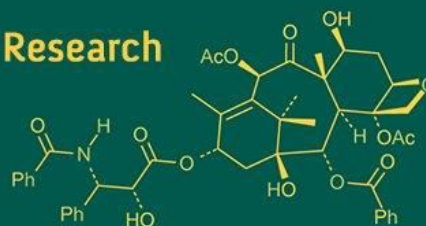


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Evaluation of physical quality and *in vitro* fermentation parameters of paddy straw-based pellets treated with urea and indigenous cow urine

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Abstract

The present study evaluated the potential of paddy straw-based complete feed pellets treated with indigenous cow urine (5%, 7.5%, and 10%) and urea (1%) as alternative nitrogen sources by assessing pellet physical quality and *In-vitro* rumen fermentation characteristics. Pellet length and diameter were not significantly affected by treatment ($p>0.05$). However, nitrogen source significantly influenced pellet integrity, with urea-treated pellets (UREA P) exhibiting superior physical quality ($p<0.001$), characterized by the highest pellet durability index (89.61%) and bulk density (696.60 kg/m³). In contrast, higher urine inclusion (10%) adversely affected pellet structure, resulting in a significantly greater proportion of fines (2.87%). *In vitro* rumen fermentation revealed a significant reduction ($p<0.05$) in total gas and methane production in both urea- and urine-supplemented pellets compared to the control. Methane production declined from 6.22 mL/200 mg DM in the control to 4.15 mL/200 mg DM in UREA P and 4.20 mL/200 mg DM in the 7.5% urine treatment. Despite reduced gaseous output, *In-vitro* dry matter digestibility and total digestible organic matter remained comparable across all treatments ($p>0.05$). Nitrogen supplementation significantly enhanced fermentation efficiency, as evidenced by increased partitioning factor and microbial biomass production ($p<0.05$), with the highest microbial biomass yields recorded for UREA P (58.77 mg) and 7.5% urine pellets (57.26 mg). These findings indicate that inclusion of 1% urea or 7.5% indigenous cow urine effectively optimizes the physical integrity and fermentative efficiency of paddy straw-based pellets by reducing methane-associated energy losses and enhancing microbial protein synthesis. The use of cow urine as a nitrogen source offers a sustainable and low-cost strategy for valorizing paddy straw and improving ruminant feed efficiency.

Keywords: Paddy straw, complete feed pellets, *In vitro* rumen fermentation, indigenous cow urine

Introduction

India's livestock sector, a crucial component of the agricultural economy, grapples with a critical shortage of feed resources. Despite supporting a large portion of the global livestock population, the country faces significant deficits in dry fodder (23.4%), green fodder (11.24%), and concentrate feed (28.9%). This scarcity is largely due to the limited land allocated for fodder cultivation, which has stagnated at a mere 5.4% of the total cultivated area for two decades (ICAR-IGFRI, 2022) [10]. Consequently, livestock productivity is suboptimal, hindering the sector's growth. Simultaneously, India's crop production generates vast amounts of agricultural residues, with approximately 585 million tonnes produced annually. Of this, about 151 million tonnes, primarily from rice, wheat, and sugarcane, are either underutilized due to imbalanced nutrient composition or wasted sometimes. The global emphasis on resource efficiency and waste reduction, as highlighted by the UN Sustainable Development Goals (SDGs), necessitates innovative solutions for valorizing such agricultural residues. Paddy straw presents a unique paradox. While its direct use as a ruminant feed is limited due to its poor nutritive value containing low levels of Total Digestible Nutrients (TDN) and almost no Digestible Crude Protein (DCP) and the presence of anti-nutritional factors like lignin and silica, its high carbohydrate content (approximately 70%) makes it a viable energy source if its utilization can be enhanced (Hartley *et al.*, 1974) [8]. Chemical treatments, such as using ammonia or urea, have shown promise in breaking

