enzymes such as ALT, AST, and GGT in the blood. The consistent levels of these enzymes suggest that incorporating BTRD up to 30% in the diet did not have a negative impact on liver function. The current study's findings on

AST and ALT activity were in line with a previous investigation involving Holstein steers[47]. Elevated levels of these blood markers are indicative of hepatocyte damage and impaired liver function. AST and GGT activities are commonly assessed in dairy cows during early lactation, particularly in cases of fatty liver syndrome, reduced appetite, and the onset of ketosis. Oxidative stress triggers peroxidation, leading to damage to the fatty acids in hepatocytes' cell membranes.

Conclusion

Despite the necessity of considering the limited observations per treatment in the trial for accurate interpretation of the results, it remains feasible to draw certain conclusions from the experimental evidence. The incorporation of BTRD into the diet has emerged as a viable option for Holstein beef cattle farmers. By including BTRD in the diet, the intake of NDF and ADF improved without negatively impacting rumen fermentation. Additionally, when BTRD was included in the diet by 20%, there was a significant increase in DM and OM digestibility, average daily gain, and feed efficiency ($P < 0.05$), while the feed conversion ratio decreased during the 60 to 120-day period. To enhance average daily gain and reduce feed conversion ratios, it is recommended to include BTRD up to 20% in the diets of beef steer calves. The positive effects of BTRD supplementation on oxidative parameters, as well as the performance and health status of Holstein steer calves, cannot be denied. Therefore, BTRD holds great potential as a valuable source of nutrients for the ruminant livestock industry.

Data availability

The data that support the findings of this study are available from the corresponding authors, upon reasonable request.

Received: 12 May 2024; Accepted: 5 December 2024
Published online: 28 December 2024

