



**EVALUATION OF CONCENTRATE DIET CONTAINING UREA AND MULTI-
ENZYME PRETREATED CORN COBS FOR RUMINANT NUTRITION USING *IN*
VITRO GAS PRODUCTION TECHNIQUE**

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ABSTRACT

The effective and efficient utilization of agricultural residues remains critical for sustainable ruminant production, particularly in regions that generate substantial corn processing waste. This study evaluated how urea and multi-enzyme pretreatment of corn cobs affect the in vitro fermentation characteristics of corn cob-containing concentrate diets. Ground corn cobs were pretreated with two urea levels (0 and 5%) and three multi-enzyme levels (0, 0.5, and 1.0 mg/g) in a 2×3 factorial arrangement. Chemical analysis showed that 5% urea pretreatment increased ($p<0.05$) crude protein of corn cobs from 4.22% to 6.21%, while multi-enzyme reduced hemicellulose content from 40.27% to 32.18%. In vitro fermentation showed that urea reduced total gas production at 24-48 h of incubation. Multi-enzyme enhanced neutral detergent fibre degradability, with 0.5 mg/g level achieving 60.19% degradability compared to 54.18% in the control. However, multi-enzyme pretreatment increased methane production by 37%, raising environmental concerns. The interaction effects of urea and multi-enzyme pretreatment revealed synergistic responses between treatments. These findings show that combined urea and multi-enzyme pretreatment substantially improved the chemical composition of corn cobs and the fermentation characteristics of a concentrate diet containing pretreated corn cobs, although

methane mitigation strategies may be needed to balance nutritional gains with environmental sustainability in practical feeding programs.

Keywords: Crop byproduct; dietary fibre; exogenous enzymes; fibre degradation; Natuzyme®

INTRODUCTION

The growing demand for animal protein in recent years has been putting considerable pressure on livestock producers to develop feeding strategies that make both economic and nutritional sense. In ruminant nutrition, the rising costs of conventional protein sources, combined with the need to better utilize agricultural by-products, pose a major challenge (Kazemi, 2025), prompting exploration of alternative nitrogen supplements and low-cost feed resources. The use of urea as a non-protein nitrogen source in ruminant diets is gaining traction because it is cheap, nitrogen-dense, readily available (Wahyono *et al.*, 2022), and it effectively provides nitrogen for microbial protein synthesis in the rumen, thereby supporting animal growth while reducing dependence on expensive supplements like soybean meal (Xu *et al.*, 2019). However, efficient urea use requires good synchronisation between ammonia release and available fermentable energy, without which nitrogen loss or ammonia toxicity occurs.

Corn cobs, which are available in large quantities after maize processing, could serve as a fibre and energy source for ruminants (Mierliță, 2001). Unfortunately, their use is limited by their poor nutritional value, primarily due to their high lignocellulose content, which reduces fermentation efficiency and digestibility. While ruminants are naturally equipped to digest lignocellulosic biomass, the presence of lignin significantly limits the utilization of cellulose and hemicellulose (van Kuijk *et al.*, 2015; Islamiyati *et al.*, 2025; Zheng *et al.*, 2025). To address the nutritional limitations of corn cobs, physical, chemical and biological treatment methods have been employed. Urea treatment is a widely adopted chemical treatment method for corn cobs, serving the dual purpose of increasing crude protein content while breaking down lignocellulosic

structures through ammonia action. The ammonia released from urea hydrolysis cleaves ester bonds linking lignin with hemicellulose and cellulose, thereby improving carbohydrate accessibility to rumen microbes (Supriyati *et al.*, 2018). The use of enzymes as a biological approach has also gained attention, with exogenous fibrolytic enzymes targeting specific components of the plant cell wall to enhance carbohydrate release (Amaro-Reyes *et al.*, 2016).

