



Effect of supplementation of postbiotics on dry matter intake, nutrient digestibility and rumen fermentation pattern in early lactating crossbred COWS[#]

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Abstract

A 90-day feeding trial was conducted to evaluate the effects of *Saccharomyces cerevisiae* postbiotics on dry matter intake, nutrient digestibility and rumen fermentation pattern in early-lactating crossbred cows. Twenty-four crossbred cows (within 10 days of calving) were randomly assigned to three groups: a control group fed a standard diet and two treatment groups receiving the standard diet supplemented with either 3g or 6g of postbiotic per animal. All animals were managed uniformly and fed according to ICAR (2013) standards. Weekly average daily dry matter intake was similar across three groups ($P>0.05$). A five-day digestibility trial conducted at the end of the experimental period revealed no significant differences ($P>0.05$) among the groups in the digestibility coefficients of dry matter, crude protein, ether extract, crude fibre, nitrogen-free extract, neutral detergent fibre and acid detergent fibre. Rumen pH and ammonia nitrogen levels were similar and remained within the normal range for all three treatment groups. However, the acetate proportion was significantly higher ($P<0.05$) in T1, while propionate was higher ($P<0.05$) in T3 compared to T1 and similar to T2. The results indicate that supplementation of *S. cerevisiae* postbiotics at 3 g or 6 g per animal does not significantly influence dry matter intake and nutrient digestibility in early lactating crossbred cows. However, it altered the proportion of ruminal volatile fatty acids.

Keywords: *Saccharomyces cerevisiae* postbiotics, early lactation, nutrient digestibility, rumen fermentation.

Early lactation is one of the most metabolically demanding phases in a dairy cow's lifecycle, marked by a sharp rise in nutrient and energy requirements to sustain milk production (Nayeri and Stothard, 2016). Feed intake often fails to meet these demands, resulting in negative energy balance and predisposing cows to immune suppression, oxidative stress, reduced fertility, and metabolic or infectious disorders such as ketosis, metritis, and mastitis (Esposito *et al.*, 2014). Although ruminants efficiently utilise fibre-rich forages, the decline in voluntary feed intake during this period limits nutrient supply. Energy-dense concentrates are commonly used to offset the deficit, but their rapid ruminal fermentation increases the risk of acidosis and related disturbances (Vicente *et al.*, 2024).

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Conventional nutritional management has focused on balancing energy, protein and minerals, but recent strategies include functional feed additives to enhance rumen function, immunity, and gut health. Microbial-derived products, particularly probiotics, have shown benefits by modulating the gut microbiota (Zhong *et al.*, 2022). However, their application is constrained by issues of stability, infection risk, antimicrobial resistance transfer and inconsistent responses. To address these limitations, interest has shifted to postbiotics. These are bioactive compounds and cell components produced by probiotics, which include short-chain fatty acids, extracellular polysaccharides and peptidoglycan fragments (Aggarwal *et al.*, 2022). Postbiotics offer better safety, stability, and shelf life by promoting gut health, pathogen inhibition, immune stimulation and fibre digestion when compared to live microorganisms.

Based on these considerations, the study was designed to investigate the effect of *Saccharomyces cerevisiae* postbiotic in improving dry matter intake, nutrient digestion and rumen fermentation of early-lactating crossbred cows.

Materials and methods

Experimental animals

Twenty-four healthy crossbred cows in early lactation, within 10 days post-calving, were selected from University Livestock Farm and Fodder Research Development Scheme (ULF & FRDS), Mannuthy. The cows were divided into three groups of eight each in a completely randomized design and allocated to one of the three dietary treatments T1, T2 and T3. The animals were maintained under a uniform system of feeding and management throughout the experimental period of 90 days.

Experimental feed

All experimental cows were fed a compound feed mixture (CFM) in pellet form according to three treatments: 1) control (CON), basal diet containing 20% crude protein (CP) and 68% total digestible nutrients (TDN) without *Saccharomyces cerevisiae* fermentation product (SCFP), 2) basal diet + 3 g/head/day SCFP; and 3) basal diet + 6 g/head/day SCFP, top-dressed. Clean drinking water was provided *ad libitum*, and feeding followed ICAR (2013) standards. The *Saccharomyces cerevisiae*-derived postbiotic was procured from Church and Dwight Co. Inc., USA. The ingredient composition of the experimental diet is presented in Table 1.

Dry matter intake

All the experimental animals were fed individually with weighed quantities of feed and fodder. The residue, if any, in the manger was collected manually and weighed

Table 1. Ingredient composition of compound feed mixture offered to cows maintained on three dietary treatments.

