OPEN ACCESS
Journal of Animal Health and Production

Research Article

Apparent Nutrient Digestibility in Sheep Fed Diets Supplemented with Nano-Zeolite and Urea-Impregnated Nano-Zeolite

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Abstract | The search for sustainable strategies to improve nutrient utilization in ruminant diets has focused on zeolite-based additives, particularly nano-zeolite and urea-impregnated nano-zeolite (UINZ), for their potential to enhance digestibility and nitrogen use. This study compared the effects of nano-zeolite, UINZ, and conventional urea supplementation on the apparent digestibility of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF), nitrogen-free extract (NFE), total digestible nutrients (TDN), neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, and hemicellulose in sheep. Twenty-four male sheep (20±1.5 kg) were randomly assigned to six diets: control (natural protein), urea, inactivated nano-zeolite, thermally activated nano-zeolite, urea-impregnated inactivated nano-zeolite, and urea-impregnated thermally activated nano-zeolite. Diets consisted of dwarf elephant grass and concentrate (55:45 DM basis). Nutrient digestibility was assessed over a 14-day period, consisting of 7 days for adaptation and 7 days for digestibility measurement, using the acid-insoluble ash marker method. Statistical analysis employed a completely randomized design with GLM and Duncan's test. Results showed that all nano-zeolite diets maintained digestibility comparable to the natural protein control for DM, OM, CP, EE, CF, NFE, and TDN. In contrast, conventional urea supplementation significantly reduced digestibility ($P<0.05$) of these nutrients. Furthermore, nano-zeolite (both non-impregnated and urea-impregnated) significantly improved digestibility ($P<0.05$) of fiber fractions (NDF, ADF, cellulose, hemicellulose) compared to the urea diet. In conclusion, nano-zeolite, in both its non-impregnated and urea-impregnated forms, emerges as a promising feed additive to enhance nutrient digestibility and overall feed efficiency in ruminant diets, potentially leading to improved animal performance and more sustainable feeding practices.

Keywords | Feed additives, Nano-zeolite, Nutrient digestibility, Sheep, Urea-impregnated nano-zeolite

Received | May 23, 2025; **Accepted** | July 31, 2025; **Published** | December 05, 2025
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Citation | Kardaya D, Sudrajat D, Wahyuni D, Gopar RA, Pramartaa IQ (2025). Apparent nutrient digestibility in sheep fed diets supplemented with nano-zeolite and urea-impregnated nano-zeolite. J. Anim. Health Prod. 13(4): 1288-1298.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.4.1288.1298
ISSN (Online) | 2308-2801

INTRODUCTION

The growing demand for efficient and sustainable livestock production systems has spurred extensive research into feed additives that can enhance nutrient utilization and reduce environmental impacts. In ruminant nutrition, one of the persistent challenges is improving the digestibility of feed components to ensure optimal nutrient absorption and efficient conversion into animal products. Among the feed additives studied, zeolites naturally occurring crystalline aluminosilicates have shown promise due to their unique physicochemical properties, including high surface area, porosity, and cation-exchange capacity (Valpotić *et al*., 2017).

Previous research has indicated that urea-impregnated zeolite (UIZ) can effectively reduce ruminal ammonia, stabilized pH, reduced methane production, improving rumen fermentation efficiency, and improved most nutrient digestibility (Kardaya *et al*., 2012, 2018, 2021, 2023). In a study focusing on Awassi lambs, the results showed that the combination of urea and zeolite significantly enhanced final live weight, daily weight gain, nutrient digestion, and feed conversion ratio compared to diets with urea alone or zeolite alone (Alrez *et al*., 2024).

In recent years, the application of nano-sized zeolites has emerged as a novel strategy to enhance the efficacy of zeolite supplementation. Nanotechnology has enabled the production of zeolite particles at a nano scale, significantly increasing their surface area and reactivity (de Jesús Ruíz-Baltazar, 2024; Kianfar, 2019). These properties allow nano-zeolites to interact more efficiently with digestive enzymes and microbial populations in the rumen, potentially improving the digestibility of nutrients. Nano-zeolites can also be used to protect nutrients such as amino acids from ruminal degradation, ensuring their availability post-ruminal. This is crucial for meeting the dietary amino acid requirements of high-production ruminants (Albuquerque *et al*., 2020).

Furthermore, the impregnation of nano-zeolites with urea, forming urea-impregnated nano-zeolite (UINZ), introduces a dual-purpose function: Nitrogen supplementation and controlled release. Urea is a common non-protein nitrogen (NPN) source in ruminant diets; however, its rapid hydrolysis in the rumen often leads to ammonia toxicity or inefficient nitrogen use. By impregnating urea into nano-zeolites, it is expected that the release mechanism of urea will be more controlled so as to further improve the efficiency of rumen fermentation, thereby enhancing nutrient digestibility.

These studies collectively highlight the potential benefits of incorporating zeolite-based additives, including nano-zeolite and urea-impregnated nano-zeolite, in ruminant diets to enhance nutrient digestibility, improve rumen fermentation, and boost animal performance. However, further research is warranted to fully elucidate the mechanisms underlying these effects and to optimize their application in sheep nutrition.

Although the use of zeolite and nano-zeolite in animal feed has been studied, there remains a significant gap in the literature regarding the direct effects of nano-zeolite and UINZ on apparent nutrient digestibility in sheep. Therefore, this study aims to compare the efficacy of nano-zeolite, urea-impregnated nano-zeolite, and conventional urea supplementation in improving the apparent digestibility of key nutrients, including dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF). This research contributes to the growing body of knowledge on nano-additives in animal nutrition and offers insights into their practical utility for improving feed efficiency and sustainable sheep production systems.