Enzyme cocktails containing cellulases, hemicellulases, and xylanases can selectively degrade cellulose and hemicellulose fractions without harsh chemical conditions, making the carbohydrate more accessible for ruminal fermentation. The combined application of urea and multi-enzyme pretreatment to corn cobs has received limited research attention and represents an innovative dual-action approach that may offer synergistic benefits by simultaneously addressing nitrogen deficiency and structural recalcitrance. The resulting pretreated corn cobs could serve as an improved feed ingredient in ruminant concentrate formulations. However, little is known about the combined effect of urea and multi-enzyme pretreatment on corn cobs, particularly in terms of fermentation kinetics, digestibility and metabolizable energy. This research is particularly relevant in regions where corn cobs accumulate after harvest but go largely unused; therefore, turning the waste into an improved feed ingredient could provide both economic and environmental benefits through sustainable agricultural resource utilization. The *in vitro* gas production technique is a feed evaluation method that provides detailed information on fermentation kinetics, digestibility and metabolizable energy under controlled conditions (Kumar *et al.*, 2015; Wang *et al.*, 2025). Compared with *in vivo* trials, it is cost-effective, faster, and yields consistent results, enabling simultaneous testing of multiple samples (Dhanoa *et al.*, 2000).

MATERIALS AND METHODS

Study location

This study was carried out at the laboratory of the Department of Animal Nutrition, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria. The site is located in southwestern Nigeria at coordinates 7°13'58" N and 3°26'14" E (Google Earth, 2025).

Pretreatment of corn cobs

Corn cobs were collected from the maize processing unit of the Directorate of University Farms after removal of grains (kernels) with a mechanical sheller. The collected cobs were ground through a 4mm sieve and treated with two levels of urea (0 and 5%) and three levels of multi-enzyme (0, 0.5 and 1 mg/g). The multi-enzyme used in this study is Natuzyme®, a commercially available exogenous enzyme blend in powder form produced by Bioproton PTY Ltd, Queensland, Australia, with cellulase (6000 u/g), xylanase (10,000 u/g), beta-glucanase (700 u/g), phytase (1500 u/g), protease (700 u/g) and amylase (400 u/g) enzyme activities. Urea was made into a solution by dissolving 0 and 50 g urea/ 1 kg corn cobs (w/w) in 50% (500mL) water (w/v) by continuous stirring (Ben Salem *et al.*, 2005), and the appropriate multi-enzyme dose rate (0, 0.5 and 1mg/g) was added and mixed. The mixture was then homogeneously mixed with the milled corn cobs, and the treated corn cobs were then kept in airtight containers at room temperature for 14 days. The pretreated corn cobs were then incorporated into a concentrate diet. Chemical composition of pretreated corn cobs was determined using standard methods (Van Soest *et al.*, 1991; AOAC, 2012).

Experimental substrates, treatments and experimental design

The experimental substrate in this study consisted of 40% *Megathyrus maximus* and 60% concentrate diet on a dry matter basis. The concentrate diet contained 13% pre-treated corn cobs, 20% cocoa seed testa, 11% groundnut cake, 20% rice bran, 13% wheat offal, 8.5% cassava peels, 10% palm kernel cake, 3% bone meal, 1% common salt and 0.5% premix. The experiment used a 2 x 3 factorial design (2 urea levels and 3 multi-enzyme levels) in a completely randomized design, with 6 treatments and 15 replicates per treatment.

Incubation procedure

The *in vitro* gas production technique employed was that described by Menke and Steingass (1988). Rumen content was collected from the rumen of one White Fulani bull weighing approximately 250Kg immediately after slaughter at the abattoir into an insulated flask pre-

warmed to 39°C and used as the source of rumen fluid. This was then transported immediately to the laboratory, strained through layers of cheesecloth, and mixed with mineral buffer solution (1:2 v/v) to form the inoculum used for digestion. Approximately 500mg each of incubation substrate was weighed separately into fibre bags of known weight and inserted into 100 mL calibrated syringes fitted with a silicon tube at the tip. About 30 mL of buffered inoculum was then added to each syringe, and the piston was pushed upward to expel air. The silicon tube was then secured with a metal clip to prevent gas from escaping during incubation. The syringes were arranged in an incubator, and incubation was carried out at 39°C. The volume of gas produced was measured at three-hour (h) intervals from 0 to 48 hours. Three blank syringes containing 30 mL of inoculum were included in the run to correct for gas production from fermentation of residual feed particles in the buffer inoculum. The syringes were shaken at regular intervals during the reading of the gas produced to simulate rumen motility. Measurement of methane gas production and degradation of dry matter and neutral detergent fibre.

