Development of Slow-Release Urea Additives Using Autoclaved Oil Palm Empty Fruit Bunches and Urea via Wet Granulation for Ruminants

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Abstract. This study developed slow-release urea (SRU) additives using autoclaved oil palm empty fruit bunches (OPEFB) and urea via wet granulation to improve nitrogen utilization in ruminants. OPEFB was autoclaved at 121°C and 1 atm pressure to create a stable matrix, which was then combined with urea in different proportions to form SRU. In the first phase, the physical and morphological properties of autoclaved and non-autoclaved OPEFB were analysed using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR) spectroscopy. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were measured, with hemicellulose calculated as the difference between NDF and ADF. In the second phase, SRU formulations included varying percentages of urea and autoclaved OPEFB: SRU 100 (100% urea), SRU 98 (2% OPEFB), SRU 96 (4% OPEFB), SRU 94 (6% OPEFB), SRU 92 (8% OPEFB), and SRU 90 (10% OPEFB). SEM and FTIR descriptively showed surface changes in OPEFB after autoclaving, including increased porosity. Autoclaving also descriptively reduced NDF, ADF, lignin, cellulose, and hemicellulose contents. *In vitro* rumen incubation revealed that the addition of autoclaved OPEFB in SRU reduced urea release and pH in the rumen at various time intervals: 3, 6, 9, and 24 hours. SRU with autoclaved OPEFB optimizes urea use and controls nitrogen release.

Keywords: Autoclave, Empty fruit bunch, Feed additive, *in vitro*, Slow-release urea

Abstrak. Studi ini mengembangkan aditif urea pelepas lambat (UPL) menggunakan tandan kosong kelapa sawit (TKKS) yang diautoklaf dan urea melalui granulasisasi basah untuk meningkatkan efisiensi pemanfaatan nitrogen pada ruminansia. TKKS diautoklaf pada suhu 121°C dan tekanan 1 atm untuk menciptakan matriks yang stabil, yang kemudian dicampurkan dengan urea dalam berbagai proporsi untuk membentuk UPL. Pada fase pertama, sifat fisik dan morfologi TKKS yang diautoklaf dan yang tidak diautoklaf dianalisis menggunakan *Scanning Electron Microscopy* (SEM) dan *Fourier Transform Infrared* (FTIR) spectroscopy. Kandungan *neutral detergent fiber* (NDF) dan *acid detergent fiber* (ADF) diukur, dengan hemiselulosa dihitung sebagai selisih antara NDF dan ADF. Pada fase kedua, formulasi SRU mencakup berbagai persentase urea dan TKKS yang diautoklaf: SRU 100 (100% urea), SRU 98 (2% TKKS), SRU 96 (4% TKKS), SRU 94 (6% TKKS), SRU 92 (8% TKKS), dan SRU 90 (10% TKKS). SEM dan FTIR menunjukkan secara deskriptif perubahan signifikan pada permukaan TKKS setelah diautoklaf, termasuk peningkatan porositas. Autoklaf juga secara deskriptif mengurangi kandungan NDF, ADF, lignin, selulosa, dan hemiselulosa. Inkubasi rumen *in vitro* menunjukkan bahwa penambahan TKKS yang diautoklaf pada SRU menurunkan pelepasan urea dan pH di dalam rumen pada berbagai interval waktu 3, 6, 9, dan 24 jam. SRU dengan OPEFB yang diautoklaf mengoptimalkan penggunaan urea dan mengontrol pelepasan nitrogen.

Kata kunci: Autoklaf, Tandan kosong kelapa sawit, Aditif pakan, *in vitro*, Urea pelepas lambat.

Introduction

Due to its amino acid profile and availability, soybean meal is a universal protein source, but it significantly raises the cost of ruminant diets (Dinani et al., 2020). The integration of urea as a non-protein nitrogen (NPN) source is a viable solution to this challenge. However, conventional urea is rapidly hydrolysed in the rumen, releasing ammonia at rates that often exceed the capacity of rumen microbes to assimilate it. This imbalance leads to nitrogen losses, reduced feed efficiency, and heightened environmental concerns, such as ammonia emissions (Mentz et al., 2016). Effective synchronization of carbohydrate and protein degradation in the rumen is essential for

optimizing microbial protein synthesis (MPS) and improving nitrogen utilization (Seo et al., 2013). To address these challenges, slow-release urea (SRU) formulations have been developed to provide a gradual nitrogen release, ensuring better synchronization of nutrient availability, enhancing microbial activity, and improving overall MPS efficiency (Gardinal et al., 2016).

The formulation of SRU products is crucial for their efficiency, as variations in nitrogen release rates and interactions with dietary components significantly affect efficacy. The nitrogen release rate from SRU products can be optimized using technologies such as biopolymers and geopolymers as coating materials, improving nitrogen's durability and controlled release properties (Azeem et al., 2020). *In vitro* investigations have contrasted polymer-coated urea (PCU) with gelatinized starch urea (GSU) regarding nitrogen retention. PCU enhanced rumen fermentation, elevating total VFAs and their proportions, including acetic acid, butyric acid, and propionic acid, while promoting the abundance of Firmicutes in rumen microflora (Fan et al., 2024). SRU can substitute conventional protein sources such as soybean meal in ruminant diets without impairing rumen function or animal health, thereby decreasing the carbon footprint of feed by as much as 54% (Salami et al., 2021). The advancement of coating technologies and the refinement of dietary formulas are essential for enhancing the efficacy of SRU products across diverse agricultural settings.