MATERIALS AND METHODS

Animals

This study utilized 24 healthy male sheep, each with an initial mean weight of 20 ± 1.5 kg. The animals were sourced from a local farm and were acclimatized to the experimental environment prior to the commencement of the study. Each sheep was housed in an individual pen measuring 90 cm in width and 120 cm in length, designed to provide adequate space for movement and comfort while facilitating precise monitoring of feed intake and fecal output. The pens were equipped with separate feeding and drinking stations to prevent dietary contamination. The flooring of each pen was constructed from wooden slats with 1.5 cm gaps to allow debris to fall through. The floors were regularly cleaned, and appropriate ventilation was maintained to minimize stress and enhance the animals' comfort.

Experimental diets

The sources of food ingredients used and the nutritional composition are presented in Table 1. The basal diet was formulated using dwarf elephant grass (Pennisetum purpureum cv. Mott) and concentrates, combined in a 55:45 ratio on a dry matter (DM) basis (Table 2). The dwarf elephant grass was freshly harvested at 40 days post-regrowth, chopped into approximately 3–5 cm lengths to enhance intake and digestibility, and sun-dried to ensure uniform moisture levels before feeding. The concentrate primarily comprised pollard, coarsely milled corn kernels, soybean meal, coconut meal, and cassava pulp, all formulated to meet the growing nutritional needs of sheep (NRC, 2007).

Six experimental diets were prepared to evaluate the effects of nano-zeolite and urea-impregnated nano-zeolite supplementation on nutrient digestibility. The treatments were as follows: R1 (control): Basal diet containing natural protein sources without supplementation, as a positive control. R2 (urea): basal diet supplemented with conventional urea to provide non-protein nitrogen, as a negative control. R3 (Inactivated Nano-Zeolite): Basal diet supplemented with nano-sized zeolite particles that were thermally inactivated to assess the effect of zeolite alone. R4 (Thermally Activated Nano-Zeolite): Basal diet supplemented with nano-zeolite that underwent thermal activation to enhance its adsorptive properties. R5 (Urea-Impregnated Inactivated Nano-Zeolite): Basal diet supplemented with inactivated nano-zeolite impregnated with urea, aimed at controlled release of nitrogen. R6 (Urea-Impregnated Heat-Activated Nano-Zeolite):

Table 1: Nutrient composition of feed sources (Dry Matter Basis).

Feed sources	DM	CP	TDN
		%	
Dwarf elephant grass (*Pennisetum purpureum* cv. Mott)	24.4	8.2	56.2
Pollard	88.5	18.5	71.5
Yellow corn	87.8	10	82.3
Soybean meal	88.1	46.9	87.74
Cocconut meal	88.6	21.3	78.7
Molasses	62.57	1.29	88.38
Nonactivated nano-zeolite	98	-	-
Thermal activated nano-zeolite	100	-	-
Urea	100	281	-
Urea impregnated nonactivated nano-zeolite	-	106.87	-
Urea impregnated pre-thermal activated nano-zeolite	-	106.87	-
Cassava dregs	79.8	1.87	78.3

DM= dry matter, CP= crude protein, TDN= total digestible nutrient.

Table 2: Experimental diet formula.

Feed ingredients	R1	R2	R3	R4	R5	R6
	Dry matter (%)					
Dwarf elephant grass (*Pennisetum purpureum* cv. Mott)	55	55	55	55	55	55
Pollard	4	11	2	2	6.6	6.6
Yellow corn	14	15	15	15	16	16
Soybean meal	16.5	10.2	17	17	14	14
Cocconut meal	9.5	5	9	9	4.8	4.8
Molasses	-	1	-	-	1	1
Nonactivated nano-zeolite	-	-	1	-	-	-
Thermal activated nano-zeolite	-	-	-	1	-	-
Urea	-	0.8	-	-	-	-
Urea impregnated nonactivated nano-zeolite	-	-	-	-	1.6	-
Urea impregnated pre-thermal activated nano-zeolite	-	-	-	-	-	1.6
Cassava dregs	1	2	1	1	1	1
Total	100	100	100	100	100	100

R1 = basal diets; R2 = basal diets + urea; R3 = basal diets + non-activated nano-zeolite; R4 = basal diets + thermal activated nano-zeolite; R5 = basal diets + urea impregnated non-activated nano-zeolite; R6 = basal diets + urea impregnated pre-thermal activated nano-zeolite.

Basal diet supplemented with thermally activated nano-zeolite impregnated with urea, hypothesized to optimize nitrogen utilization through improved surface activity.

The supplementation levels were calculated to provide equivalent nitrogen content across urea-containing treatments, ensuring comparability (Kardaya *et al*., 2025). All feed ingredients were thoroughly mixed to ensure homogeneity before feeding.

Feeding management and experimental setup

Feeding was conducted twice daily at 8:00 AM and 4:00 PM, with the total daily feed allowance set at 2.5% of each sheep's body weight on a dry matter basis. Feed refusals were collected and weighed daily to calculate actual intake. Water was always freely available.

Prior to the start of the feeding trial, all sheep received a standard deworming treatment using ivermectin (0.2 mg/kg body weight, subcutaneous injection) to eliminate internal parasites, and a vitamin-mineral injection to support health and immune function during the experimental period. The feeding trial lasted for 14 days, divided into a 7-day adaptation period to allow animals to adjust to the diets and housing conditions, followed by a 7-day nutrient digestibility measurement phase during which fecal samples were collected for analysis. Body weights were recorded at the beginning and end of the experiment (day 0 and day 14) using a calibrated digital scale to monitor changes in live weight and assess the impact of dietary treatments on growth performance.