Ingredient	Per cent composition of compound feed mixture
Maize	16
Corn gluten fibre	25
De oiled rice bran	16
Rice polish	8
Coconut oil cake	10
Black gram husk	8
Alfalfa	13
Tapioca starch waste	1
Calcite	1.5
Salt	1
Mineral mixture	0.5
Total	100

daily to analyse the moisture content for estimating daily dry matter intake.

Digestion trial

A digestibility trial of five-day duration was carried out at the end of the feeding trial using the total collection method. Quantities of daily feed offered, residues, and dung voided were recorded, and the dry matter content of feed and residues was determined daily. Feed samples were collected in double-lined polyethylene bags and stored at -20°C for analysis. Dung voided by each animal was quantitatively collected in individual containers on a continuous 24-hour basis, ensuring freedom from urine contamination. The total dung output of the previous 24 hours was weighed daily at 8 A.M, thoroughly mixed, and a one per cent representative sample was collected and stored at -20°C . At the end of the trial, dung samples from five consecutive days were pooled, mixed, and representative subsamples were taken for chemical analysis.

Rumen fermentation parameters

Rumen liquor (RL) was collected from all twenty-four animals towards the end of the feeding trial using a stomach tube, four hours after the morning feeding. The pH was determined immediately after collection. Rumen liquor was collected in a container containing 50 per cent H_2SO_4 (0.2 mL per 10 mL of RL) for the estimation of individual volatile fatty acids (VFA) (Gas chromatography) and ammonia nitrogen ($\text{NH}_3\text{-N}$) (Beecher and Whitten, 1970). Rumen liquor stored was centrifuged at 4°C at 5500 rpm for 10 min, filtered through muslin cloth, and stored at -20°C for the analysis of volatile fatty acids (VFA) and ammonia nitrogen ($\text{NH}_3\text{-N}$). For individual VFA estimation, 5 mL of the supernatant was mixed with 1 mL of metaphosphoric acid, refrigerated for 3-4 h, centrifuged again at 5500 rpm

for 10 min, and the resulting supernatant was stored at -20°C for subsequent analysis.

Analysis of feed and dung

The proximate analysis of concentrate feed, grass, and dung samples was conducted (AOAC, 2016). Calcium and Phosphorus content of feed and fodder were estimated by AOAC, 2016. Fibre fractions were estimated by the detergent method (Goering and Van Soest, 1970).

Statistical analysis

Data obtained on the experiment were analysed statistically as per Snedecor and Cochran (1994) by analysis of variance (ANOVA) technique, using the software Statistical Product and Service Solutions (SPSS) version 24.0.

Results and discussion

Chemical composition

The per cent chemical composition of the experimental ration, green fodder and paddy straw offered

to experimental animals are given in Table 2. The dung samples collected from the experimental animals during the digestibility trial are presented in Table 3.

Dry matter intake

The data on weekly average daily dry matter intake of animals fed the three dietary treatments are presented in Table 4. The average daily dry matter intake (DMI, kg/animal/day) were 13.87 ± 0.50 , 14.17 ± 0.33 and 14.42 ± 0.44 for T1, T2 and T3, respectively, with no significant difference ($P > 0.05$) between the groups.

In agreement with this study, Acharya *et al.* (2017) reported that there was no difference in DMI of cows fed *Saccharomyces cerevisiae* fermentation product (SCFP; Diamond V Original XPC) as a supplement during early lactation. Similarly, Zhu *et al.* (2017) conducted studies in eighty mid-lactation Holstein cows supplemented with 0, 60, 120, or 180 g/day of SCFP and found that SCFP supplementation had no significant effect on dry matter intake. Fernandez *et al.* (2023) evaluated the effects of a postbiotic in ten goats during late lactation and reported no significant difference in total DMI between diets.

Table 2. Chemical composition¹ of the concentrate feed, green fodder and paddy straw fed to the experimental animals, %

Parameters	Concentrate	Green fodder	Paddy straw
Moisture	9.01±0.27	84.95±1.15	9.98±1.42
Dry matter (DM)	90.99±0.27	15.05±1.15	90.02±1.42
Crude protein (CP)	20.78±0.10	12.58±0.63	2.98±0.35
Ether extract (EE)	4.36±0.13	2.10±0.11	1.14±0.04
Crude fibre (CF)	8.85±0.21	30.26±0.56	32.72±0.46
Total ash (TA)	10.55±0.17	10.10±0.29	15.22±0.20
Nitrogen free extract (NFE)	55.46±2.51	44.96±0.94	47.94±0.53
Calcium	0.96±0.25	0.60±0.32	0.28±0.22
Phosphorus	0.62±0.12	0.26±0.01	0.09±0.07
Neutral detergent fibre (NDF)	38.85±1.16	60.78±0.56	68.23±0.76
Acid detergent fibre (ADF)	14.24±0.19	37.00±0.55	46.37±0.68

