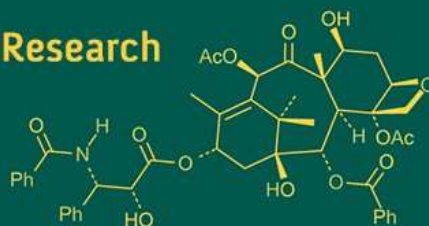
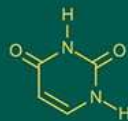
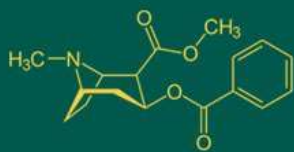


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Biochemical characterization and *In vitro* fermentation of bakery waste as an alternative energy source for ruminant feeding

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Abstract

The main aim of this study was to investigate the effect of bakery waste inclusion in the concentrate mixture on *in vitro* evaluation and fermentation characteristics. The Biochemical evaluation of bakery waste revealed that 39.9% starch, 22.1% sugars, 19.8% ether extract, 94.1% total digestible nutrients and low fibre fractions. *In vitro* incubation of bakery waste showed net gas production of 264 ml/g at 24 h, 96.6% true organic matter degradability, 66.0% neutral detergent fibre degradability, 13.0 MJ/kg metabolizable energy and microbial biomass production of 731 mg/g digested OM, indicating improved fermentability. Nine concentrate mixtures were formulated, replacing grain with bakery waste, included at 0%, 5%, 10%, 15%, 20%, 25%, 30%, 35% and 40% on an equivalent basis. *In vitro* analysis indicated significant ($P < 0.001$) linear improvement in gas production, digestibility and metabolizable energy with increased level of bakery waste, while microbial biomass production remained unaffected ($P > 0.05$). Metabolizable energy value significantly ($P < 0.001$) increased from 9.20 MJ/kg at 0% inclusion to 11.1 MJ/kg at 40% inclusion. These results indicate that bakery waste can effectively replace grain sources up to 40% with enhanced ruminal fermentation, improved nutrient utilization and greater energy availability without any negative effect on nutrient digestibility. Overall, findings support the bakery waste act as an alternative energy source, circular feed resource utilization and environmentally sustainable livestock production.

Keywords: Bakery waste, Concentrate, Grain replacement, Rumen fermentation

Introduction

By 2050, the global demand for animal products will rise by 60-70% (Derbyshire, 2022; Kırkpınar *et al.*, 2025) ^[4, 16] along with reduction in conventional cereal grain resources (Kilama *et al.*, 2023) ^[15]. Simultaneously, global food waste is a major part of the socio-economic and ecological crisis (Soria *et al.*, 2025) ^[30], with substantially contribution to greenhouse gas emissions and excessive landfill burdens (Soria *et al.*, 2025) ^[30]. While cereal grains are highly palatable, energy-dense, and rich in starch (Humer and Zebeli, 2017) ^[12], their intensive use is associated with metabolic and ruminal fermentation disorders in high-yielding ruminants. Consequently, exploring alternative, non-human-edible by-products like bakery waste, noodle waste, and snack processing leftovers offers a viable strategy for waste disposal and resource recycling (Narasimmalu and Ramasamy, 2020) ^[20]. From a regulatory and sustainability perspective, utilizing these industrial streams in animal nutrition helps valorize food waste within a circular bioeconomy framework (Nath *et al.*, 2023; Shurson *et al.*, 2023; Bytyqi *et al.*, 2025) ^[2, 21, 29].

Bakery waste is highly energy-dense ingredient characterized by higher amount of digestible non-fibrous carbohydrate, gelatinized starch and lipid; and provide a cost-effective substitute for cereal grains like barley and maize (Nayohan *et al.*, 2024; Obeidat, 2025) ^[22, 24]. So, use of industrial by-product, mainly bakery waste provide sustainable circular economy strategy to reduce feed wastage and environmental pollution with alternative to grain (Pinotti *et al.*, 2021) ^[26].

However, inclusion of bakery by-products in ruminant feed is limited by variation in biochemical composition, processing effects (i.e., time and temperature) and effective inclusion level of bakery waste (Pastorelli *et al.*, 2023; Obeidat, 2025) ^[24, 25].