down complex fiber structures in straw and increasing protein levels. However, these methods may involve costs or require specialized handling, posing challenges for smallholder farmers. One potential alternative is the use of animal urine as a treatment for rice straw. In India, a vast network of gaushalas (cow shelters) and numerous unproductive animals contribute to a significant supply of cow urine, a resource that holds potential for various applications. Research indicates that treating rice straw with animal urine can increase its crude protein content and digestibility in sheep. Coxworth and Kullman (1978)^[7] have demonstrated under laboratory conditions that cattle urine is as efficient as other sources of ammonia for straw treatment. As urine contains urea, it can be used as a source of urea and ammonia to improve the quality of rice straw. Urine can be sprayed on the straw similarly to urea solutions (Dias da Silva, 1993)^[8] and can provide a nearly equal improvement in degradability and nitrogen content as other methods of ammonia treatment (Dias da Silva, 1993)^[8]. The abundance of rice straw as an agricultural by-product has spurred research into methods for improving its digestibility and nutritional content for livestock. Methods such as fermentation, creating complete feed blocks, and pelleting have all been shown to improve digestibility and efficiency (Bakshi and Wadhwa, 2017)^[3]. Pelleting is especially promising as it transforms the bulky straw into a compact, uniform product, making it much easier to store, transport, and feed to livestock. Pelleting paddy straw with other ingredients taking urine and urea as nitrogenous source may help to provide balanced nutrition to dairy animals and utilize paddy straw for efficiently.

The present study was conducted to evaluate the suitability of paddy straw based pellets, treated with Urea/Urine and supplemented with other essential ingredients (maize, soybean meal, distillers dried grains with soluble, molasses, salt, mineral mixture). This present research aims to bridge the gap between two major challenges in India's agricultural sector, the shortage of livestock feed and the unsustainable disposal of agricultural residues.

Materials and Methods

Ethical considerations

The ethical conduct of this study was paramount, and all procedures were carried out in strict adherence to the guidelines laid down by the Institutional Animal Ethics Committee (IAEC) of the ICAR-National Dairy Research Institute, Karnal. The study protocol was formally approved under reference number 55-IAEC-25-03, with the ICAR-National Dairy Research Institute holding the registration number 1705/GO/ReBi/SL/13/CCSEA. The study's design and execution upheld the highest standards of animal care and ethical practice as required by the overseeing committees.

Collection and Safety Evaluation of Indigenous Cow Urine for Use in Feed Pellets

Urine samples (50-100 mL) were aseptically collected from healthy Sahiwal cows following thorough cleaning of the perineal area. Midstream urine was obtained either during spontaneous or induced urination, immediately labeled, chilled on ice for transport, and stored at 2-8 °C for no more than 24 hours prior to analysis. Urine pH was measured using a calibrated digital pH meter. For assessment of microbial safety, aliquots were cultured on CLED agar and

nutrient agar plates, with aerobic incubation at 35-37 °C for 18-24 hours. Colony counts were recorded and converted to CFU/mL, and colony morphology evaluated as described by Karah *et al.* (2020)^[14]. Urine sediment was examined microscopically post-centrifugation, with quantification of cell types and crystalline structures per high-power field, following the methodology of Swenson *et al.* (2011). Bacterial load was categorized as occasional, few, moderate, or many. Total nitrogen content was determined through micro-Kjeldahl digestion and distillation with titration against 0.1N H₂SO₄, calculating nitrogen content by accounting for sample volume, acid normality, and dilution factor. This comprehensive approach ensured that the urine used as a nitrogen source in feed pellets met stringent standards for microbiological safety and chemical composition.

Pellet Formulation and Preparation

The initial phase focused on the conception, sourcing, and production of test pellets. Raw ingredients including maize, paddy straw, mustard oil cake, distillers dried grains, molasses, mineral mixture, salt, oil, and water were procured from institutional and reputed local suppliers to assure quality and homogeneity. Nitrogen supplementation was provided via two sources: urea (at 1% inclusion) and freshly collected indigenous cow urine, added at 5%, 7.5%, and 10% levels, respectively. This tiered approach was specifically designed to evaluate the maximum permissible or beneficial inclusion rate of urine without compromising pellet quality or animal health, while effectively utilizing its nitrogen content for microbial protein synthesis in the rumen. Urine was aseptically collected from healthy Sahiwal cows using sterile containers after cleaning the perineal area with warm water and antiseptic, ensuring avoidance of contamination and prompt storage in ice and refrigeration at 2-8 °C until used within 24 hours. All solid feed ingredients were weighed precisely using electronic balances. Grinding was performed using a hammer mill set to produce particles sized approximately 4-5 mm, optimizing mixability and pellet compaction. After grinding, the components were transferred to a ribbon-type horizontal mixer with a batch capacity of 150 kg, operating for a standardized 30-minute cycle to ensure uniformity of the mash. The blended feed was conveyed to a pellet mill (commercial model with an 8-mm die diameter and 4-inch rollers; throughput 250-300 kg/h). Here, pellets were formed under mechanical pressure and mild heat, simulating commercial feed production conditions. Completed pellets were conveyed for cooling and then packed in 50 kg gunny bags, labeled, and transported to experimental storage areas, ensuring minimal exposure to moisture and pests.