Preparation of nano-zeolite and urea-impregnated nano-zeolite supplements

Nano-zeolite particles were synthesized from natural zeolite minerals through a top-down milling process to achieve particle sizes in the nanometer range, enhancing surface area and adsorptive properties. The inactivated nano-zeolite (used in R3 and R5) was prepared by washing with distilled water and drying at ambient temperature to remove impurities and deactivate surface activity. Thermally activated nano-zeolite (used in R4 and R6) was produced by heating the nano-zeolite at 600°C for 3 hours in a muffle furnace to increase pore volume and surface reactivity. For urea impregnation (R5 and R6), micronized zeolite was put into liquid urea at 80-110 °C following the procedure of (Kardaya *et al.*, 2012). Furthermore, urea-impregnated nano-zeolite was prepared from urea-impregnated micronized zeolite.

Experimental design

A completely randomized design (CRD) was employed, consisting of six dietary treatments (R1 to R6) with four replicates each (one sheep per replicate). The sheep were individually housed in pens (90 cm × 120 cm) and fed twice daily at 8:00 AM and 4:00 PM. The daily feed allowance was fixed at 2.5% of body weight on a dry matter basis.

Procedure for conducting digestibility trial using AIA marker method

Studies comparing AIA marker digestibility with total fecal collection have shown high correlations ($r > 0.9$) for dry matter digestibility in sheep and cattle, confirming the method's validity when properly applied (Block *et al.*, 1981). The feeding trial lasted 14 days, including a 7-day adaptation period to the diets and housing conditions, followed by a 7-day digestibility measurement phase.

Diet preparation and feeding: During the digestibility phase, sheep are fed experimental diets containing a natural level of acid-insoluble ash, which is indigestible and passes through the digestive tract unabsorbed. Feed intake is recorded, and animals are fed at fixed levels at 2.5% of body weight on a dry matter basis.

Sample collection: Representative samples of the feed offered are collected daily, dried, and ground for analysis. Grab samples of feces are collected directly from the animals over seven days during the digestibility measurement period. Care is taken to avoid contamination of samples with soil or dust, which can artificially increase AIA content, especially in grazing animals.

Laboratory analysis

The AIA content of feed, refusals, and feces is determined gravimetrically. This involves: (1) Drying samples to constant weight; (2) Ashing samples at high temperature to remove organic matter; (3) Boiling the ash in hydrochloric acid to dissolve acid-soluble minerals, leaving acid-insoluble ash (mainly silica); and (4) Filtering, washing, and re-ashing the residue to quantify AIA.

Proximate analyses of feed and feces for dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), and ash were conducted according to AOAC standard methods (AOAC, 2016). Organic matter (OM) was calculated by subtracting ash from DM. Nitrogen-free extract (NFE) was calculated as NFE = DM – (CP + EE + CF + Ash).

Fiber fractions including neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose, and hemicellulose were determined using the Van Soest sequential fiber analysis method (Van Soest *et al.*, 1991).

Apparent nutrient digestibility calculation

Digestibility (%) for any nutrient is calculated using the AIA concentrations in feed and feces and the nutrient concentrations in feed and feces, according to the formula (Van Keulen and Young, 1977):

$$\text{Apparent Nutrient Digestibility} = 100 - (100 \times AIA_{feces} \times Nutrient_{feed} / AIA_{feed} \times Nutrient_{feces}) \quad \ldots(1)$$

Where; AIA_{feed} and AIA_{feces} are the acid-insoluble ash concentrations in feed and feces, respectively, and $Nutrient_{feed}$ and $Nutrient_{feces}$ are the nutrient concentrations refer to any of DM, OM, CP, CF, EE, NFE, NDF, ADF, cellulose, or hemicellulose in feed and feces.

Statistical analysis

All data were analyzed using IBM SPSS Statistics (version 26). A generalized linear model (GLM) multivariate analysis was performed to evaluate the effect of dietary treatments on nutrient digestibility parameters. When significant treatment effects were detected ($p < 0.05$), Duncan's multiple range test was used for pairwise comparison of means. The statistical model used is as follows: $Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$, where i = 1,2,3,4,5,6 and j = 1,2,3,4; Y_{ij} = Observation for the i-th treatment and the j-th replicate; μ = Overall mean; τ_i = Effect of the i-th treatment (fixed effect); ε_{ij} = Experimental error, assumed to be independently and normally distributed with mean 0 and constant variance: $\varepsilon_{ij} \sim N(0, \sigma^2)$.

RESULTS AND DISCUSSION

The results of proximate analysis showed a range of energy and protein content that was almost the same for each treatment, namely energy ranging from 3,210 - 3,273 kcal/kg and protein ranging from 16.41 - 17.48% (Table 3).

Table 3: Nutrient content of diets based on proximate analysis results.

Nutrient contents	R1	R2	R3	R4	R5	R6
Dry matter (DM, %)	91.24	89.23	91.83	92.00	91.08	90.63
Crude protein (CP, %)	16.86	16.41	17.48	17.44	16.90	16.92
Extract ether (EE, %)	2.11	2.05	2.00	1.89	1.98	1.90
Crude fiber (CF, %)	19.72	19.51	19.78	19.55	19.42	19.34
Ash (%)	9.98	9.81	10.92	10.86	10.74	10.68
NFE (%)*	42.57	41.45	41.66	45.26	42.04	41.79
DE (Kcal/Kg)**	3,275	3,243	3,266	3,216	3,229	3,210

R1 = basal diets; R2 = basal diets + urea; R3 = basal diets + non-activated nano-zeolite; R4 = basal diets + thermal activated nano-zeolite; R5 = basal diets + urea impregnated non-activated nano-zeolite; R6 = basal diets + urea impregnated pre-thermal activated nano-zeolite. *Nitrogen free extracts (NFE) = DM – (CP + EE + CF + Ash); Digestible Energy. **DE = Gross Energy (GE) × Energy Digestibility Coefficient.