¹Mean of six values with SE; values from third row onwards in dry matter basis

Table 3. Chemical composition¹ of dung of experimental animal maintained on three experimental rations, %

Nutrients	Experimental rations		
	T1	T2	T3
Moisture	80.50±0.29	80.35±0.16	80.19±0.32
Dry matter (DM)	19.50±0.29	19.65±0.16	19.81±0.32
Crude protein (CP)	15.65±0.60	14.99±0.55	14.84±0.65
Ether extract (EE)	2.07±0.25	2.19±0.29	2.23±0.12
Crude fibre (CF)	21.53±0.39	21.01±0.53	20.81±0.53
Total ash (TA)	15.68±0.22	15.52±0.30	15.92±0.28
Nitrogen free extract (NFE)	45.08±0.67	46.30±0.75	46.21±0.65
Neutral detergent fibre (NDF)	52.96±0.69	53.71±0.89	53.20±0.34
Acid detergent fibre (ADF)	36.19±0.73	34.18±0.65	35.40±0.70

¹Mean of six values with SE; values from third row onwards in dry matter basis

Table 4. Weekly average daily dry matter intake¹ of the experimental animals maintained on the three experimental rations, kg

Weeks	Dietary treatments			P value
	T1	T2	T3	
1	13.59±0.56	14.01±0.28	14.36±0.39	0.457 ^{ns}
2	13.66±0.52	14.00±0.29	14.38±0.41	0.486 ^{ns}
3	14.06±0.51	14.36±0.37	14.63±0.42	0.659 ^{ns}
4	14.19±0.54	14.37±0.36	14.72±0.42	0.697 ^{ns}
5	14.34±0.50	14.45±0.37	14.50±0.43	0.965 ^{ns}
6	14.44±0.51	14.49±0.36	14.68±0.43	0.921 ^{ns}
7	14.18±0.53	14.31±0.36	14.57±0.46	0.830 ^{ns}
8	13.97±0.54	14.28±0.37	14.45±0.50	0.771 ^{ns}
9	13.84±0.52	14.23±0.36	14.39±0.50	0.695 ^{ns}
10	13.67±0.50	14.10±0.35	14.32±0.50	0.588 ^{ns}
11	13.61±0.50	14.03±0.36	14.30±0.49	0.558 ^{ns}
12	13.44±0.50	13.86±0.34	14.17±0.47	0.512 ^{ns}
13	13.35±0.52	13.69±0.25	13.94±0.47	0.628 ^{ns}
Mean±SE	13.87±0.50	14.17±0.33	14.42±0.44	0.675 ^{ns}

¹Mean values are based on eight replicates with SE, ns- non-significant (P>0.05)

Table 5. Digestibility coefficient¹ of nutrients in the experimental rations fed to the experimental animals, %

Nutrients	Experimental rations			P value
	T1	T2	T3	
Dry matter	62.19±1.12	63.93±2.07	65.34±2.30	0.512 ^{ns}
Crude protein	61.51±2.20	64.50±2.93	66.49±1.96	0.355 ^{ns}
Ether extract	75.45±3.30	74.21±5.31	76.01±1.28	0.939 ^{ns}
Crude fibre	56.12±1.10	58.36±2.39	61.28±3.35	0.350 ^{ns}
Nitrogen free extract	66.88±1.19	67.58±1.93	68.89±2.03	0.718 ^{ns}
Neutral detergent fibre	59.80±0.80	60.46±2.76	63.18±2.43	0.518 ^{ns}
Acid detergent fibre	47.10±1.02	51.18±3.66	53.09±3.35	0.352 ^{ns}

¹Mean values are based on eight replicates with SE, ns- non significant (P>0.05)

Nutrient digestibility

The nutrient digestibility coefficients of animals subjected to the three dietary regimens during the digestibility trial are presented in Table 5. The results indicated that there were no significant differences (P>0.05) among the experimental groups for DM, CP, EE, CF, NFE, NDF, and ADF.