Pellet Quality Assessment

Physical quality assessment of the pellets was conducted following standard and widely accepted methods. Pellet durability index (PDI) was measured according to Pfof (1976), utilizing a tumbling box where a 500 g sample of pellets was rotated in a metal box (30×30×12 cm) with a diagonal baffle at 50 rpm for 10 minutes, after which pellets were sieved and the PDI calculated as the percentage of pellets remaining intact. Pellet dimensions (length and diameter) were measured using the ISO 17829 standard with a digital vernier caliper providing high precision measurements. Bulk density and single pellet density were

also determined as per ISO 17829, where bulk density involved gently filling a container of known volume with pellets and measuring mass to calculate density, and single pellet density was calculated by weighing individual pellets and calculating volume from diameter and length using the formula for a cylinder ($V = \pi r^2 h$, $V = \pi r^2 h$). Fines percentage was evaluated following ASAE (2008) methods by dry sieving 100 to 500 g of pellets through a 1.0 mm mesh for 5 to 10 minutes; fines were collected and weighed to express as a percentage of the original sample. These methodologies ensured a comprehensive and standardized assessment physical quality of pellets.

Chemical Composition Analysis

Chemical composition analysis employed standard Association of Official Analytical Chemists (AOAC, 2016) procedures. Moisture content was determined by drying 2-5 g of ground pellet samples at 65 °C for 72 hours in a hot air oven, with moisture calculated from the loss in mass. Dry matter (DM) was calculated on the basis of difference after oven drying. Crude protein (CP) was determined using a micro-Kjeldahl method, which involved digestion, distillation, and titration of the nitrogen content; the total N value was multiplied by 6.25 to obtain CP. Ether extract (EE) was measured by continuous extraction in a Soxhlet apparatus with petroleum ether as the solvent. Total ash (TA) was obtained by combusting 2.5-5 g of sample in a muffle furnace at 550 °C for 2-3 hours, with ash content calculated as a percentage of sample weight. Organic matter (OM) was derived from the difference between 100 and the ash percentage. Fiber fractions, specifically neutral detergent fiber (NDF) and acid detergent fiber (ADF), were analyzed using the Van Soest sequential method. For NDF, samples (0.5 g) were boiled in neutral detergent solution and filtered through pre-weighed crucibles, then dried and ashed. Likewise, ADF determination used acid detergent solution; residue weights before and after ashing were used to compute fiber percentages.

In vitro Rumen Fermentation Assessment

Rumen liquor was collected prior to feeding via the oral stomach tube method from a donor animal maintained on a standard mixed diet. The liquor was handled anaerobically in a thermos flask to preserve microbial activity. Pelleted feed samples (~200 mg) were weighed and incubated in triplicate within 100 mL glass syringes containing a buffered rumen fluid mixture. The incubation was carried out at 39 °C for 24 hours. The buffered medium was prepared with precise volumes of distilled water, micro- and macro-mineral solutions, buffer, resazurin, and a reducing solution; it was pre-warmed and bubbled with CO₂ immediately before addition of rumen liquor. Gas production was measured periodically during incubation, and total gas volume was corrected for blanks to ensure accuracy, following the procedure described by Menke and Steingass (1988) [16].

The standard gas used for methane estimation (Spantech House Surrey, England) is composed of 50% methane and 50% CO₂. The peak of methane gas was identified based on the retention time of standard methane gas and the response factor obtained was used to calculate methane percentage in the gas sample. The methane produced from the substrate during 24 h incubation was corrected for the blank values. The proportion of methane (%) and the volume of methane (mL) was calculated as follows:

$$\text{Methane (\%)} = \frac{\text{Area covered by the sample}}{\text{Area covered by the standard of methane}} \times 50$$

$$\text{Methane production (mL)} = \text{Total gas produced (mL)} \times \% \text{ methane in the sample}$$

In-vitro Digestibility

At the end of incubation, the contents of syringes were transferred to centrifuge tubes and centrifuged at 3000 rpm for 10 minutes. The solid residues were treated with neutral detergent solution (NDS), refluxed at 100 °C for 1 hour, filtered through pre-weighed crucibles, dried at 65 °C for 48 hours, and finally ashed at 550 °C. These procedures allowed measurement of residues used to calculate digestibility indices.