Currently, there remains a gap in the research on the *in vitro* study of specific fermentation kinetics, net gas production profile and selection of a proper level of inclusion of bakery waste in the ruminant feed formulation. Therefore, present study aimed to investigate the biochemical *in vitro* analysis of bakery wastes as ruminant feed. The primary objective was to analyse biochemical composition, *in vitro* digestibility with determination of rumen fermentation characteristics.

Materials and Methods

Biochemical characterization of bakery waste

After receiving the bakery waste sample were dried in hot air oven at 65 °C for 48 h. Then samples were ground through mm sieve in a Wiley mill. Then sample analyzed for crude protein (CP), ether extract (EE), total ash, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (AOAC, 2007; Robertson and Van Soest, 1981; Shilwant *et al.*, 2022) [27, 28] along with gross energy (Bomb Calorimeter) as well as aflatoxin level (Aflatoxin analyzer). Total digestible nutrient (TDN) and Net energy for lactation (NE_L) of samples were estimated (NRC, 2001) according to equations:

$TDN (\% DM) = 40.2625 + 0.1969 \times \text{total protein } \% + 0.4228 \times \text{NFE } \% + 1.1903 \times (EE \%) - 0.1379 \times CF \%$ (Wardeh, 1981) [31]

$NE_L (\text{Mcal/kg}) = 0.0245 \times TDN (\%) - 0.12$ (NRC, 2001; Marchi *et al.*, 2013) [17]

The moisture-free samples were sent to Punjab Biotechnology Incubator (PBTI), Laboratory (NABL accredited), Mohali-160059 and were analyzed for starch, sugar, major minerals, micro-minerals, heavy metals, rancidity and microbial parameters as presented in Table 1.

In Vitro gas production and fermentation analysis

For in-vitro analysis, nine dietary levels were prepared with bakery waste inclusion as the substitute for cereal grain at 0%, 5%, 10%, 15%, 20%, 25%, 30%, 35% and 40%, while the level of grain was proportionally reduced from 35% to 0%. Ingredient composition of the concentrate mixture containing different levels of Bakery waste is presented in Table 2.

The nutritional value of wastes was assessed by *in vitro* gas production (IVGP) technique described by Menke and Steingass (1988). For this trial, rumen liquor was collected from fistulated bulls fed on conventional diet consisting of roughage and concentrate to meet their requirement as per NRC (2001), before feeding at 09:00 h in a thermos flask flushed with CO₂ and maintained at 39°C. About 200 mg of the dried ground sample of each waste was incubated at 39°C for 24 h in triplicate in 100 mL calibrated glass syringes (Haberle Labortechnik, Germany) with buffered rumen fluid for assessing the net gas production, ME availability, true organic matter (OM) and NDF digestibility (Menke *et al.*, 1979; Menke and Steingass, 1988; Hundal *et al.*, 2019) [18, 19]. The microbial biomass production (MBP) was estimated according to Grings *et al.* (2005) [9]. $MBP = (IVTDOM, \text{mg/g}) - (\text{net gas production, ml/g}) \times 2.2$ The metabolizable energy (ME) was estimated as per Menke *et al.* (1979) formula [19]:

$ME (\text{MJ/kg DM}) = 1.54 + 0.145 \times \text{net gas production (ml/200 mg DM)} + 4.12 \times CP (\text{g/g DM}) + 6.5 \times CP (\text{g/gDM}) \times CP (\text{g/g}) + 20.6 \times \text{ether extract (g/g DM)}$ (Shilwant *et al.*, 2022) [28].

Statistical analysis

Data were analysed by using one-way ANOVA with inclusion of bakery waste as the fixed effect. Tukey's test used for comparing mean of different treatment. Linear and quadratic effects of bakery waste inclusion were evaluated using orthogonal polynomial contrast. Significant difference were considered at $P < 0.05$. Statistical analyses were done by using SPSS 20.0.