The crude protein content of each diet was slightly higher (16-17%) than the upper limit of the NRC recommended minimum requirement of 14-16%. Similarly, the energy content of the diets (3200-3275 kcal/kg) was higher than the NRC recommendation (2800-3000 kcal/kg DM). The DE and CP contents in R1–R6 meet or exceed (NRC, 2007) recommendations for growing 20 kg sheep. The diets are nutritionally adequate and may even support faster growth based on higher protein and energy content. The ether extract contents of diets R1, R2, R3 and R5 were within or close to the ideal range (2-4% of dry matter). Diet R4 and R5 showed rather low extract content (<2% of dry matter), but since the energy was high, this was acceptable. The NFE values in R1–R6 have appropriate NFE content (41–45%), and within 38–45% ideal range as recommended by (NRC, 2007). The crude fiber (CF) all diets close to 20%, within the ideal range (15–20% of DM) for growing sheep. The ash content of R1 and R2 is within the acceptable range (5–10%) while the ash content of R3-R6 is rather high (>10%), but still acceptable because diets R3 - R6 are supplemented with zeolite minerals.

Neutral detergent fiber (NDF) includes hemicellulose, cellulose, and lignin it reflects the total fiber content that limits feed intake because it is slowly digested and fills the rumen. R1 (48.49%), R2 (47.42%), R3 (47.66%), R4 (46.88%), R5 (49.32%), and R6 (45.56%) are all above the recommended NRC (2007) range (30–45%) for growing sheep (Figure 1). ADF includes cellulose and lignin it predicts the indigestible fiber portion. Higher ADF means lower digestibility of the diet. ADF for all treatments (R1–R6) ranged from 25.64% to 26.81%, well within the recommended range (17–30%). Moderate ADF indicates that a reasonable portion of the fiber is digestible, which is important for energy availability. Cellulose is a major polysaccharide in plant cell walls, relatively digestible by rumen microbes, although more slowly than hemicellulose. Cellulose values (20.50%–21.68%) are within optimal digestibility range. Good cellulose content supports microbial fermentation, providing volatile fatty acids (VFAs) needed for energy. Hemicellulose is a polysaccharide that is more easily degraded by rumen microbes than cellulose. Hemicellulose values (19.72%–23.33%) are high and favorable for sheep. High hemicellulose levels are associated with better fiber digestibility and more energy availability. Lignin is an indigestible fiber component that binds cellulose and hemicellulose, preventing their digestion. Lignin contents (2.60%–3.25%) are low and well below the maximum recommended level (<7%). Low lignin improves fiber digestibility because lignin physically restricts microbial access to cellulose and hemicellulose

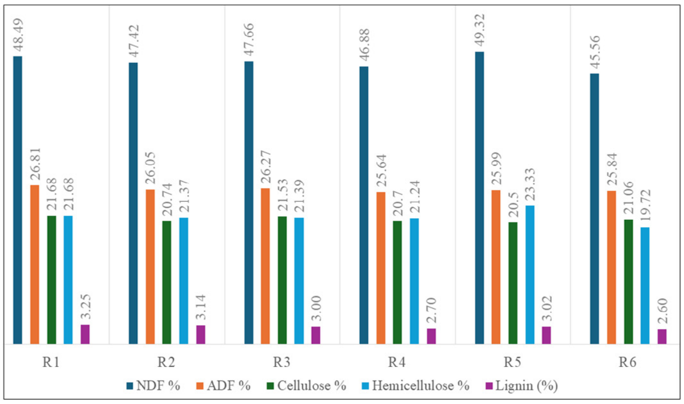

Figure 1: Fiber fraction content in experimental diets.

Apparent nutrient digestibility

The digestibility of various nutrients in sheep diets is a critical factor in understanding their nutritional efficiency and energy utilization. The provided study outlines a range of digestibility values for dry matter, organic matter, crude protein, ether extracts, crude fiber, nitrogen-free extract, and energy (Table 4). Comparing these values with other research reveals both consistencies and variations, which can be attributed to differences in diet composition, sheep.

The dry matter digestibility in this study ranged from 63.10% to 78.33% (Table 4) which comparable to the previous study (Kardaya *et al.*, 2023) but with a narrow range (72.43-76.74%). Dry matter digestibility reported by some researchers ranged from 62.14 - 74.81% in sheep fed rye straw and agricultural waste-based silage (Seok *et al.*, 2016), supplemented with various selenium sources (Ibrahim and Mohamed, 2018), oat hay without or with

Table 4: Nutrient digestibility in sheep fed experimental rations.

Digestibility metrics (%)	R1	R2	R3	R4	R5	R6
Dry matter	72.90±2.61^{bc}	63.10±6.09^{a}	78.33±3.45^{c}	73.46±3.05^{bc}	68.35±3.32^{ab}	74.23±1.72^{bc}
Organic matter	74.10±2.88^{bc}	65.44±6.23^{a}	79.49±3.34^{c}	74.85±2.93^{bc}	70.34±3.31^{ab}	75.96±1.80^{bc}
Crude protein	75.06±3.27^{bc}	69.71±3.76^{a}	80.72±3.27^{c}	72.98±3.30^{ab}	73.94±3.09^{abc}	78.41±1.58^{bc}
Ether extracts	72.14±2.91^{b}	63.00±5.73^{a}	75.59±3.84^{b}	69.20±3.59^{ab}	67.73±3.09^{ab}	71.65±0.77^{b}
Crude fiber	66,16±4,58^{bc}	47,90±10,12^{a}	73,03±4,26^{c}	66,92±4,18^{bc}	59,15±4,61^{a}	65,83±3,24^{bc}
Nitrogen free extract	78.61±3.64^{bc}	72.15±5.97^{a}	83.73±2.58^{c}	80.75±3.44^{bc}	75.77±2.90^{ab}	80.92±1.77^{bc}
Total Digestible Nutrient	69.27±3.05^{bc}	59.26±5.63^{a}	75.90±3.78^{c}	70.13±3.44^{bc}	65.21±3.87^{ab}	71.78±2.19^{bc}