The volume of methane gas produced was determined using the carbon-dioxide absorption method with sodium hydroxide as the alkaline solution for trapping carbon-dioxide, following the procedure outlined by Fievez *et al.* (2005). After 48 h of incubation, fibre bags in the syringes were removed and washed properly under a continuous flow of water to terminate fermentation. The bags were then oven-dried at 105°C for 12 h. The weight of residues in the bags was estimated by subtracting the known weight of the bags from the weight of the bags plus residues. Substrate degradation was determined by subtracting the un-degraded residue for each sample from the initial quantity incubated. For the determination of neutral detergent fibre (NDF) degradability, samples of substrate incubated and the residues were analyzed for NDF content following procedures of Van Soest *et al.* (1991). NDF degradation was calculated by subtracting the undegraded NDF in the residue from the initial quantity incubated.

Statistical analysis

Data generated from this study were analyzed using the general linear model (GLM) procedure of SAS (2002). Significant differences between treatment means were separated using Duncan's multiple range test or a t-test for the main effect of urea with 2 levels (SAS 2002).

RESULTS AND DISCUSSION

The pretreatment of corn cobs with 5% urea resulted in improvements ($p < 0.05$) in their dry matter and crude protein (CP) contents (Table 1). The CP increase reflects the effective incorporation of non-protein nitrogen into the corn cobs via urea hydrolysis, in which urea is converted to ammonia, which then becomes incorporated into the treated material and subsequently serves as a readily available nitrogen source for rumen microorganisms. The role of urea in enhancing nitrogen availability when added to low-quality fibrous feeds is well-established (Sari *et al.*, 2018; Yulistiani *et al.*, 2024). Multi-enzyme pretreatment had an effect on hemicellulose concentration ($p < 0.05$) of corn cobs, with the concentration reduced linearly with an increase in the level of multi-enzyme from 0.5 mg/g to 1.0 mg/g when compared to the control. The multi-enzyme used has cellulase and xylanase activities, which have been reported to exert substrate-specific synergistic effects, resulting in enhanced degradation of lignocellulosic materials (Haung *et al.*, 2018). Results from this study therefore suggest targeted enzymatic hydrolysis of β -1,4-glycosidic bonds in xylan polymers of corn cobs, which are more susceptible to enzymatic attack than cellulose or lignin (Dagaew *et al.*, 2025). Results of the interaction effect of urea and multi-enzyme pretreatment of corn cobs showed improved concentrations ($p < 0.05$) of dry matter and crude protein, indicating synergistic benefits, with the optimal combination of 5% urea and 0.5 mg/g multi-enzyme achieving 6.65% crude protein. This compositional change aligns with the strategic upgrading of crop residues to improve their feeding value for ruminants. The results of *in vitro* fermentation in Table 2 show that both urea and multi-enzyme pretreatment of corn cobs significantly ($p < 0.05$) influenced total gas production, fibre degradation, and methane output from the incubation substrate, indicating pronounced effects on rumen microbial activity and substrate utilization. Pretreatment of corn cobs with 5% urea resulted in reduction in gas production during the later stage of fermentation compared to the control, rather than indicating impaired fermentation, this response likely reflects changes in fermentation efficiency to favour efficient microbial growth and improved nitrogen synchrony (He *et al.*, 2022; Simeonidis *et al.*, 2025), by allowing a greater portion of the fermentable energy to be incorporated into microbial biomass rather than being lost in gas. Gas production represents fermentation end-products rather than total substrate disappearance,