Result

The biochemical characterization of bakery waste (on a dry matter basis) is presented in Table 1. Nutrient composition of bakery waste contains 96.0% dry matter (DM), 39.9% starch, 22.1% sugars, 94.1% of total digestible nutrients with an ether extract (EE) content of 19.8% while NE_L value of 2.19 Mcal/kg DM (9.16 MJ/kg DM) with 5.097 kcal/g gross energy value was observed. Conversely, structural carbohydrates contain very low NDF and ADF, which were 3.0 and 0.95, respectively. Biochemical composition of the concentrate mixture containing different levels of Bakery waste is presented in Table 3. The nutrient composition of the concentrate mixtures indicated that crude protein remained constant (25.0-25.9%) across different levels of inclusion. The increasing level of bakery waste increased ether extract content from 6.19-13.50%, while fibre fractions, including NDF, ADF, hemicellulose, cellulose and crude fibre and starch content, slightly decreased. Total digestible nutrient (74.1-75.8%), rumen undegradable protein (36.4-36.9% of CP) and metabolizable energy (2.643-2.676 Mcal/kg) remained same with different levels of inclusion of bakery waste. The biochemical evaluation of *in vitro* fermentation of bakery waste is presented in Table 4. The net gas production was 264 ml/g at 24 h with 96.6% *in vitro* true organic matter degradability (IVTOMD), 66.0% *in vitro* neutral detergent fibre degradability (IVNDFD), while metabolizable energy (ME) was 13.0 MJ/kg and microbial biomass production (MBP) 731 mg/g digested OM. The effect of the inclusion of Bakery waste on biochemical evaluation of *in vitro* fermentation is presented in Table 5. As the inclusion of bakery waste increased from 0 to 40%, a significant linear improvement ($P < 0.001$) was reported in all *in vitro* fermentation parameters except microbial biomass production (MBP). NGP increased from 159.2 ml/g at 0% inclusion to 182.5 ml/g at 40% inclusion ($P < 0.001$), with significant difference observed between the 20% to 40% inclusion levels. A significant difference was observed in IVNDFD ($P = 0.003$) and IVTOMD ($P < 0.05$) from 25 to 40% inclusion of bakery waste. ME increased from 9.20 MJ/kg at 0% inclusion to 11.1 MJ/kg at 40% inclusion ($P < 0.001$), with significant differences observed across all inclusion levels. Microbial biomass production (MBP) ranged from 549.4 to 681 mg/g digested OM, remains unaffected ($P > 0.05$) across different levels of bakery waste.

Discussion

Biochemical composition of bakery waste and experimental diets

The result of high starch, sugar and ether-extract content of bakery waste reported in the present study is aligned with previous literature, which classified bakery waste as energy-rich feed ingredients (El-Haysha *et al.*, 2017) [6]. High energy content (ME and NE_L) in bakery waste was observed due to the addition of oil and butter during the processing of

the product. A similar high NE_L value (9.3 MJ/ kg of DM) for bakery by-products was observed in a previous study of Humer *et al.* (2018) ^[11]. The results for sugar, starch and NDF are closely matched with Nayohan *et al.* (2024) ^[22], who reported that bakery by-products contain sugar, starch and NDF at 210 to 590 g/kg DM, 260 to 320 g/kg DM and 15 to 43 g/kg DM, respectively. Similarly, Humer *et al.* (2018) ^[11] also noted the low NDF and ADF levels in bakery by-products. In addition to this, microbiological and toxicological parameters were measured for bakery waste with total viable counts (7.8×10^3 cfu/g) and aflatoxin (8.8 ppb) remains within safe limit, which is very essential because high moisture in industrial by-products which lead to susceptible for rapid fungal deterioration and mycotoxin contamination (Kilama *et al.*, 2023) ^[15]. In study of Humer *et al.* (2018) ^[11], increase inclusion of bakery by-products from 0 to 45%, increased ether extract content from 2.5 to 7.1% while decreasing NDF from 34.3 to 31.5% and starch from 29.8 to 24.0% were reported. In various previous studies, Similar variation was observed in EE in bakery by-products which ranging from less than 35 to more than 80 g/kg DM, depending upon sources and composition of bakery waste (Champe and Church, 1980; França *et al.*, 2012; Pinotti *et al.*, 2021; Nayohan *et al.*, 2024) ^[3, 7, 22, 26].

Biochemical evaluation of *in vitro* fermentation

The linear improvement in nutrient digestibility and increase gas production of bakery waste due to gelatinization of starch (Han *et al.* 2022; Nayohan *et al.*, 2024) ^[10, 22]. Additionally, high metabolizable energy (13.0 MJ/kg) with microbial biomass production of 731 mg/g digested OM of bakery waste indicates it high energy and high digestible feed ingredient.