References

1. FAO U (2009) Global agriculture towards 2050. FAO Rome.
2. Godfray, H. C. J. et al. Food security: The challenge of feeding 9 billion people. *Science* **327**, 812–818 (2010).
3. Roslan, M. Y., Debbra, M. & Tan, T. L. Characterisation of wastewater quality from a local ruminant abattoir in Banting, Selangor, Malaysia, Malaysian. *J. Vet. Res.* **10**, 76–86 (2019).
4. Abouhief, M. A., Kraidees, M. S. & Al-Selbood, B. A. The utilization of rumen content-barley meal in diets of growing lambs. *Asian-Australas J. Anim. Sci.* **12**, 1234–1240 (1999).
5. Cherdthong, A. et al. Effects of replacing soybean meal with dried rumen digesta on feed intake, digestibility of nutrients, rumen fermentation and nitrogen use efficiency in Thai cattle fed on rice straw. *Livestock Sci.* **169**, 71–77 (2014).
6. Seankamsorn, A. et al. Effect of dried rumen digesta pellet levels on feed use, rumen ecology, and blood metabolite in swamp buffalo. *Trop. Animal Health Prod.* **49**, 79–86 (2017).
7. Fajemisin, A. N., Fadiyimu, A. & Alokan, J. A. Dry matter, fiber consumption and body weight gain of West African Dwarf sheep fed sun-dried of fermented rumendigesta- poultry droppings mixed diets. *Appl. Trop. Agricult.* **15**, 84–89 (2010).
8. Olafadehan, O. A., Okunade, S. A. & Njidda, A. A. Evaluation of bovine rumen contents as a feed for lambs. *Trop. Animal Health Prod.* **46**, 939–945 (2014).
9. Osman AAMO and Abass HAM. Processed animal waste as feed for Sudanese desert sheep. *Int. J. Adv. Multidiscipl. Res.* **2**, 12–17 (2015).
10. Okpanachi, U., Aribido, S. O. & Daikwo, I. S. Growth and haematological response of growing rabbits to diets containing graded levels of sun dried bovine rumen content. *Afr. J. Food Agricult. Nutr. Dev.* **10**, 11 (2010).
11. Ghosh, T. K. & Dey, A. Availability of rumen contents and their utilization in diets for goats. *Indian J. Anim. Sci.* **63**, 1194–1196 (1993).
12. Al-Wazeer, A. M. Effect of different levels of dried rumen content on nutrient intake, digestibility and growth performance of Awassi lambs. *Int. J. Adv. Res.* **4**, 2106–2113 (2016).
13. Khattab, H. M., Abdelmawla, S. M. & Singer, A. M. Nutritional evaluation of rumen contents as a slaughterhouse waste in sheep rations. *Egypt. J. Anim. Prod.* **33**, 173–186 (1996).
14. Jovanović, M. & Čuperlović, M. Nutritive value of rumen contents for monogastric animals. *Anim. Feed Sci. Technol.* **2**, 351–360 (1997).
15. Goodrich, R. D. & Meiske, J. C. The value of dried rumen contents as a ration ingredient for finishing steers. *Univ. Minnesota Beef Cattle Feeders Day Res. Rep.* **24**, 31–36 (1969).
16. Ireland-Perry, R. L. & Stallings, C. C. Fecal consistency as related to dietary composition in lactating Holstein cows. *J. Dairy Sci.* **76**, 1074–1082 (1993).
17. Woolsoncroft, M. A. et al. Effects of exercise and roughage source on the health and performance of receiving beef calves. *Prof. Animal Sci.* **34**, 183–191 (2018).
18. Association of Official Analytical Chemists International (2005) Official methods of analysis. 18th ed. AOAC Int., Gaithersburg, MD.
19. Van Soest, P. J., Robertson, J. B. & Lewis, B. A. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **74**, 3583–3597 (1991).
20. Van Soest, P. J. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *J. Assoc. Off. Agricult. Chem.* **46**, 829–835 (1963).
21. Van Keulen, J. & Young, B. A. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Animal Sci.* **44**, 282–287 (1977).
22. Broderick, G. A. & Kang, J. H. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* **63**, 64–75 (1980).
23. Saxton, A. M. A macro for converting mean separation output to letter groupings. In *Proceedings of 23rd SAS User Group International*. SAS Institute, Cary, NC. 1243–1246 (1998).
24. Mondal, S., Haldar, S., Samanta, I., Samanta, G. & Ghosh, T. K. Exploring nutritive potential of undigested rumen contents as an ingredient in feeding of goats. *Animal Nutr. Feed Technol.* **13**, 79–88 (2013).
25. Tan, N. D., Wanapat, M., Uriyapongson, S., Cherdthong, A. & Pilajun, R. Enhancing mulberry leaf meal with urea by pelleting to improve rumen fermentation in cattle. *Asian-Austral. J. Anim. Sci.* **25**, 452–461 (2012).
26. Hung, L. V., Wanapat, M. & Cherdthong, A. Effects of Leucaena leaf pellet on bacterial diversity and microbial protein synthesis in swamp buffalo fed on rice straw. *Livestock Sci.* **151**, 188–197 (2013).