R1 = basal diets; R2 = basal diets + urea; R3 = basal diets + non-activated nano-zeolite; R4 = basal diets + thermal activated nano-zeolite; R5 = basal diets + urea impregnated non-activated nano-zeolite; R6 = basal diets + urea impregnated pre-thermal activated nano-zeolite. Different superscripts on the same row indicate significant differences ($p<0.05$).

micronutrient fertilization (Singh *et al*., 2020), various protein sources (El-Nomeary *et al*., 2021), and various energy sources (Yateem *et al*., 2021). The use of slow-release urea in diets also showed comparable dry matter digestibility, ranging from 63.28-77.88% in sheep (Geron *et al*., 2016; Hashem and Tayeb, 2023) but was still below the dry matter digestibility in pasundan cattle fed a diet containing urea-impregnated zeolite, which ranged from 82.55 - 83.25% (Kardaya *et al*., 2021).

The control diet containing only natural protein without supplementation (R1) produced dry matter digestibility comparable ($p>0.05$) to that of the nano-zeolite diets, either non-heat-activated (R3), heat-activated (R4), or urea-impregnated (R5 and R6), but higher ($p<0.05$) than the conventional urea diet (R2). It was clear that the conventional urea diet resulted in lower dry matter digestibility ($p<0.05$) than almost all experimental diets. These data indicate that all the nano-zeolite diets performed comparably to diets containing only natural protein sources without supplementation as the positive control in maintaining high dry matter digestibility. In contrast, the use of conventional urea in the diet as a non-protein nitrogen source to partially replace natural protein sources was found to reduce dry matter digestibility.

Organic matter digestibility in this study ranged from 65.44% to 79.49% (Table 4) and still comparable with the previous study (Kardaya *et al*., 2023) but with a narrow range (74.4-79.10%). The digestibility of organic matter from this study is slightly higher than the digestibility of organic matter (53.05-73.93%) from previous research (Ftiwi and Tadess, 2018; Geron *et al*., 2016; Tekliye *et al*., 2018). The digestibility of organic matter reported by some researchers (ranged from 64.55-75.05% in sheep fed rye straw and agricultural waste-based silage (Seok *et al*., 2016), supplemented with various selenium sources (Ibrahim and Mohamed, 2018), oat hay without or with micronutrient fertilization (Singh *et al*., 2020), various protein sources (El-Nomeary *et al*., 2021), and various energy sources (Yateem *et al*., 2021). The use of slow-release urea in the di*et al*so showed comparable organic matter digestibility, ranging from 68.18 - 80.02% in sheep (Geron *et al*., 2016; Heidari *et al*., 2022) but still below the organic matter digestibility in pasundan cattle fed a diet containing urea-impregnated zeolite, which ranged from 86.95-87.30% (Kardaya *et al*., 2021).

The effect of experimental diets on organic matter digestibility showed the same pattern as dry matter digestibility. The control diet containing only natural protein without supplementation (R1) produced organic matter digestibility comparable ($p>0.05$) to that of non-heat-activated (R3), heat-activated (R4), or urea-impregnated (R5 and R6), but higher ($p<0.05$) than that of the conventional urea diet (R2). It was clear that the urea diet resulted in lower organic matter digestibility ($p<0.05$) than almost all experimental diets. These data indicate that all the nano-zeolite diets performed comparably to diets containing only natural protein sources without supplementation as the positive control in maintaining high organic matter digestibility. In contrast, the use of conventional urea in the diet as a non-protein nitrogen source to partially replace natural protein sources decreased organic matter digestibility.

Crude protein digestibility in this study ranged from 69.71% to 80.72.33% (Table 4), which the previous result (75.01-77.4%) was within the range of the recent study (Kardaya *et al*., 2023). Crude protein digestibility reported by some researchers ranged from 59.28-78.8% in sheep fed rye straw and agricultural waste-based silage diets (Seok *et al*., 2016), supplemented with various selenium sources (Ibrahim and Mohamed, 2018), oat hay without or with micronutrient fertilization (Singh *et al*., 2020), various protein sources (El-Nomeary *et al*., 2021), various energy sources (Yateem *et al*., 2021), and unpolished rice and polished rice (Kim *et al*., 2019) which reached the upper range of percent digestibility. The use of slow-release urea in diets also showed higher crude protein digestibility, ranging from 63.8 - 92.55% in sheep (Hashem and Tayeb, 2023; Tekliye *et al*., 2018). In comparison, Pasundan cattle fed diets containing urea-impregnated zeolite showed crude protein digestibility in the upper range, ranging from 91.75 - 92.01% (Kardaya *et al*., 2021).

The control diet containing only natural protein without supplementation (R1) produced crude protein digestibility comparable ($p>0.05$) to that of the nano-zeolite diets, either non-heat-activated (R3), heat-activated (R4), or urea-impregnated (R5 and R6), but higher ($p<0.05$) than the conventional urea diet (R2). It was clear that the urea diet resulted in lower crude protein digestibility ($p<0.05$) than almost all experimental diets. These data indicate that all the nano-zeolite diets performed comparably to diets containing only natural protein sources without supplementation as the positive control in maintaining high crude protein digestibility. In contrast, the use of conventional urea in the diet as a non-protein nitrogen source to partially replace natural protein sources decreased crude protein digestibility.