As the inclusion level from 20% to 40% of bakery waste increased, significant difference was noted in net gas production (NGP) at 24 hrs. Increase utilization of easily fermentable substrate during *in vitro* fermentation causes the highest net gas production as seen at 40% inclusion levels (Kilama *et al.*, 2023) ^[15]. A similar result of linear increase in gas production was reported in various studies (Humer *et al.*, 2018; Kaltenegger *et al.*, 2020; Nayohan *et al.*, 2024) ^[11, 14, 22]. In cereal grains, starch is enclosed within a protein matrix and a crystalline granule structure that limited the amylolytic process which cause slower fermentation lead to lower NGP (Pastorelli *et al.*, 2023) ^[25], whereas the starch in bakery products undergoes gelatinization during baking, which disrupts the granule and increases its susceptibility to microbial and enzymatic attack (Han *et al.*, 2022) ^[10]. In addition to gelatinized starch, the sugar fraction of bakery waste also contribute to gas production, since simple sugars are fermented several-fold faster than starch in the rumen, with reported fractional degradation rates of approximately 300%/h for sugar compared with only 5-50%/h for starch (Nayohan *et al.*, 2024) ^[22]. Additionally, Dong *et al.* (2021) ^[5] also reported that increasing dietary sugar concentration significantly increased gas production independent of starch levels. Additionally, increase in IVTOMD and IVNDFD with increasing bakery waste level indicates that the improved fermentability. Gelatinized starch is degraded more rapidly by amylolytic bacteria resulting increase in microbial fermentative activity and provide more favorable environment for fibrolytic population. In contrast to this, Firkins *et al.* (2025) ^[8] reported that high concentration of

starch was suppress the fiber digestibility and reduce ruminal pH lead to acidosis conditions. In present study, this inhibitory effect did not appear to occur in the fiber digestibility mainly due to very low level of NDF and ADF in bakery waste which significantly reduce load of fiber and prevent acidosis conditions. This is supported by Nayohan *et al.* (2024) ^[22], who reported that the low residual fiber and high gelatinized-starch content of bakery by-products favored rather than compromised fiber disappearance *in vitro*.

The significantly higher ME availability of 11.1 MJ/kg was observed at the 40% level of inclusion mainly due to higher ME value of bakery waste, while microbial biomass production remain unaffected. This indicated that bakery waste increases ME availability with NGP while the efficacy of microbial protein synthesis is maintained across different levels. Previous studies have shown that high dietary fat concentrations (>60-70 g/kg DM) may depress ruminal fibre digestion by coating feed particles and inhibiting cellulolytic microorganisms (NRC 2001). However, despite containing 13.5% EE at the highest level of bakery waste inclusion, no negative effects on *in vitro* organic matter or fibre degradability were observed in the present study. This response may due to the low-NDF and highly digestible nature of bakery waste, which is rich in gelatinized starch and readily fermentable carbohydrates (França *et al.*, 2012; Kaltenegger *et al.*, 2021; Morais *et al.*, 2024) ^[7, 13]. The abundance of rapidly fermentable substrates likely stimulated microbial activity and fermentation, thereby offsetting any potential inhibitory effects of the lipid fraction. Consequently, organic matter and fibre degradability remained unaffected.

Table 1: Biochemical Characterization of Bakery waste, % DM

Parameter	Bakery waste
Nutrient composition	
Dry matter	96.0
Crude protein	7.41
Ether extract	19.8
Neutral detergent fiber	3.0
Acid detergent fiber	0.95
Crude fiber	1.78
Hemi-cellulose	2.05
Ash	1.98
Organic matter	98.0
Starch	39.9
Sugar	22.1
Total digestible nutrients	94.1
NE _L , Mcal/kg	2.19
Gross energy, kcal/g	5.097
Aflatoxins, ppb	8.8
Rancidity	
Free fatty acids, %	3.83
Peroxide value	ND
Microbiological parameters	
Total viable count, cfu/g	7.8×10^3
Coliform count, cfu/g	ND (MDL 10)
Salt	
Sodium, mg/kg	268.16
Chloride, %	0.35
Heavy metals	
Mercury	ND (MDL 10)
Cadmium	ND (MDL 10)
Lead, mg/kg	0.32
Arsenic	ND (MDL 10)

