Journal of Animal and Feed Sciences, 34, 4, 2025, xxx–xxx, https://doi.org/10.22358/jafs/203875/2025
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

ARTICLE IN PRESS

ORIGINAL PAPER

Effect of polyol supplementation in forage-based total mixed rations on *in vitro* rumen gas production and fermentation in beef cattle

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KEY WORDS: fatty acids, *in vitro* fermentation, polyol

Received: 24 January 2025
Revised: 7 April 2025
Accepted: 7 April 2025

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ABSTRACT. The study evaluated the effects of a polyol blend (equal parts xylitol, arabinitol, and sorbitol) added to beef cattle total mixed rations (TMR) on *in vitro* rumen fermentation, total gas production, digestibility, and organic acid profiles. Polyols were supplemented at 0, 2, 4, 8, and 12% of dry matter. Results showed no significant differences in total gas production, metabolic energy, lactation net energy, or organic matter digestibility (OMd) between polyol-supplemented groups and the control ($P < 0.05$). However, the addition of 2, 4 and 8% polyols increased *in vitro* rumen pH ($P < 0.05$). All polyol levels reduced the proportions of acetic acid (AA), butyric acid (BA), propionic acid (PA), iso-butyric acid (IBA), valeric acid (VA), iso-valeric acid (IVA), and branched short chain fatty acids (BSCFA) in total short-chain fatty acids (T-SCFA), while only 12% supplementation elevated straight-chain SCFA concentrations ($P < 0.05$). Overall, polyol addition at 2, 4, 8, and 12% did not significantly alter *in vitro* rumen fermentation parameters (total gas production, OMd, energy values and SCFAs) in beef cattle TMR. However, the increase in rumen fluid pH observed with moderate polyol doses (2–8%) suggests a potential buffering effect, which may be beneficial in starch-rich diets used during intensive fattening or early lactation in dairy cattle.

Introduction

Global food production faces significant challenges in meeting the demands of a rapidly growing population. Projections indicate that the world population will rise by approximately 1 bn by 2050 (Alexandratos and Bruinsma, 2012). With the recommended daily protein intake for adults established at 66 g (WHO, 2007), this population growth will correspondingly escalate global protein requirements. Research suggests that ruminants, particularly beef cattle, which occupy an important place in food production, have sufficient potential to meet this growing protein demand (Hubbart et al., 2023). Improving animal productivity, rather than increasing livestock numbers has emerged as a more sustainable approach in food production. A key factor in rising productivity lies in optimising energy supply in animal rations. Considering the problems associated with high-level inclusion of fats and carbohydrates in cattle rations, polyols have emerged as a potential alternative energy source. As a result,

polyols, i.e. hydrogenated carbohydrates, are commonly used as sugar substitutes due to their various health benefits and functional properties.

Polyols, classified as sugar alcohols, are derived from the reduction of aldehyde or ketone groups in sugars into corresponding hydroxyl groups. These polyhydroxy compounds, also referred to as polyalcohols or polyhydric alcohols, are formed when sugars gain additional alcohol groups (Bieleski et al., 1982). Polyols are non-cariogenic osmotic carbohydrates characterised by low digestibility, and minimal glycaemic and insulinemic effects (Livesey, 2003). The simplest form, glycerol, represents the only tritol sugar alcohol (Bieleski et al., 2023). Due to these properties, polyols are widely used as sugar substitutes, particularly in reduced-calorie and diabetic-friendly food products (Ortız et al., 2013).

The glycaemic index (GI) measures how much a carbohydrate-containing food raises blood glucose, while the insulin index (II) reflects the impact of all foods on insulin response (Caferoğlu et al., 2018). From this perspective, polyols exhibit significantly lower GI and II values compared to conventional sugars. Reported GI/II values for selected polyols are as follows: mannitol – 0/0, erythritol – 0/2, lactitol – 6/4, isomalt – 9/6, sorbitol – 9/11, xylitol – 13/11, maltitol – 13/11, and polyglycitol – 39/23. These values are markedly lower than those calculated for sucrose (65/43) and glucose (100/100). These significantly lower glycaemic and insulinemic responses make polyols a preferred ingredient in food products, particularly for individuals managing blood glucose and insulin levels (Livesey, 2003). A study on dairy cows comparing sugar beet pulp, grain and polyol, each with equivalent energy content, reported a reduction in feed intake in animals fed sugar beet (Tuori and Poutiainen, 1977), suggesting no adverse effects from polyol supplementation in cattle. Weaned piglets fed polyol had lower mortality rates and better growth performance than those receiving sucrose (Näsi and Alaviuhkola, 1981). In another trial with weaned calves, average daily live weight gains were recorded at 452 g, 479 g and 425 g for groups fed glucose, xylitol and mixed polyol, respectively. Haemoglobin and haematocrit levels were higher in the glucose group, while xylitol was associated with lower plasma nitrogen and higher plasma phosphorus levels (Tuori, 1984). Sheep studies revealed that ruminal polyol administration increased the acetate-to-propionate ratio (Lister and Smithard, 1984). Meanwhile in rats, polyol supplementation led to the formation of a stable polyol-Cu complex, thereby reducing copper utilisation (Hämäläinen and Mäkinen, 1989). These findings suggest that polyol may be a viable and manageable energy source for high-yielding animals.