In vitro Dry Matter Digestibility (IVDMD) was calculated as the percentage of dry matter that disappeared during incubation. Total digestible organic matter (TDOM) was calculated similarly, based on organic matter

$$\text{IVDMD(\%)} = \frac{(\text{DM taken for incubation} - \text{NDF residue})}{(\text{DM taken for incubation})} \times 100$$

$$\text{TDOM(\%)} = \frac{(\text{OM taken for incubation} - \text{residue OM})}{(\text{OM taken for incubation})} \times 100$$

Partitioning factor (PF) and microbial biomass production (MBP) were calculated based on truly degraded organic matter (TDOM) as described by Blummel *et al.*, (1997) [16]

$$\text{PF} = \frac{\text{mg TDOM}}{\text{Net gas production}}$$

Microbial Biomass Production (MBP) was calculated using Blummel's equation:

$$\text{MBP} = \text{TDOM} - (\text{Net gas volume} \times 2.20)$$

Where, 2.20 is the strichocetric factor for roughages (Blummel *et al.*, 1997) [5] and mixed diets (Blummel and Lebzien, 2001) [4]

Metabolizable Energy (ME) content of the pellets was estimated using the Menke *et al.*, 1988 [16] equation:

$$\text{ME(MJ/kg DM)} = 1.24 + 0.146 \times \text{GP} + 0.007 \times \text{CP} + 0.0224 \times \text{EE}$$

Where GP is the 24-hour gas production (mL/200 mg DM), CP is crude protein (%), and EE is ether extract (%).

Statistical Analysis

Data from all phases was subjected to rigorous statistical analyses. The general linear model (GLM) of SPSS software was used to perform one way ANOVA appropriate to each experimental design, followed by Tukey's HSD test for multiple means comparisons, with statistical significance declared at $p < 0.05$.

Results

Safety Assessment of Cow Urine

Urine samples collected from Dry Sahiwal Cows are presented. The urine pH values of Dry Sahiwal cows ranged from 7.27 to 7.52, confirming a mildly alkaline nature typical of bovine urine. Sahiwal urine showed slightly lower pH values (7.27-7.52) compared to crossbred Karan Fries samples (7.62-7.86). Total nitrogen content in indigenous

Sahiwal urine, varied from 0.48 to 0.55%, while Microbial colony counts on CLED and nutrient agar revealed a microbial load in Sahiwal urine (1000 CFU/mL), indicating better hygienic quality. Similarly Sediment analysis showed no pathogenic bacteria in Sahiwal urine samples.

Proximate Composition of Pellets

Proximate analysis indicated significant differences in the dry matter content, with the lowest level in pellets containing 10% urine (82.58%) and the highest in urea-treated pellets (88.67%). Organic matter content showed similar trends, with highest value in urea pellets (89.24%). Crude protein levels were statistically similar among all treatments, indicating diets were formulated to be isonitrogenous. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and ether extract (EE) contents fell within acceptable ranges, showing no adverse effect from urine inclusion.

Physical Quality of Pellets

Physical quality assessment demonstrated that pellet length and diameter were consistent across all treatments, averaging approximately 40 mm and 7.7 mm respectively. However, bulk density and single pellet density differed significantly, with urea-treated pellets exhibiting the highest density (696.60 kg/m³ bulk, 0.770 kg/m³ single pellet) and 10% urine pellets showing the lowest. Pellet durability index and sieve percentage followed a similar pattern, highlighting the superior mechanical quality of urea-treated pellets and the relatively good, though numerically inferior, quality of 7.5% urine pellets.