Ether extract digestibility in this study ranged from 63.00% to 75.59% (Table 4). The ether extract digestibility reported by several researchers ranged from 55.74 - 89.2% in sheep fed rye straw and silage diets based on agricultural waste (Seok *et al*., 2016), supplemented with various selenium sources (Ibrahim and Mohamed, 2018), oat hay without or with micronutrient fertilization (Singh *et al*., 2020), various protein sources (El-Nomeary *et al*., 2021), various energy sources (Yateem *et al*., 2021), and unpolished rice and polished rice (Kim *et al*., 2019). The use of slow-release urea up to 1.2% in diets (Geron *et al*., 2016) showed higher extract digestibility (82.37 - 89.01%) compared to the use of SLU up to 1.8% (58.23 - 65.61%) reported by (Hashem and Tayeb, 2023). Meanwhile, the use of urea-impregnated zeolite in pasundan cattle diets showed that the digestibility of ether extract remained high, ranging from 89.71 - 90.47% (Kardaya *et al*., 2021).

A control diet containing only natural protein sources without supplementation (R1) resulted in ether extract digestibility comparable ($p>0.05$) to that of the nano-zeolite diets, either non-heat-activated (R3), heat-activated (R4), or urea-impregnated (R5 and R6), but higher ($p<0.05$) than the conventional urea diet (R2). It was clear that the urea diet resulted in lower ether extract digestibility ($p<0.05$) than almost all experimental diets. These data indicate that all the nano-zeolite diets performed comparably to diets containing only natural protein sources without supplementation as the positive control in maintaining high ether extract digestibility. In contrast, the use of conventional urea in the diet as a non-protein nitrogen source to partially replace natural protein sources was found to reduce ether extract digestibility.

Crude fiber digestibility of the study ranged from 47.90% to 73.03% (Table 4). Crude fiber digestibility reported by some researchers was lower, ranging from 29.13-66.29% in sheep fed rye straw and agricultural waste-based silage diets (Seok *et al*., 2016), supplemented with various selenium sources (Ibrahim and Mohamed, 2018), various protein sources (El-Nomeary *et al*., 2021), and various energy sources (Yateem *et al*., 2021). The use of SLU up to 1.8% also resulted in low fiber digestibility, ranging from 30.58 - 47.60% (Hashem and Tayeb, 2023).

A control diet containing only natural protein sources without supplementation (R1) resulted in crude fiber digestibility comparable ($p>0.05$) to that of the nano-zeolite diets, either non-heat-activated (R3), heat-activated (R4), or urea-impregnated (R6), but higher ($p<0.05$) than the conventional urea diet (R2) or urea-impregnated non-heat activated nano-zeolite (R5). It was clear that the urea diet resulted in lower crude fiber digestibility ($p<0.05$) than almost all experimental diets. These data indicate that all the nano-zeolite diets performed comparably to diets containing only natural protein sources without supplementation as the positive control in maintaining high crude fiber digestibility. In contrast, the use of conventional urea in the diet as a non-protein nitrogen source to partially replace natural protein sources was found to reduce crude fiber digestibility.

Nitrogen free extracts (NFE) digestibility ranged from 72.15% to 83.73% (Table 4). NFE digestibility reported by several researchers ranged from 65.67-83.47% in sheep fed rye straw and agricultural waste-based silage diets (Seok *et al*., 2016), various selenium sources (Ibrahim and Mohamed, 2018), and various protein sources (El-Nomeary *et al*., 2021).

A control diet containing only natural protein sources without supplementation (R1) resulted in NFE digestibility comparable ($p>0.05$) to that of the nano-zeolite diets, either non-heat-activated (R3), heat-activated (R4), or urea-impregnated (R5 and R6), but higher ($p<0.05$) than the conventional urea diet (R2). It was clear that the urea diet resulted in lower NFE digestibility ($p<0.05$) than almost all experimental diets. These data indicate that all the nano-zeolite diets performed comparably to diets containing only natural protein sources without supplementation as the positive control in maintaining high NFE digestibility. In contrast, the use of conventional urea in the diet as a non-protein nitrogen source to partially replace natural protein sources was found to reduce NFE digestibility.

Total digestible nutrient (TDN) of this study ranged from

59.26% to 75.90% (Table 4). These results are comparable to those of (Seok *et al*., 2016) which ranged from 66.6 - 66.7% in sheep fed rye straw and agricultural waste-based silage, 65.67-71.48% of diets supplemented with various selenium sources (Ibrahim and Mohamed, 2018), 65.82 - 73.51% of diets contained various protein sources (El-Nomeary *et al*., 2021), and 60.42-72.80% of sheep fed diets contained different energy levels (Yateem *et al*., 2021).

A control diet containing only natural protein sources without supplementation (R1) resulted in TDN digestibility comparable ($p>0.05$) to that of the nano-zeolite diets, either non-heat-activated (R3), heat-activated (R4), or urea-impregnated (R5 and R6), but higher ($p<0.05$) than the conventional urea diet (R2). It was clear that the urea diet resulted in lower TDN digestibility ($p<0.05$) than almost all experimental diets. These data indicate that all the nano-zeolite diets performed comparably to diets containing only natural protein sources without supplementation as the positive control in maintaining high TDN digestibility. In contrast, the use of conventional urea in the diet as a non-protein nitrogen source to partially replace natural protein sources was found to reduce TDN digestibility.

The digestibility of the fiber fraction consisting of NDF, A DF, cellulose and hemicellulose (Table 5) showed the same pattern as the digestibility of crude fiber (Table 4).