The lack of effect on nutrient digestibility in the present study is consistent with earlier reports. Hristov *et al.* (2010) observed that inclusion of SCFP in the diets of rumen-cannulated Holstein cows did not significantly alter apparent total-tract digestibility, with all P-values exceeding 0.45. More recently, Odunfa *et al.* (2024) reported similar findings in a crossover trial with Holstein steers, while Jiang *et al.* (2025) also found that supplementation of SCFP in rumen-cannulated Holstein steers did not significantly influence nutrient digestibility.

In contrast, Izuddin *et al.* (2019) reported significantly higher (P < 0.05) digestibility of DM, CP, and

NDF in newly weaned male lambs supplemented with postbiotics compared with controls. Likewise, Fernandez *et al.* (2023) observed a significant improvement in the apparent digestibility of NDF (by 4.7%) and ADF (by 5.2%) in late-lactation goats fed postbiotics, although digestibility of DM, OM, CP, and EE remained unaffected (P > 0.05). Vicente *et al.* (2024) also documented variable responses in digestibility parameters in dairy cows receiving SCFP supplementation. Such inconsistencies across studies may be attributed to multiple factors, including differences in basal diet composition, animal species and physiological stage, feed intake levels, as well as variations in the production technologies and formulations of SCFP products. These findings highlight the complexity of responses to postbiotic supplementation and emphasise the need for further controlled studies to elucidate the conditions under which improvements in nutrient digestibility can be reliably achieved.

Rumen fermentation parameters

The rumen fermentation parameters like rumen

Table 6. Rumen fermentation parameters¹ of the experimental animals maintained on the three experimental rations

Parameters	Dietary treatments			P value
	T1	T2	T3	
Rumen pH	6.45±0.06	6.41±0.05	6.38±0.07	0.746 ^{ns}
NH ₃ N(mg/dL)	14.18±1.23	15.29±0.43	15.10±0.70	0.622 ^{ns}
Acetic acid (mmol/L)	69.76±3.37	63.76±2.67	62.62±3.79	0.282 ^{ns}
Propionic acid (mmol/L)	11.95±0.88	15.69±1.30	16.00±1.67	0.078 ^{ns}
Butyric acid (mmol/L)	6.88±0.69	7.68±0.67	8.03±0.82	0.534 ^{ns}
Total volatile fatty acids (mmol/L)	88.59±3.42	87.13±3.55	86.65±5.23	0.943 ^{ns}
Acetate %	78.61 ^a ±1.53	73.23 ^b ±1.20	72.31 ^b ±1.69	0.014*
Propionate %	13.56 ^a ±1.00	17.98 ^{ab} ±1.38	18.48 ^b ±1.64	0.036*
Butyrate %	7.83±0.81	8.79±0.64	9.21±0.73	0.406 ^{ns}
Acetate: propionate ratio	6.05 ^a ±0.49	4.28 ^b ±0.40	4.18 ^b ±0.43	0.011*

¹Mean values are based on eight replicates with SE; ns- non significant (P>0.05);

*Means with different superscripts within a row differ significantly (P<0.05)

pH, rumen ammonia nitrogen and volatile fatty acid (VFA) of the experimental animals maintained on three experimental rations are depicted in Table 5. The average rumen pH values were 6.45±0.06, 6.41±0.05 and 6.38±0.07, respectively, for T1, T2 and T3. The results were similar among all the treatment groups (P>0.05). The stability of rumen pH may be attributed to the balanced concentrate-to-roughage ratio in all experimental diets, which likely prevented excessive acid accumulation. The findings also suggested that postbiotics are unlikely to induce appreciable changes in ruminal pH. Supplementation of XP did not alter ruminal pH in an *in vitro* study (Mao *et al.*, 2013) and in lactating cows (Hristov *et al.*, 2010). Similarly, Izuddin *et al.* (2018) observed that postbiotic supplementation in goat rumen fluid and in newly weaned lambs did not significantly affect rumen pH. Supplementation of SCFP did not produce any effect on rumen pH in Holstein bull calves over the 56-day study period (Xiao *et al.*, 2016) and in animals under both normal and SARA conditions (Li *et al.*, 2016). Unlike these results, Jiang *et al.* (2025) observed a significant increase in ruminal pH with SCFP supplementation (6.29 vs. 6.01; P=0.01) in high-grain-fed steers.