ND: not detected, BDL: below detection limit, MDL: method detection limit

Table 2: Ingredient composition of the concentrate mixture containing different levels of Bakery waste

S. No.	Ingredients	Inclusion level of Bakery waste, %								
		0	5	10	15	20	25	30	35	40
1	Maize	35.0	30.0	25.0	20.0	15.0	10.0	5.00	0.00	0.00
2	Rice Kanni	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	0.00
3	Bakery Waste	0.00	5.00	10.0	15.0	20.0	25.0	30.0	35.0	40.0
4	Mustard Cake (De-Oiled)	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
5	Mustard Cake (Oiled)	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
6	Soya Cake (De-Oiled)	10.0	10.0	10.0	9.00	9.00	8.00	8.00	8.00	8.00
7	Rice Bran (20%)	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70
8	De Oiled Rice Bran	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
9	Roasted Soybean	0.00	0.00	0.00	1.00	1.00	2.00	2.00	2.00	2.00
10	Guar Korma	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
11	Wheat Bran	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
12	By Pass Fat	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
13	Urea	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
14	Limestone Powder	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
15	Trace Mineral	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
16	Magnesium Oxide	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
17	Salt	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
18	Sodium Bicarbonate	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
19	Toxin Binder	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
20	Mineral mixture	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
21	Yeast	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
22	Vitamin AD ₃	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10

Table 3: Biochemical composition of concentrate mixture containing different levels of Bakery waste (% DM basis)

Parameter	Inclusion level of Bakery waste, %								
	0	5	10	15	20	25	30	35	40
Crude protein	25.7	25.6	25.5	25.9	25.4	25.2	25.2	25.1	25.0
Ether extract	6.19	7.10	8.00	8.90	9.80	10.7	11.6	12.5	13.5
Ash	9.13	9.20	9.28	9.34	9.41	9.47	9.55	9.62	9.67
Acid insoluble ash	1.25	1.29	1.33	1.39	1.41	1.46	1.50	1.54	1.57
Organic matter	90.87	90.8	90.72	90.66	90.59	90.53	90.45	90.38	90.33
Neutral detergent fibre	15.4	15.1	14.8	14.4	14.0	13.70	13.4	13.0	12.7
Acid detergent fibre	9.36	9.30	9.24	9.20	9.16	9.12	9.07	9.00	8.94
Acid detergent lignin	3.15	3.12	3.10	3.07	3.05	3.02	3.00	2.97	2.95
Hemicellulose	6.04	5.8	5.56	5.20	4.84	4.58	4.33	4.00	3.76
Cellulose	6.21	6.18	6.14	6.13	6.11	6.1	6.07	6.03	5.99
Starch	33.5	32.6	31.8	30.9	30.1	29.3	28.4	27.6	26.9
Crude fibre	6.71	6.60	6.50	6.38	6.20	6.00	5.90	5.78	5.60
Total digestible nutrients	74.1	74.3	74.4	75.4	74.7	74.9	75.0	75.2	75.8
RUP (% of CP)	36.9	36.7	36.5	36.8	36.4	36.6	36.4	36.4	36.4
ME (MCal/kg)	2.661	2.658	2.656	2.676	2.651	2.648	2.646	2.643	2.661

RUP – Rumen Undegradable Protein

Table 4: Biochemical Evaluation of *In vitro* fermentation of bakery waste

Parameter	Bakery waste
NGP, ml/g at 24 h	264
IVNDFD, %	66.0
IVTOMD, %	96.6
ME, MJ/kg	13.0
MBP, mg/g digested OM	731

NGP: net gas production; IVTOMD: *in vitro* true organic matter degradability; IVNDFD: *in vitro* neutral detergent fiber degradability; MBP: microbial biomass production; ME: metabolizable energy

Table 5: Effect of inclusion of Bakery waste on biochemical evaluation of *in vitro* fermentation