The study hypothesised that the sugar alcohol polyol could serve as an energy source in ruminant forages. The primary objective was to evaluate, under *in vitro* conditions, the effects of adding 2%, 4%, 8%, and 12% polyol to the total mixed ration (TMR) for beef cattle on total gas production, pH, metabolic energy, organic matter digestibility, net energy for lactation and volatile fatty acid profiles.

Material and methods

Rumen fluid was collected from five Brown Swiss-Simmental males, with an average age of 18 months, in a slaughterhouse settings. Since no live animals were used in the study, ethical approval was not required. A total mixed ration (TMR) based on one kg of beef cattle feed was prepared under controlled research conditions (dry matter (DM) basis), taking into account the rumen content collected from the animals. The TMR used in the study was formulated for 10-month-old Brown Swiss-Simmental breed males, with an initial live weight of 250 kg, an expected daily live weight gain of 1.5 kg, and a final live weight of 500 kg at the end of the fattening period. The TMR was ground to a particle size of 1 mm using an IKA MF10 Basic Microfine Grinder (Staufen im Breisgau, Germany). Feed composition analysis included ash, crude protein, ether extract, neutral detergent fibre, and acid detergent fibre, was conducted using standard methods (Van Soest et al., 1991; AOAC, 1995; Wang et al., 2015), with all samples analysed in triplicate. The ingredients and nutrient composition of the TMR formulated for *in vitro* fermentation are shown in Table 1.

Table 1. Feed ingredients and chemical composition of the beef cattle ration (% DM basis)

Feed		Chemical composition	
Ingredient	% as in DM	Chemical parameter	Value, % DM
Maize silage	42.44	CP	12.01
Barley grain	33.95	EE	2.01
Wheat straw	8.48	Ash	6.52
Wheat bran	5.94	ADFom	26.00
Alfalfa herbage	4.24	aNDFom	45.00
Cottonseed meal	4.24	HemS	19.00
Limestone	0.67	NFC	34.47

CP – crude protein, EE – diethyl ether extract, ADFom – acid detergent fibre (ash-free, α-amylase treated), aNDFom – neutral detergent fibre (ash-free, α-amylase treated), HemS – haemicellulose, NFC – non-fibre carbohydrate (calculated as: 100 − (% NDF + % CP + % EE + % ash), DM − dry matter

The polyol mixture used in the study was a commercial veterinary preparation intended for postpartum energy supplementation. It was incorporated into beef cattle total mixed rations at five inclusion levels: 0, 2, 4, 8, and 12% of DM, following a 2 × 4 factorial experimental design. *In vitro* ruminal fermentation parameters of TMRs were analysed using the gas production technique described by Menke (1988). Rumen fluid for the *in vitro* assays was collected from two Brown Swiss-Simmental crossbreed cattle. For each analysis, 0.20 g of TMR sample (DM basis) was incubated in an 100-ml anaerobic glass fermenter (Model Fortuna, Haberle Labortechnik, Lonsee, Germany) containing 30 ml of a rumen fluid-buffer mixture (1:2, v/v). All samples were analysed in quadruplicate. After 24 h of incubation, total gas volume was measured using the calibrated scale of the anaerobic fermenters, as described by Menke et al. (1979). The metabolic energy (ME), net energy lactation (NE_L) and organic matter digestion (OMd) values were calculated using established equations (Menke, 1988). The fermentation fluid was analysed for the concentrations of short-chain fatty acids (SCFA) and branched chain fatty acids (BCFA) using a Thermo Trace 1300 gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a flame ionisation detector (FID) and polyethylene glycol columns (TG-WAXMS, 60 m length × 0.25 mm internal diameter × 0.25 µm film thickness) following the methodology of Ersahince and Kara (2017). The pH of the fermentation fluid was measured with a digital pH meter (Mettler Toledo, Columbus, OH, USA). All analyses were performed in triplicate. Methane production was estimated both through direct measurement using an infrared methane analyser and by calculation from SCFA molarities using the equations of Moss et al. (2000). The ME, NE_L, and OMd values for all TMR treatments (with and without polyol) were determined based on gas production data and nutrient composition, applying equations of Menke (1988).