The average values of rumen ammonia nitrogen for the experimental lactating cows on the three different rations, T1, T2 and T3 were 14.18±1.23 mg/dL, 15.29±0.43 mg/dL and 15.10±0.70 mg/dL, respectively. Similar values were observed among the three treatment groups (P>0.05). Similarly, observations were recorded in the studies of Li *et al.* (2016) and Zhu *et al.* (2017). Jiang *et al.* (2025) also noted that NH₃-N concentrations were unaffected by SCFP supplementation (12 g/head/day) in high-grain-fed steers. In contrast, Izuddin *et al.* (2019) found that postbiotic supplementation in lambs significantly increased ruminal NH₃-N (14.37 vs. 11.28 mg/100 mL; P<0.05). Hristov *et al.* (2010) observed a tendency for XP to reduce ruminal ammonia (P=0.08). Mao *et al.* (2013) demonstrated a reduction in ammonia-N in mixed diet *in vitro* fermentations with SCFP (XP; Diamond V, Cedar Rapids, IA).

The concentrations of individual volatile fatty acids (VFAs, mmol/L) recorded in the study were as follows: acetate, 69.76±3.37, 63.76±2.67, and 62.62±3.79; propionate, 11.95±0.88, 15.69±1.30, and 16.00±1.67; and butyrate, 6.88±0.69, 7.68±0.67, and 8.03±0.82, respectively, in T1, T2, and T3. The total VFA concentrations were 88.59±3.42, 87.13±3.55 and 86.65±5.23 mmol/L for T1, T2 and T3, respectively. No significant differences were observed among the three treatment groups (P>0.05). The molar proportions of acetate, propionate, and butyrate were 78.61±1.53, 13.56±1.00 and 7.83±0.81 per cent in T1, 73.23±1.20, 17.98±1.38 and 8.79±0.64 per cent in T2 and 72.31±1.69, 18.48±1.64 and 9.21±0.73 per cent in T3, respectively. Statistical analysis revealed that the molar proportion of acetate was significantly (P<0.05) higher in T1 compared to T2 and T3, while propionate were significantly (P<0.05) higher in T3 compared to T1 and was comparable to T2. These findings indicate a shift in the rumen fermentation pattern. Furthermore, the acetate-to-propionate ratio was significantly (P<0.05) higher in T1 than in T2 and T3, with no significant difference between T2 and T3. These observations were in accordance with findings of Hristov *et al.* (2010) and Li *et al.* (2016), who found no significant changes in total VFA concentration of major VFAs in Holstein cows. Acharya *et al.* (2017) reported that cows fed Product 2 (19 g/day SCFP) exhibited lower acetate and higher propionate proportions. Shi *et al.* (2019) evaluated the effects of SCFP during the periparturient period on the Holstein cows and noticed no effects on VFA profiles. Jiang *et al.* (2025) reported no significant changes in total VFAs or the major VFA molar proportions in high-grain-fed steers, although minor VFAs such as isobutyrate and isovalerate increased.

Contrary to this study's observations, Mao *et al.* (2013) reported that XP supplementation (0–3 g/L) enhanced total VFA concentrations, particularly propionate, while reducing acetate. Zhu *et al.* (2017) demonstrated that SCFP supplementation in mid lactation Holstein dairy

cows significantly increased absolute concentrations of total VFAs and individual VFAs, including acetate, propionate, and butyrate, although molar proportions remained unchanged. Izzudin *et al.* (2018) observed a linear increase in acetate, propionate, butyrate, and total VFAs with increasing levels of *Lactobacillus plantarum* RG14-derived postbiotics in *in vitro*, although the acetate-to-propionate ratio remained unchanged.

In vivo studies in lambs and goats also indicated comparable trends. Izzudin *et al.* (2019) found that postbiotic supplementation increased propionate without significantly affecting total VFAs or other individual VFAs. Fernandez *et al.* (2023) reported higher propionic acid concentrations in postbiotic-supplemented Murciano-Granadina goats, with acetate and butyrate remaining unaffected. In calves Xiao *et al.* (2016) reported higher butyrate concentrations in SCFP-supplemented without affecting total VFAs.

Conclusion

Supplementation of postbiotics derived from *Saccharomyces cerevisiae* in the diets of early lactating crossbred cows did not exert a significant effect on dry matter intake and nutrient digestibility of DM, CP, EE, CF, NFE, NDF, or ADF but modulated the rumen fermentation by increasing propionate and reducing acetate proportion favouring a more glucogenic fermentation pattern under the present experimental conditions. These findings are in line with several earlier reports in cattle but contrast with studies in small ruminants where improvements in fibre or protein digestibility have been noted. The variability in response across species and experimental settings suggests that the influence of postbiotics on digestibility is context-dependent, being modulated by factors such as basal diet composition, physiological stage, and postbiotic preparation. Further targeted investigations are warranted to clarify the conditions under which *S. cerevisiae* postbiotic may positively influence dry matter intake, nutrient utilisation and rumen fermentation in dairy cows.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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