Variable	Inclusion level of bakery waste									SEM	P-value	Contrast P-value	
	0	5	10	15	20	25	30	35	40			Linear	Quadratic
NGP, ml/g at 24 h	159.2 ^a	162.5 ^{ab}	165.0 ^{abc}	169.2 ^{abc}	170.8 ^{cd}	174.2 ^{cd}	180.0 ^{de}	181.7 ^e	182.5 ^e	1.7088	0.000	0.000	0.764
IVNDFD, %	51.3 ^a	51.4 ^a	52.7 ^{ab}	53.7 ^{abc}	54.8 ^{abc}	56.2 ^{bcd}	57.7 ^{bcd}	59.0 ^{cd}	61.9 ^d	0.814	0.003	0.000	0.288
IVTOMD, %	90.0 ^a	90.4 ^{ab}	91.2 ^{abc}	92.6 ^{abcd}	93.1 ^{abcd}	93.6 ^{bcd}	93.6 ^{bcd}	94.1 ^{cd}	94.7 ^d	0.414	0.036	0.000	0.462
MBP, mg/g digested OM	549.9	661.8	549.4	676.7	675.8	679.3	672.2	675.4	681	32.78	0.976	0.362	0.675
ME, MJ/kg	9.20 ^a	9.44 ^b	9.66 ^b	9.96 ^c	10.1 ^c	10.4 ^d	10.7 ^e	10.9 ^{ef}	11.1 ^f	0.122	0.000	0.000	0.748

Means bearing different superscripts with in a row differ significantly ($P < 0.05$); NGP: net gas production; IVTOMD: *in vitro* true organic matter degradability; IVNDFD: *in vitro* neutral detergent fiber degradability; MBP: microbial biomass production; ME: metabolizable energy; DM: dry matter

Conclusion

Replacing grains with bakery waste improved gas production, starch degradability and metabolizable energy availability with maintaining microbial biomass production. Improved fermentation

response was mainly attributed to the high content of fermentable sugars and low fibre concentration of bakery waste. Overall, the result indicate that bakery waste can effectively replace cereal grains up to 40% inclusion in the concentrate mixture with improved rumen fermentation characteristics and energy

availability without compromising nutrient digestibility, indicating that bakery waste can serve as a sustainable alternative energy source in ruminant feeding systems.