In vitro Fermentation Characteristics

In vitro fermentation studies indicated that total gas and methane production were significantly reduced ($p < 0.05$) in pellets supplemented with either urea or urine compared to the control. The lowest gas volumes were observed in the 7.5% urine (22.04 mL) and UREA P (22.09 mL) treatments, while methane production was lowest in the UREA P group (4.15 mL/200 mg DM), closely followed by the 7.5% urine group (4.20 mL). Despite these reductions, IVDMD and TDOM remained statistically consistent across all groups, suggesting that neither urea nor urine supplementation hindered feed degradability. Furthermore, the partitioning factor and microbial biomass production (MBP) were significantly enhanced in all treatment groups, with the UREA P recording the highest MBP (58.77 mg). These results demonstrate that both urea and urine pellets optimize fermentation efficiency by shifting nutrient utilization toward microbial protein synthesis rather than gaseous energy loss.

Discussion

The urine safety assessment was a critical first step to ensure the hygienic and nutritional suitability of using cow urine as a feed ingredient. The study compared urine samples from indigenous (Sahiwal) analyzing key parameters like pH, nitrogen content, total viable microbial count, and the presence of urinary sediments. The results showed that Sahiwal cow urine was possessing a lower pH (7.25), higher nitrogen content (0.83%), and a significantly lower

microbial load (2.49×10^3 cfu/mL). Additionally, it had very little sediment. Based on these findings, indigenous cow urine was deemed the safer for incorporating into the feed pellets.

Regarding the proximate composition of paddy straw-based pellets, urea-treated pellets demonstrated the highest dry matter and organic matter content, primarily because urea does not contribute additional moisture, enhancing pellet stability and storability. In contrast, pellets with 10% urine inclusion contained increased moisture, which predisposed them to spoilage risks. The crude protein content remained consistent across all treatments, reflecting the iso-nitrogenous formulation approach employed to isolate nitrogen source effects. Minor variations in fiber fractions likely stem from variability in straw batches but remain within acceptable ranges for ruminant feed. The physical quality parameters indicated that urea-treated pellets had superior bulk density, pellet density, and durability compared to urine-treated pellets. This performance disparity is mainly due to the higher moisture content in urine-treated pellets, particularly at 10% inclusion, which compromises pellet compaction and structural integrity, resulting in greater pellet breakage. Pellet dimensions remained consistent across all treatments, suggesting that modifications in nitrogen source and concentration do not influence pellet size but do affect mechanical strength and handling properties.

The lower gas production from urine-supplemented pellets indicates a shift in rumen fermentation favoring more efficient microbial growth instead of gas release. This is because the added nitrogen from urine and urea supports the proliferation of beneficial microbes, directing more energy towards microbial protein synthesis. However, the total digestible organic matter (TDOM) remains similar across treatments due to the intrinsic nature of paddy straw, which contains large amounts of indigestible components such as lignin. While nitrogen supplementation boosts microbial activity, these lignin-rich fractions remain inaccessible, limiting any significant increase in organic matter breakdown. The microbial ecosystem in the rumen likely adapts to the nitrogen source without altering the overall extent of digestion, with improvements observed more in fermentation efficiency than the total organic matter digested. Methane reduction observed in treated pellets results directly from enhanced rumen fermentation efficiency mediated by nitrogen supplementation. A higher partitioning factor (PF) alongside increased microbial biomass production (MBP) demonstrates that more feed energy is converted into microbial cells rather than lost as methane gas. The 7.5% urine pellet showed the most balanced improvements—low methane emissions, high MBP, and superior PF—making it the most promising formulation for optimizing feed utilization and environmental impact. This balance justified selecting the 7.5% urine pellet for detailed animal feeding trials. *In vitro* fermentation thus reflects an optimized rumen microbial environment driven by accessible nitrogen sources from urine and urea, facilitating increased microbial growth and suppressed methane production without altering overall digestibility or metabolizable energy, which are limited by the fundamental composition of paddy straw.

Table 1: Formulations of Paddy Straw Pellets with Different Nitrogen Sources for in-vitro studies

Components	PARTS IN KG PER 100 KG			
	5% P	7.5% P	10% P	UREA P
MAIZE	24	27	30	28
RICE STRAW	45	45	45	45
MOC	8	5.5	3	8
URINE	5	7.5	10	-
UREA	-	-	-	1
DDGS	7.5	4.5	1.5	7.5
MOLASSES	8	8	8	8
OIL	1	0.5	1	1
SALT	0.5	1	0.5	0.5
MINERAL MIX	1	1	1	1

Table 2: Safety Analysis of Indigenous and Crossbred Cow Urine

Ani no	Breed	pH	Urine N (%)	Colony count (CFU/mL)	Growth type	Urine sediment
2738	Sahiwal	7.27	0.48	1000	Insignificant	0
2476	Sahiwal	7.51	0.51	1000	Insignificant	0
2630	Sahiwal	7.52	0.55	1000	Insignificant	0