and the observed similar substrate dry matter degradation with urea pretreatment in this study further corroborates this assumption. The two multi-enzyme levels (0.5 and 1.0 mg/g) reduced gas production ($p < 0.05$) during the early stage of fermentation (the first 3-12 h of incubation). These reductions likely reflect enzymatic modifications of the fibre matrix prior to extensive microbial colonization, which could have rather thaned fermentation rates rather than suppressed microbial activity. The *in vitro* neutral detergent fibre degradation (IVNDFD) of substrate was enhanced by multi-enzyme pretreatment. This response demonstrates the effectiveness of enzymatic pretreatment in breaking down recalcitrant cell wall components, thereby improving fibre utilization by rumen microorganisms. Methane production exhibited a contrasting response, underscoring a key trade-off between nutrition and the environment. Multi-enzyme pretreated substrates produced more methane, with an increase of approximately 37% relative to the control. This increase suggests greater hydrogen availability resulting from enhanced fibre degradation, which in turn favoured methanogenesis when alternative hydrogen sinks were insufficient (Li *et al.*, 2025; Dagaew *et al.*, 2025). The increase in methane production will increase greenhouse gas (GHG) emissions, resulting in a larger carbon footprint. There may be a need to redirect the available hydrogen via nitrate supplementation, although this may reduce fibre digestibility, creating a trade-off between digestion efficiency and emission reduction (Hiristov *et al.*, 2013). The positive interaction effect between urea and multi-enzyme pretreatment across incubation periods further indicates that fermentation responses were not simply additive but depended on specific treatment combinations.

Table1: Chemical composition (%DM) of urea and multi-enzyme pretreated corn cobs

Urea (%)	Multi-enzyme (mg/g)	Parameters								
		Dry matter	Crude protein	Ether extract	Ash	NDF	ADF	ADL	Hemic.	Cell.
<i>Main effect of urea level</i>										
0		77.27 ^b	4.22 ^b	3.85	3.48	74.48	38.44	12.01	36.04	26.43
5		83.83 ^a	6.21 ^a	3.83	3.43	77.17	41.01	13.24	36.15	27.77
SEM		0.93	0.26	0.07	0.12	0.92	0.79	0.36	1.23	0.65
<i>P</i>		<0.001	<0.001	0.878	0.845	0.150	0.103	0.089	0.967	0.314
<i>Main effect of multi enzyme level</i>										
	0.0	79.57	4.87	3.86	3.43	78.21	37.94	11.51	40.27 ^a	26.43
	0.5	79.77	5.54	3.87	3.41	75.64	39.80	12.86	35.84 ^{ab}	26.94
	1.0	82.32	5.22	3.79	3.54	73.63	41.45	13.51	32.18 ^b	27.94
SEM		0.93	0.26	0.07	0.12	0.92	0.79	0.36	1.23	0.65
	<i>p</i>	0.427	0.598	0.882	0.911	0.121	0.197	0.062	0.016	0.653
<i>Interaction effect of urea and multi-enzyme level</i>										
0	0.0	76.87 ^d	3.79 ^d	3.78	3.39	76.76	36.73	10.76	40.03	26.89
	0.5	74.99 ^d	4.44 ^c	3.91	3.48	74.29	38.52	12.04	35.78	27.41
	1.0	79.96 ^c	4.44 ^c	3.85	3.58	72.40	40.08	13.22	32.33	29.02
5	0.0	82.26 ^b	5.95 ^b	3.94	3.48	79.67	39.15	12.26	40.52	25.97
	0.5	84.56 ^{ab}	6.65 ^a	3.82	3.34	76.98	41.08	13.67	35.89	26.48
	1.0	84.67 ^a	6.01 ^b	3.73	3.49	74.85	42.81	13.79	32.04	26.85
SEM		0.93	0.26	0.07	0.12	0.92	0.79	0.36	1.23	0.65
	<i>p</i>	<0.001	<0.001	0.960	0.997	0.287	0.325	0.095	0.191	0.866

^{abcd} Means in the same column with different letters are different at $p < 0.05$; NDF – neutral detergent fibre, ADF – acid detergent fibre, ADL – acid detergent lignin, Hemic – hemicellulose,

Cell – cellulose.