Statistical analysis was performed using SPSS 22.0 software package. A multivariate general linear model was employed to examine the effects of polyol supplementation and dose (2, 4, 8, and 12%) on measured parameters, with polynomial contrast analysis (linear, quadratic, and cubic) used to assess dose-response relationships. Significant differences were identified using Tukey's post hoc test at a significance level of $P < 0.05$.

Results

The proportions of feed ingredients used in the formulated ration (% DM basis) were as follows: maize silage (42.44%), barley grain (33.95%), wheat straw (8.48%), wheat bran (5.94%), alfalfa hay (4.24%), cottonseed meal (4.24%) and limestone (0.67%). Chemical analysis revealed that the ration contained 12.01% crude protein, 2.01% fat, 6.52% crude ash, 26% acid detergent fibre (ADF), 45% neutral detergent fibre (NDF), 19% haemicellulose, and 34.47% non-fibre carbohydrates (NFC) (Table 1). Polyol supplementation at 2–12% of TMR DM did not significantly affect ($P > 0.05$) total gas production, ME, OMd, or NE_L values. However, quadratic increases in rumen pH were observed at 2, 4, and 8% inclusion levels ($P < 0.05$; Table 2). Fatty acid analysis showed that polyol addition decreased ($P < 0.05$) the proportions of butyric acid (BA), valeric acid (VA), iso-valeric acid (IVA) and iso-butyric acid (IBA) ($P < 0.05$), while increasing amino acid (AA) percentage in total fatty acids ($P < 0.05$), with no effect on propionic acid (PA) proportion ($P > 0.05$; Table 3). Furthermore, polyol

Table 2. *In vitro* fermentation parameters of fattening feed supplemented with polyol after 24 h

	Polyol addition, %	Total gas production, ml/0.2 g DM	pH	ME, MJ/kg DM	OMd, %	NE_L, MJ/kg DM	Methane, ml/0.2 g DM
Addition rates	0	74.35	5.83^{ba}	12.99	86.54	8.22	23.64^{ab}
	2	76.30	5.75^{a}	13.26	67.85	8.45	15.86^{a}
	4	64.75	6.17^{cb}	11.69	76.49	7.12	17.83^{bc}
	8	63.19	6.16^{cb}	11.47	77.88	6.94	19.34^{bc}
	12	53.46	6.37^{c}	10.15	88.15	5.82	27.45^{a}
	SD	11.96	0.26	1.62	10.65	1.37	4.93
	SE	3.08	0.06	0.42	2.75	0.35	1.27
*P*value	L	0.013	<0.001	0.012	0.429	0.012	0.055
	Q	0.546	0.620	0.546	0.024	0.546	<0.001
	combined *P*-value	0.099	0.001	0.099	0.098	0.098	0.003

ME – metabolic energy, OMd – organic matter digestion, NE_L – net energy lactation, L – linear, Q – quadratic, DM – dry matter, SD – standard deviation, SE – standard error; ^{abc} – means with different superscripts in each column are significantly different at $P < 0.05$

Table 3. Individual short-chain fatty acid (SCFA) proportions in *in vitro* fermentation fluid (% of total SCFA)

	Addition rates, %	AA	BA	PA	VA	IVA	IBA
		% in T-SCFA					
Polyol	0	58.64^{b}	15.81^{a}	21.75	1.84^{a}	1.41^{a}	0.53^{a}
	2	63.68^{a}	11.53^{c}	22.56	1.13^{c}	0.75^{b}	0.32^{b}
	4	63.25^{a}	12.46^{bc}	21.86	1.21^{bc}	0.87^{b}	0.33^{b}
	8	63.61^{a}	12.17^{c}	21.81	1.23^{bc}	0.83^{b}	0.33^{b}
	12	62.26^{a}	13.94^{b}	21.21	1.39^{b}	0.86^{b}	0.32^{b}
	SD	2.08	1.65	1.16	0.27	0.25	0.08
	SE	0.53	0.42	0.30	0.07	0.06	0.02
P-value	L	0.001	<0.001	0.446	<0.001	<0.001	<0.001
	Q	<0.001	<0.001	0.447	<0.001	<0.001	<0.001
	combined *P*-value	<0.001	<0.001	0.784	<0.001	<0.001	<0.001