Table 3: Proximate Composition of Paddy Straw-Based Pellets (% Mean±SEM)

Attributes (%)	Treatments				p-value
	5% P	7.5% P	10% P	UREA P	
Dry matter	87.56 ^c ±0.15	86.51 ^b ±0.25	82.58 ^a ±0.05	88.67 ^d ±0.07	0.001
Organic matter	88.30 ^a ±0.14	88.16 ^a ±0.29	88.09 ^a ±0.09	89.24 ^b ±0.06	0.026
Crude protein	11.98±0.04	12.07±0.03	12.06±0.01	12.15±0.02	0.09
Neutral detergent fiber	43.00±0.26	41.96±0.98	39.50±0.49	44.46±2.45	0.09
Acid detergent fiber	24.00±0.01	26.50±0.5	24.50±0.5	22.50±1.5	0.108
Ether extract	9.84±0.86	8.90±0.07	11.50±0.95	11.39±2.00	0.44

Table 4: Pellet quality parameters of paddy straw based pellets

Attributes (%)	Treatments				p-value
	5% P	7.5% P	10% P	UREA P	
Length (mm)	40.52±1.20	41.04±2.15	39.60±1.12	41.878±0.93	0.727
Diameter (mm)	7.70±0.83	7.86±0.40	7.66±0.14	7.80±0.08	0.361
PD Bulk (kg/m ³)	663.39 ^b ±0.93	662.00 ^b ±0.48	641.40 ^a ±0.56	696.60 ^c ±0.44	<0.001
PD single (kg/m ³)	0.59 ^b ±0.008	0.542 ^{ab} ±0.006	0.54 ^a ±0.016	0.77 ^c ±0.013	<0.001
Sieve (%)	2.68 ^c ±0.008	2.48 ^b ±0.10	2.87 ^d ±0.004	2.08 ^a ±0.048	<0.001
Pellet Durability Index (%)	87.41 ^b ±0.87	86.82 ^b ±0.39	84.44 ^a ±0.47	89.61 ^c ±0.65	<0.001

Table 5: *In vitro* analysis of paddy straw based pellets

Attributes	Treatments					p-value
	CON	5% P	7.5% P	10% P	UREA P	
Gas (mL/200 mg DM)	27.55 ^b ±0.19	23.80 ^{ab} ±0.23	22.04 ^a ±0.57	22.48 ^a ±1.35	22.09 ^a ±1.33	0.011
IVDMD %	53.09±0.06	57.28±1.14	58.03±1.18	57.30±0.57	58.39±1.61	0.085
TDOM %	54.48±0.84	58.36±1.43	59.20±1.82	58.39±0.87	59.81±1.73	0.14
Methane (mL/200 mg)	6.22 ^b ±0.01	4.29 ^a ±0.01	4.20 ^a ±0.13	4.22 ^a ±0.28	4.15 ^a ±0.24	0.001
Methane (%)	22.20 ^c ±0.05	18.63 ^b ±0.01	18.52 ^a ±0.013	18.33 ^a ±0.02	18.26 ^a ±0.02	<0.01
Partitioning factor	1.98 ^a ±0.04	2.45 ^{ab} ±0.04	2.68 ^b ±0.03	2.61 ^{ab} ±0.18	2.74 ^b ±0.21	0.019
ME (MJ/kg DM)	6.76±0.04	6.26±0.07	6.31±0.08	6.08±0.08	6.34±0.09	0.186
Microbial biomass production (mg)	35.87 ^a ±1.80 ^a	51.49 ^{ab} ±2.49	57.26 ^b ±2.10	54.76 ^b ±2.78	58.77 ^b ±1.87	0.01

Conclusion

The study identifies the use of 7.5% indigenous cow urine as the most effective method for utilizing paddy straw, as it transforms a low-quality agricultural residue into a high-value, nutrient-dense feed pellet. This inclusion significantly enhances fermentation efficiency by shifting energy utilization from gaseous loss toward microbial protein synthesis, reducing methane emissions by approximately 32%. By combining bulky straw with local nitrogen sources, this strategy provides a compact, durable, and eco-friendly solution to bridge India's fodder deficit.

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