NDF digestibility showed the highest trend in diet R3 (74.18%) and the lowest in R2 (56.11%). The results of this study exceeded the upper range of NDF digestibility (64.26-70.39%) in sheep fed micronized zeolite diets studied previously (Kardaya *et al*., 2023). Diet R3, R4, R5, and R6 produced the same high NDF digestibility ($p>0.05$) as diet R1. Only diet R2 had a lower digestibility ($p<0.05$) than diet R1. This NDF digestibility was higher than the NDF digestibility (40.72-52.27%) of a study that used slow-release urea in the diet (Geron *et al*., 2016) but still within the range of NDF digestibility values (49.6-78.4%) that used rice straw diet treated with urea (Tekliye *et al*., 2018).

ADF digestibility followed a similar trend, highest in R3 (69.65%) and lowest in R2 (51.23%). The results of this study slightly exceed the upper range of ADF digestibility (53.02-63.17%) in sheep fed micronized zeolite diet studied previously (Kardaya *et al*., 2023); Diet R3, R4, R5, and R6 produced similarly high ADF digestibility ($p>0.05$) as diet R1. Only diet R2 had a lower digestibility ($p<0.05$) than diet R1. This ADF digestibility was higher than the ADF digestibility (31.34-50.47%) of studies that used slow-release urea in their diet (Ftiwi and Tadess, 2018; Geron *et al*., 2016), but still within the range of ADF digestibility values (53.1- 81.1%) that used rice straw diet that had been treated with urea (Tekliye *et al*., 2018). Diet R3 showed higher ADF digestibility ($p<0.05$) than diet R2 and R5, presumably because it benefited from the cation exchange properties of nano-zeolite, which modulated ammonia levels to support rumen microbial activity. In contrast, diet R2 had the lowest ADF digestibility, presumably due to energy and nitrogen synchrony and unregulated ammonia release, which impaired ADF fermentability in the rumen.

Cellulose digestibility also showed the highest trend in R3 (77.94%) and the lowest in R2 (61.39%). Cellulose 66.65 -73.11% (Kardaya *et al*., 2023). Diet R3, R4, R5 and R6 produced the same high cellulose digestibility ($p>0.05$) as diet R1. Only diet R2 had lower digestibility ($p<0.05$) than diet R1. The high digestibility of cellulose in diet R3-R6 is thought to be due to the role of nano-zeolite as a medium that can absorb, hold, and release urea and ammonia in a page and more controlled manner so as to create a synchronous atmosphere between the utilization of energy and ammoniacal nitrogen which in turn can support the fermentation of feed fiber in the rumen optimally.

Hemicellulose digestibility also showed the highest trend in R3 (79.73%) and the lowest in R2 (62.05±7.60). Hemicellulose 69.68-77.75% (Kardaya *et al*., 2023). Diet R3, R4, R5, and R6 produced the same high hemicellulose digestibility ($p>0.05$) as diet R1. Only diet R2 had lower digestibility ($p<0.05$) than diet R1. The higher hemicellulose digestibility in diet R3-R6, which matched

Table 5: Fiber digestibility in sheep fed experimental diets.

Fiber digestibility metrics (%)	R1	R2	R3	R4	R5	R6
NDF (%)	$68.46±2.59^{bc}$	$56.11±7.66^{a}$	$74.18±3.97^{c}$	$68.73±2.48^{bc}$	$63.08±5.15^{ab}$	$68.36±4.49^{bc}$
ADF (%)	$63.83±2.70^{bc}$	$51.23±7.72^{a}$	$69.65±3.63^{c}$	$62.84±2.30^{bc}$	$54.93±5.62^{ab}$	$64.22±4.32^{bc}$
Cellulose (%)	$72.94±2.70^{bc}$	$61.39±7.87^{a}$	$77.94±3.54^{c}$	$73.61±1.90^{bc}$	$65.38±5.48^{ab}$	$74.64±4.40^{bc}$
Hemicellulose (%)	$74.19±3.86^{b}$	$62.05±7.60^{a}$	$79.73±4.41^{b}$	$75.84±2.76^{b}$	$72.16±5.45^{ab}$	$73.78±4.73^{b}$

R1 = basal diets; R2 = basal diets + urea; R3 = basal diets + non-activated nano-zeolite; R4 = basal diets + thermal activated nano-zeolite; R5 = basal diets + urea impregnated non-activated nano-zeolite; R6 = basal diets + urea impregnated pre-thermal activated nano-zeolite. Different superscripts on the same row indicate significant differences ($p<0.05$). NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber.

the hemicellulose digestibility in R1, was probably due to the role of nano-zeolite in optimizing feed fiber fermentation in the rumen. Heat activation of nano-zeolites used in diet R4 and R6 did not significantly increase hemicellulose digestibility compared to non-heat activated nano-zeolites (R3 and R5). These data indicate that the use of nano-zeolite as a medium for slow-release urea in diet does not require prior heat activation.

The results of this study demonstrate that, except for diet R5, diet R2 exhibited a significant reduction in the digestibility of fiber components, including neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, and hemicellulose ($p<0.05$). Diet R2, which incorporates conventional urea, undergoes rapid fermentation by rumen microbes into ammonia, which is subsequently absorbed from the rumen into the liver. This process results in suboptimal utilization of ammonia by the microbes to meet their nitrogen requirements for growth, as the ammonia concentration in the rumen fluid is comparatively lower than in other diet. Additionally, the efficiency of energy utilization derived from the fermentation of carbohydrates into volatile fatty acids (eVFA) in this conventional urea diet was also reduced (74.85±1.00%), as reported by (Kardaya *et al*., 2025), in comparison to the diet containing natural protein (R1). This situation led to a decrease in the digestibility of fiber fractions (NDF, ADF, cellulose, hemicellulose).