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References

- Association of Official Analytical Chemists. Official methods of analysis. 18th ed. Gaithersburg (MD): Association of Official Analytical Chemists; 2001.
- Bytyqi H, Kunili IE, Mestani M, Antoniak MA, Berisha K, Dinc SO, *et al.* Consumer attitudes towards animal-derived food waste and ways to mitigate food loss at the consumer level. *Trends Food Sci Technol.* 2025;159:104898.
- Champe KA, Church DC. Digestibility of dried bakery product by sheep. *J Anim Sci.* 1980;51(1):25-27.
- Derbyshire E. Food-based dietary guidelines and protein quality definitions—time to move forward and encompass mycoprotein? *Foods.* 2022;11(5):647.
- Dong JN, Li SZ, Chen X, Qin GX, Wang T, Sun Z, *et al.* Effects of different combinations of sugar and starch concentrations on ruminal fermentation and bacterial-community composition in vitro. *Front Nutr.* 2021;8:727714.
- El-Haysha MS, Abdel-Razek GA, El-Sayed AA. Growing ruminants fed rations containing bread wastes: a review. *J Anim Vet Sci.* 2017;4:1-3.
- França AB, Morenz MJ, Lopes FC, Madeiro AS, Morenz DA, Faria BM, *et al.* Bakery waste in sheep diets: intake, digestibility, nitrogen balance and ruminal parameters. *R Bras Zootec.* 2012;41(1):147-153.
- Firkins JL, Henderson EL, Duan H, Pope PB. International Symposium on Ruminant Physiology: current perspective on rumen microbial ecology to improve fiber digestibility. *J Dairy Sci.* 2025;108(7):7511-7529.
- Grings EE, Blümmel M, Südekum KH. Methodological considerations in using gas production techniques for estimating ruminal microbial efficiencies for silage-based diets. *Anim Feed Sci Technol.* 2005;123:527-545.
- Han C, Guo Y, Cai X, Yang R. Starch properties, nutrients profiles, in vitro ruminal fermentation and molecular structure of corn processed in different ways. *Fermentation.* 2022;8(7):315.
- Humer E, Aditya S, Kaltenegger A, Klevenhusen F, Petri RM, Zebeli Q. Graded substitution of grains with bakery by-products modulates ruminal fermentation, nutrient degradation, and microbial community composition in vitro. *J Dairy Sci.* 2018;101(4):3085-3098.
- Humer E, Zebeli Q. Grains in ruminant feeding and potentials to enhance their nutritive and health value by chemical processing. *Anim Feed Sci Technol.* 2017;226:133-151.
- Kaltenegger A, Hummer E, Pacífico C, Zebeli Q. Feeding dairy cows bakery by-products enhanced nutrient digestibility, but affected fecal microbial composition and pH in a dose-dependent manner. *J Dairy Sci.* 2021;104(7):7781-7793.
- Kaltenegger A, Hummer E, Stauder A, Zebeli Q. Feeding of bakery by-products in the replacement of grains enhanced milk performance, modulated blood metabolic profile, and lowered the risk of rumen acidosis in dairy cows. *J Dairy Sci.* 2020;103(11):10122-10135.
- Kilama J, Yakir Y, Shaani Y, Adin G, Kaadan S, Wagali P, *et al.* Chemical composition, in vitro digestibility, and storability of selected agro-industrial by-products: alternative ruminant feed ingredients in Israel. *Heliyon.* 2023;9(3):e14302.
- Kırkpınar F, Açıkgoz Z, Niyonshuti E. Current situation, future goals, and strategies of the global feed and feed additives industries. In: *Animal Feeds and Additives.* London: IntechOpen; 2025.
- Marchi FE, Figueiroa FJ, Santos GT, Santos WB, Kazama DC, Branco AF, *et al.* Intake, digestibility and ruminal parameters of dairy cows fed pelleted diets and treated with lignosulfonate-containing sunflower seeds. *R Bras Zootec.* 2013;42:656-663.
- Menke KH. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim Res Dev.* 1988;28:7-55.
- Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. *J Agric Sci.* 1979;93(1):217-222.
- Narasimmalu A, Ramasamy R. Food processing industry waste and circular economy. *IOP Conf Ser Mater Sci Eng.* 2020;955(1):012089.
- Nath PC, Ojha A, Debnath S, Sharma M, Nayak PK, Sridhar K, *et al.* Valorization of food waste as animal feed: a step towards sustainable food waste management and circular bioeconomy. *Animals (Basel).* 2023;13(8):1366.
- Nayohan S, Sekoguchi M, Nishikawa Y, Matamura M, Jayanegara A, Matsui H, *et al.* In vitro rumen fermentation characteristics of bakery by-products containing high starch and sugar. *Anim Sci J.* 2024;95(1):e14000.
- National Research Council. Nutrient requirements of dairy cattle. 7th rev ed. Washington (DC): National Academies Press; 2001. 405 p.
- Obeidat BS. Bakery waste inclusion in the diet of growing black goat kids: evaluation of performance and health aspects. *Animals (Basel).* 2025;15(3):383.
- Pastorelli G, Simeonidis K, Faustini M, Le Mura A, Cavalleri M, Serra V, *et al.* Chemical characterization and in vitro gas production kinetics of alternative feed resources for small ruminants in the Maltese islands. *Metabolites.* 2023;13(6):762.
- Pinotti L, Luciano A, Ottoboni M, Manoni M, Ferrari L, Marchis D, *et al.* Recycling food leftovers in feed as opportunity to increase the sustainability of livestock production. *J Clean Prod.* 2021;294:126290.
- Robertson JB, Van Soest PJ. The detergent system of analysis. In: James WPT, Theander O, editors. *The analysis of dietary fibre in food.* New York: Marcel Dekker; 1981. p. 123-158.
- Shilwant S, Hundal JS, Singla M, Patra AK. Ruminal fermentation and methane production in vitro, milk production, nutrient utilization, blood profile, and immune responses of lactating goats fed polyphenolic and saponin-rich plant extracts. *Environ Sci Pollut Res Int.* 2023;30(4):10901-10913.
- Shurson GC, Dierenfeld ES, Dou Z. Rules are meant to be broken—rethinking the regulations on the use of food waste as animal feed. *Resour Conserv Recycl.* 2023;199:107273.
- Soria A, Rodriguez-Huezo ME, Vernon-Carter EJ, Alvarez-Ramirez J. Upcycling food waste flour into dry wheat pasta production: impact on color, textural and structural characteristics, and in vitro protein and starch digestibility. *Cienc Agrotec.* 2025;49:e007425.
- Wardeh MF. Models for estimating energy and protein utilization for feeds [dissertation]. Logan (UT): Utah State University; 1981.