AA – acetic acid, BA – butyric acid, PA – propionic acid, VA – valeric acid, IVA – iso-valeric acid, IBA – iso-butyric acid, T-SCFA – total short-chain fatty acids, L – linear, Q – quadratic, SD – standard deviation, SE – standard error; ^{abc} – means with different superscripts in each column are significantly different at $P < 0.05$

Table 4. Molar concentrations of short-chain fatty acids (SCFA) in *in vitro* fermentation fluid of beef cattle ration (mmol/l)

	Addition rates, %	AA	BA	PA	VA	IVA	IBA	AA+BA/PA	TSCFA	SCFA	BSCFA
Polyol	0	51.48^{ab}	13.93^{a}	19.15^{a}	1.63^{a}	1.25^{a}	0.47^{a}	3.43	87.89^{ab}	86.16^{ab}	1.74^{a}
	2	37.00^{b}	6.73^{b}	12.96^{b}	0.66^{b}	0.44^{c}	0.19^{c}	3.30	58.03^{c}	57.28^{c}	0.63^{c}
	4	40.88^{b}	8.10^{b}	14.07^{b}	0.78^{b}	0.56^{bc}	0.21^{bc}	3.50	64.60^{bc}	63.80^{bc}	0.79^{bc}
	8	44.44^{b}	8.50^{b}	15.11^{b}	0.86^{b}	0.58^{bc}	0.23^{bc}	3.46	69.76^{bc}	68.96^{bc}	0.83^{bc}
	12	61.29^{a}	13.73^{a}	20.87^{a}	1.37^{a}	0.85^{b}	0.32^{b}	3.60	98.40^{a}	97.08^{a}	1.18^{b}
	SD	10.11	3.36	3.39	0.41	0.31	0.11	0.21	17.26	16.89	0.42
	SE	2.61	0.86	0.87	0.10	0.08	0.02	0.05	4.45	4.36	0.11
P-value	L	0.027	0.617	0.067	0.328	0.011	0.008	0.263	0.068	0.058	0.009
	Q	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.561	<0.001	<0.001	<0.001
	combined *P*-value	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	0.628	0.001	0.001	<0.001

AA – acetic acid, BA – butyric acid, PA – propionic acid, VA – valeric acid, IVA – iso-valeric acid, IBA – iso-butyric acid, T-SCFA – total short-chain fatty acids, BSCFA – branched short chain fatty acids, L – linear, Q – quadratic, SD – standard deviation, SE – standard error; ^{abc} – means with different superscripts in each column are significantly different at $P < 0.05$

supplementation led to a quadratic decrease in the absolute amounts of AA, BA, PA, VA, IVA, IBA, T-SCFA, BSCFA and SCFA, except for the 12% polyol treatment, which caused an increase in SCFA concentrations ($P < 0.05$). The (AA + BA)/PA ratio remained unaffected by polyol addition ($P > 0.05$; Table 4).

Methane production, estimated using established equations, showed a quadratic decrease at the 4 and 8% polyol inclusion levels ($P < 0.05$; Table 2).

Discussion

Ruminant diets incorporate various energy sources, including highly digestible forages such as maize silage and selected grasses, starch-rich like cereal grains, and lipid supplements. Fast-fermenting compounds including propylene glycol are commonly used in dairy cattle feeding during the transition period (prepartum and postpartum) due to their rapid ruminal availability. Sugar alcohols have been well characterised for human nutrition applications, but research on their use in beef cattle remains limited. Current understanding suggests that polyols may offer intermediate fermentation characteristics between rapidly degraded starch and slowly fermenting structural carbohydrates (NRC, 2001).

Recent research has explored the potential benefits of incorporating sugar alcohols like sorbitol into cattle diets during heat stress, with preliminary findings suggesting possible improvements in milk yield and feed efficiency (Anonymous, 2016). Similarly, propylene glycol supplementation has been shown to significantly alter rumen fermentation by increasing propionate production while decreasing

the acetate-to-propionate molar ratio (Wang et al., 2021). In the present study, rising levels of polyol in feedlot cattle rations did not negatively affect *in vitro* total gas production. Contrary to potential concerns, higher polyol levels may actually enhance total gas production and improve key fermentation parameters (ME, OMd, NE_L). Retaining appropriate and stable rumen pH represents a critical factor in feedlot cattle nutrition, as suboptimal acidity levels resulting from high-starch diets can disrupt microbial fermentation. The digestive efficiency of ruminants depends fundamentally on maintaining a diverse and robust microbial population. Excessively acidic conditions (pH < 5.8) suppress fibrolytic bacteria, while overly alkaline rumen environment impairs starch fermentation. On the other hand, the ideal rumen pH range of 5.8–6.2 supports optimal microbial activity and nutrient utilisation. For this reason, buffers and alkalinising agents are often used to stabilise ruminal pH. In the current study, the observed increase in *in vitro* ruminal pH after polyol supplementation, especially at the 2% inclusion level, was a desired outcome, as it may help maintain an optimal environment for rumen fermentation.