27. Wanapat, M. et al. Comparative study between swamp buffalo and native cattle in feed digestibility and potential transfer of buffalo rumen digesta into cattle. *Asian-Austral. J. Animal Sci.* **16**, 504–510 (2003).
28. Utomo, R., Yusiati, L. M., Noviandi, C. T., Aryogi, A. & Isnandar, I. Rumen contents from slaughter house as alternative feed for replacing forage in ruminant diets. In *Proceedings of the 17th Asian-Australasian Association of Animal Production Societies Animal Science Congress*, Fukuoka, Japan, 715–717 (2016).
29. Patra, U. K. & Ghosh, T. K. Nutritive value of dry rumen contents and utilization and effect of their feeding on growth performance in Black Bengal goats. *Indian J. Anim. Sci.* **61**, 328–331 (1991).
30. White, R. R. & Capper, J. L. An environmental, economic, and social assessment of improving cattle finishing weight or average daily gain within US beef production. *J. Animal Sci.* **91**, 5801–5812 (2013).
31. Osman, A. A. & Elimam, M. E. Processed animal waste as a feed for Sudanese desert lamb. *Int. J. Adv. Multidiscipl. Res.* **2**, 12–17 (2015).
32. Dey, A., Chakraborty, N. & Ghosh, T. K. Utilization of undigested rumen content in kids. *Indian J. Animal Nutr.* **9**, 97–100 (1992).
33. Allen, M. S. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *J. Dairy Sci.* **80**, 1447–1462 (1997).
34. Südekum, K. H., Wolffram, S., Ader, P. & Robert, J. C. Bioavailability of three ruminally protected methionine sources in cattle. *Animal Feed Sci. Technol.* **113**, 17–25 (2004).
35. Calabrò, S. et al. Fermentation kinetics of sainfoin hay with and without PEG. *J. Animal Physiol. Animal Nutr.* **96**, 842–849 (2012).
36. Saxena, S. K., Otterby, D. E., Donker, J. D. & Good, A. L. Effect of alkali-treated oat straw supplemented with soybean meal or non-protein on growth of lambs and certain blood and rumen liquor parameters. *J. Animal Sci.* **33**, 485–490 (1971).
37. McDonald, P., Edwards, R. A., Greenhalgh, J. F. D. & Morgan, C. A. *Aninal Nutrition* (Longman Singapore Publisher, 1996).
38. Song, M. K. & Kennelly, J. J. Ruminal fementation pattern, bacterial population and rumen degradation of feed ingredients as influenced by ruminal ammonia concentration. *J. Animal Sci.* **68**, 1110–1120 (1990).
39. Weisbjerg, M. R. & Børsting, C. F. Influence of fat and feed level on fiber digestibility in vitro and in sacco and on volatile fatty acid proportions in the rumen. *Acta Vet. Scand. Suppl.* **86**, 137–139 (1989).
40. Liu, J. X., Wang, X. Q. & Shi, Z. Q. Addition of rice straw or/and wheat bran on composition, ruminal degradability and voluntary intake of bamboo shoot shells silage fed to sheep. *Animal Feed Sci. Technol.* **91**, 129–138 (2001).
41. Weisbjerg, M. R., Gado, H., Hvelplund, T. & Jensen, B. B. The effect of easily fermentable carbohydrates and pH on fibre digestibility and VFA pattern in an in vitro continuous culture system. *South Afr. J. Animal Sci.* **29**, 112–113 (1999).
42. Rezai-Sarteshnizi, F., Babaei, M. & Hajeyan, M. The effect of adding dried rumen contents on the performance and blood parameters of Holstein dairy cows. *Animal Sci. J.* **34**, 3–16 (2021).
43. Khattab, H. M., Gado, H. M., Kholif, A. E., Mansour, A. M. & Kholif, A. M. The potential of feeding goats sun dried Rumen Contents with or without bacterial inoculums as replacement for Berseem Clover and the effects on milk production and animal health. *Int. J. Dairy Sci.* **26**, 267–277 (2011).
44. Aruwayo, A., Maigandi, S. A., Malami, B. S. & Daneji, A. I. Performance of lambs fed fore-stomach digesta and poultry litter waste. *Niger. J. Basic Appl. Sci.* **15**, 86–93 (2007).
45. Oluwafemi, R. & Iliyasu, A. Effects of graded levels of rumen digesta based diets with or without enzyme supplementation on the blood chemistry of weaner rabbits. *J. Vet. Sci. Anim. Husb.* **1**, 43–46 (2016).
46. Asghari, M. et al. Effects of emulsified essential oils blend on performance, blood metabolites, oxidative status and intestinal microflora of suckling calves. *Anim. Feed Sci. Technol.* **277**, 114954 (2021).
47. Han, Z., Yang, B., Yang, Z. & Xi, Y. Effects of 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester on growth and blood components in growing Holstein steers. *Animal Sci. J.* **88**, 286–293 (2017).
48. Cebra, C. K., Garry, F. B., Getzy, D. M. & Fettman, M. J. Hepatic lipidosis in anorectic, lactating Holstein cattle: A retrospective study of serum biochemical abnormalities. *J. Vet. Internal Med.* **11**, 231–237 (1997).
49. Steen, A. Field study of dairy cows with reduced appetite in early lactation: Clinical examinations, blood and rumen fluid analyses. *Acta Vet. Scand.* **42**, 1–10 (2001).

Author contributions

KS, FPK, RK, and MG contributed to the project idea, design and execution of the study. SZG and RF were in charge of laboratory analyses. FPK and MG were responsible for writing the manuscript. VP and RK was responsible for re-writing, scientific editing and finalizing the manuscript.

Funding

This research was supported by University of Tehran.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to F.P.-K. or R.K.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.