While diet R5 showed lower digestibility for some digestibility variables, it performed slightly better than diet R2. This can be attributed to the inclusion of urea-impregnated inactive nano-zeolite, which can reduce the negative effects of urea by slowing urea release, preventing ammonia toxicity, and creating a more stable rumen environment for microbial activity. However, the performance of diet R5, although similar in potency to the other diet, was not as good as diet R3, which both contained deactivated nano-zeolite but no impregnated urea, due to its ability to absorb, retain and release ammonia back into the rumen fluid in a more controlled manner, resulting in higher VFA (Kardaya *et al*., 2025) Thus, except for conventional urea, the use of either non-activated (R3) or heat-activated (R4) nano-zeolites, either non-activated nano-zeolites impregnated with urea (R5) or heat-activated nano-zeolites impregnated with urea (R6) has the potential to increase the digestibility of fiber (NDF, ADF, cellulose, hemicellulose) and organic matter as well as diet containing only natural protein (R1).

The use of slow-release urea up to 1.2% in sheep diet containing corn silage, ground corn, and soybean meal did not significantly affect the digestibility of organic matter, NDF, and ADF (Geron *et al*., 2016). Another study on Awassi sheep using slow-release urea up to 1.8% showed the same organic matter digestibility and fiber digestibility data as the control treatment (Hashem and Tayeb, 2023). Different from the results of the two researchers, the use of rice straw treated with urea resulted in significantly higher digestibility of organic matter, NDF, and ADF ($p<0.05$) than the control diet (Tekliye *et al*., 2018). In the R2 diet, the rapid hydrolysis of urea to ammonia resulted in an excessive increase in ammonia levels so that more ammonia was absorbed from the rumen into the liver tissue. As a result, ammonia availability in the rumen is reduced, impairing the ability of microbes to digest fiber effectively. In contrast, diet R6, heat-activated nano-zeolite stabilized ammonia release, leading to improved NDF digestibility compared to R2.

The use of nano-zeolites, both non-impregnated and urea-impregnated in diet R3-R6 significantly resulted in higher digestibility of NDF, ADF, cellulose, and hemicellulose than conventional urea diet (R2) by creating a stable rumen environment and regulating ammonia release in synchronization with energy use by rumen microbes.

CONCLUSION AND RECOMMENDATION

Nano-zeolite diets are just as effective as diets containing solely natural protein in maintaining high digestibility levels of dry matter, organic matter, crude protein, ether extract, crude fiber, nitrogen-free extract (NFE), and total digestible nutrients (TDN). In contrast, substituting natural protein with conventional urea reduces the digestibility of all these components. Incorporating both non-impregnated and urea-impregnated nano-zeolites into diets improves the digestibility of neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, and hemicellulose compared to the conventional urea diets.

The findings suggest that incorporating nano-zeolite into animal feed can significantly improve the digestibility of essential nutrients, such as dry matter, organic matter, crude protein, ether extract, crude fiber, nitrogen-free extract (NFE), and total digestible nutrients (TDN). Unlike conventional urea, which reduces digestibility, both non-impregnated and urea-impregnated nano-zeolite aid in the breakdown of fiber components, including neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, and hemicellulose. Consequently, nano-zeolite, in both its non-impregnated and urea-impregnated forms, emerges as a promising feed additive to enhance nutrient absorption and overall feed efficiency in ruminant diets, potentially leading to improved animal performance and more sustainable feeding practices.

ACKNOWLEDGEMENT

Acknowledgment is given to the Indonesian Ministry of Education, Culture, Research and Technology for their financial contribution to the 2024 Applied Research Scheme, as specified in Contract Number: 014/SP2H/RT-MONO/LL4/2024, 801/01/K-X/VI/2024.

NOVELTY STATEMENT

This study is the first to directly compare the effects of nano-zeolite, urea-impregnated nano-zeolite (UINZ), and conventional urea supplementation on the apparent digestibility of dry matter, crude protein, neutral detergent fiber, and acid detergent fiber in sheep. The research introduces the use of thermally activated urea-impregnated nano-zeolite as a dual-function feed additive, combining controlled nitrogen release with enhanced adsorptive and catalytic properties to improve nutrient utilization in ruminants. By employing nano-sized zeolite particles, the study leverages increased surface area and reactivity, showcasing the potential of nanotechnology to enhance the efficacy of mineral-based feed additives in ruminant diets. The findings reveal that thermally activated UINZ supplementation yields significantly higher nutrient digestibility than either nano-zeolite or urea alone, highlighting a synergistic effect not previously documented in sheep nutrition research. This work provides new insights into the practical application of nano-additives for improving feed efficiency and nitrogen utilization, supporting the development of more sustainable and environmentally friendly sheep production systems.

AUTHOR'S CONTRIBUTION

DK: Responsible for conceptualization, investigation, supervision, validation, and the writing review and editing.
DS: Engaged in formal analysis, methodology, investigation, and writing original draft.
DW: In charge of data curation, investigation, and writing original draft, as well as project administration.
RAG: Focused on investigation, resources, software, and validation.
IQP: Tasked with data curation, investigation, software, and project administration.

Ethical approval

The National Research and Innovation Agency's Ethics Commission for Animal Maintenance and Use has granted its approval and authorization for all procedures in this study, following the Research Ethics Clearance in the Field of Livestock and Utilization, with reference number 203/KE.02/SK/08/2024.

Generative AI and AI-assisted technology statement

Only OpenAI's ChatGPT and Microsoft Word Editor plug-in were used for grammar, spelling, and clarity checks; no AI-generated content was included, and the authors fully verified and take responsibility for the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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