The addition of 2, 4, and 8% polyol to TMR resulted in increased pH values, potentially attributed to reduced SCFA production and consequent decrease in H^+ ion release. This alkalising effect offers two key benefits for rumen health. Firstly, it might help buffer high-starch diets containing excessive grain, thereby reducing the risk of rumen acidosis. Secondly, polyol could help compensate for the insufficient fibre content and limited rumination in certain rations, which otherwise would fail to maintain adequate rumen pH. This pH-stabilising effect may support optimal microbial activity and digestion, particularly in starch-rich or fibre-deficient diets while mitigating acidosis risk. For these reasons, polyol may be considered a useful additive in feed formulations intended to promote rumen pH balance. In addition, polyol has been reported to perform other technological functions in the feed industry, including serving as an anti-caking agent (Tennant, 2014). SCFA are an important energy source for ruminants, providing approximately 60–70% of metabolizable energy (Van Soest, 1991). Low production of SCFA observed with polyol supplementation may therefore signal lower energy availability, potentially impairing fermentation and microbial efficiency and negatively affecting performance. Comprehensive *in vivo* studies are required to fully evaluate these effects and determine the practical implications of polyol supplementation in ruminant nutrition.

Based on the findings, polyol supplementation should not be used as a sole energy source in ruminant diets due to its significant reduction in SCFA production and consequent energy deficit. The current study demonstrated a dose-dependent linear decrease in IVA, IBA and VA proportions within the total rumen fatty acids, indicating polyol's capacity to modify fermentation patterns. The current findings regarding these volatile fatty acids are consistent with those reported in earlier works (Soylu et al., 2022).

The methane-reducing effect of polyol observed in this study is a positive outcome in the context of decreasing livestock methane emissions and mitigating environmental pollution—an issue that has gained increasing attention in recent years. While previous research by Soylu et al. (2022) found no impact on methane production with xylitol supplementation at 2–8% levels, the current results demonstrate the potential of polyol as an effective methane mitigation strategy. The observed reduction in VA production corroborates earlier findings by Gascoyne et al. (1988) in roughage-fed animals and is further supported by human studies (Valeur et al., 2016) showing similar VA dynamics in response to fermentable carbohydrate restriction. This consistency across species and experimental conditions strengthens the validity of the current findings regarding polyol's influence on rumen fermentation patterns. The combined evidence suggests that polyol supplementation may offer dual benefits by reducing environmental impact through methane mitigation while potentially modifying rumen fermentation toward more favourable profiles, though its effects appear dependent on both dosage and dietary context.

However, a higher acetate-to-propionate ratio was detected when polyol was added to the adapted rumen fluid (Lister and Smithard, 1984). High-dose polyol studies in laboratory animals revealed certain pathological effects, though they were considered unlikely to be significant in humans (Bär, 1985). There is increasing evidence suggesting that fermentable oligosaccharides, disaccharides, monosaccharides and polyols may contribute to digestive symptoms such as bloating, gas formation, and altered bowel habits through osmotic activity and fermentation processes.

Conclusions

The present experimental results showed that polyol supplementation at 2–8% inclusion levels in high-starch diets for intensive beef production or dairy cattle during early lactation produced several notable effects on rumen function. The addition of polyol led to decreased volatile fatty acid production while maintaining organic matter digestibility, improved pH regulation, and reduced methane emission. These findings support the consideration of polyol as a potential feed additive at 4% and 8% inclusion levels, based on its effects on rumen fermentation and methane reduction, combined with its practical advantages in feed processing. These specific concentrations exerted consistent effects across key parameters while maintaining digestibility. Lower inclusion levels (2%) exhibited some beneficial trends but require further investigation through controlled *in vitro* and *in vivo* studies to fully assess their efficacy and potential applications in different production systems.

Conflict of interest

The Authors declare that there is no conflict of interest.

Additional information

A summary of this study was presented at the 4th International Animal Nutrition Congress (29 February – 3 March 2024, Antalya, Türkiye).

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