Table2: Effect of urea and multi-enzyme pretreatment of corn cobs on total gas production, dry matter degradation, neutral detergent fibre degradation and methane production from microbial fermentation of concentrate diet-based substrate in an *in vitro* medium

Urea (%)	Multi-enzyme (mg/g)	Incubation period (hours)						IVDMD (%)	IVNDFD (%)	CH ₄ (%)
		3	6	12	24	36	48			
<i>Main effect of urea level</i>										
0		0.93	2.64	8.13	20.67 ^a	27.18 ^a	31.47 ^a	56.49	57.15	24.58
5		1.00	2.80	7.78	16.36 ^b	21.76 ^b	24.80 ^b	56.93	57.37	26.36
SEM		0.09	0.18	0.38	0.68	0.92	1.05	0.67	0.74	1.45
<i>p</i>		0.705	0.658	0.639	0.001	0.003	0.001	0.741	0.885	0.550
<i>Main effect of multi enzyme level</i>										
	0.0	1.33 ^a	3.50 ^a	9.30 ^a	19.17	26.43	30.33	56.60	54.18 ^b	20.38 ^b
	0.5	0.97 ^{ab}	2.73 ^a	8.23 ^a	18.70	24.47	28.13	56.33	60.19 ^a	28.21 ^a
	1.0	0.60 ^b	1.93 ^b	6.33 ^b	17.67	22.50	25.93	57.20	57.42 ^a	27.81 ^a
SEM		0.09	0.18	0.38	0.68	0.92	1.05	0.67	0.74	1.45
<i>p</i>		0.002	0.001	0.004	0.655	0.216	0.236	0.865	0.002	0.040
<i>Interaction effect of urea and multi-enzyme level</i>										
0	0.0	1.40 ^a	3.73 ^a	10.13 ^a	21.07 ^{ab}	30.00 ^a	34.80 ^a	57.07	54.31 ^b	14.92 ^b
	0.5	0.93 ^{abc}	2.80 ^{ab}	8.73 ^{ab}	21.20 ^a	27.53 ^{ab}	31.67 ^{ab}	56.00	60.24 ^a	29.21 ^a
	1.0	0.47 ^c	1.40 ^c	5.53 ^c	17.73 ^{abc}	24.00 ^{ab}	27.93 ^{abc}	56.40	56.92 ^{ab}	29.60 ^a
5	0.0	1.27 ^{ab}	3.27 ^{ab}	8.47 ^{ab}	17.27 ^{abc}	22.87 ^b	25.87 ^{bc}	56.13	54.05 ^b	25.83 ^a
	0.5	1.00 ^{abc}	2.67 ^{ab}	7.73 ^{abc}	16.20 ^{bc}	21.40 ^b	24.60 ^{bc}	56.67	60.15 ^a	27.21 ^a
	1.0	0.73 ^{bc}	2.47 ^{bc}	7.13 ^{bc}	15.60 ^c	21.00 ^b	23.93 ^c	58.00	57.92 ^{ab}	26.03 ^a
SEM		0.09	0.18	0.38	0.68	0.92	1.05	0.67	0.74	1.45
<i>p</i>		0.021	0.003	0.010	0.033	0.021	0.013	0.963	0.003	0.024

^{abcd} Means in the same column with different letters are different at $p < 0.05$; IVNDFD – in vitro neutral detergent fibre degradation; IVDMD – in vitro dry matter degradability; CH₄ – methane

CONCLUSION

Pretreatment of corn cobs with urea and multi-enzyme offers a practical strategy for transforming agricultural waste into nutritionally enhanced ruminant feed. The 47% increase in crude protein content and 11% improvement in fibre degradability demonstrate clear nutritional benefits. The optimal treatment combination of 5% urea and 0.5 mg/g multienzyme improved crude protein content and fibre degradability while influencing fermentation characteristics. Further *in vivo studies* should be carried out to confirm the observed improvement in fibre degradability and fermentation characteristics of corn cobs with the combined pre-treatment with urea and multi-enzyme.

Author contribution

Oguntuyo, S. A. – conceptualization, methodology, investigation, formal analysis and writing of the original draft. Isah, O. A. – methodology, resources provision, supervision and review and editing of the manuscript. Aderinboye, R.Y. – supervision, data analysis and review and editing of the manuscript. Williams, T. J.- supervision and manuscript review and editing.

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