The integration of novel feedstock materials presents a viable strategy for enhancing the performance of SRU products, with oil palm empty fruit bunch (OPEFB), a lignocellulosic byproduct of the palm oil industry, emerging as a significant candidate. Comprising roughly 21–23% of processed fresh fruit bunches (FFB), OPEFB is abundant and contains high levels of cellulose, hemicellulose, and lignin, rendering it suitable for biotechnological applications (Aktawan et al., 2020; Muryanto et al., 2022). However, its elevated lignin content presents a considerable obstacle, as lignin acts as a structural barrier that restricts the availability of cellulose and hemicellulose. Delignification procedures, such as autoclaving, are crucial for this purpose. Autoclaving utilizes high-pressure steam to effectively decompose lignin structures, thereby increasing the accessibility of cellulose and hemicellulose for subsequent processing and strengthening the physicochemical properties of OPEFB. Muthia et al. (2021) assert that autoclaving combined with urea treatment facilitates ammonia breakdown of lignocellulose, disrupting lignin-cellulose and hemicellulose linkages while improving the nutritional quality of feedstocks such as rice straw. Furthermore, higher temperatures (135°C) and pressure (2.5 atm) treatments disrupt the cell walls in oil palm fronds, facilitating the separation of components and the degradation of complex polysaccharides into simpler forms for enhanced microbial digestion in the rumen (Harahap et al., 2018). This treatment promotes the incorporation of OPEFB into wet granulation procedures for SRU manufacture by diminishing lignin concentration, thereby improving its binding and nutrient retention properties and making it a more effective nitrogen delivery matrix in ruminant diets. Cassava (*Manihot esculenta*) has demonstrated utility as a binding agent in numerous industrial applications due to its starch content (Li et al., 2017). The availability of cassava starch in different quantities and forms enhances its efficacy as a binding matrix, promising improved stability and regulated nitrogen release.

This study aimed to assess slow-release urea (SRU) additives using autoclaved oil palm empty fruit bunch (OPEFB) and urea via wet granulation, utilizing gelatinized starch from *Manihot esculenta* (cassava) as a binding agent. Fourier Transform Infrared (FTIR) spectroscopy was used to evaluate chemical functional groups to identify structural alterations in

lignocellulose, while Scanning Electron Microscopy (SEM) was employed to investigate morphological and surface modifications, both confirming the efficacy of autoclaving in enhancing OPEFB for SRU formulations. In addition, Van Soest et al. (1991) analysis was conducted to determine the chemical composition of the fibre. Furthermore, the SRU formulations were evaluated for ammonia concentration and pH levels in in vitro rumen fermentation.

Materials and Methods

Ethical approval

The research utilised rumen fluid as a medium for in vitro experiments. However, ethical approval was not required for this research as the bacteria used were obtained from slaughterhouse waste.

Study period and location

This research was conducted from April to November 2024 at the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Tanjungpura University, and the Sentarum Quality Testing Laboratory in West Kalimantan, Indonesia. Sample characterization using Fourier Transform Infrared (FTIR) spectroscopy was performed at Hasanuddin University in Makassar, Indonesia, while Scanning Electron Microscopy (SEM) analysis was conducted at the Bandung Institute of Technology in Bandung, Indonesia. The pH and NH_3 content of in vitro rumen samples were analysed at the Dairy Cattle Nutrition Laboratory, IPB University, Bogor, Indonesia.

Experiment 1

Sample Preparation

Oil palm empty fruit bunches (OPEFB) were collected from PT. Mitra Utama Bintang Korek, West Kalimantan, Indonesia, and subsequently washed with water to remove contaminants. The OPEFB was then chopped into small pieces (approximately 2–3 cm in length) and dried in an oven at 60°C for 48 hours. A portion of the OPEFB samples was autoclaved at 121°C and 15 psi for one hour. The autoclaved samples were then dried in an oven at 105°C for 4 hours. Finally, the autoclaved OPEFB was ground to a particle size of approximately 20–30 mesh.

Experimental design

Both autoclaved and non-autoclaved OPEFB were assessed for morphological and surface modifications, as well as chemical functional groups, to determine structural changes in lignocellulose. The fibre's chemical composition was analysed, and each treatment was conducted in duplicate.

Scanning electron microscopy (SEM) measurement

SEM was used to analyse the surface properties of both non-autoclaved and autoclaved OPEFB. Before examination, the specimen was mounted on a plate coated with gold and palladium to function as a conductor. The SEM analysis was conducted at an accelerating voltage of 15 kV. Images were meticulously acquired and analysed at a magnification of 2000×.

Fourier transform mid-infrared spectroscopy (FTIR) measurement

For FTIR analysis, 2 mg of both non-autoclaved and autoclaved OPEFB samples were rapidly and uniformly mixed with 200 mg of KBr in a mortar to prepare the sample. The mixture was then analysed for 60 seconds using an IRPrestige-21 FTIR spectrometer (Shimadzu, Japan) within the 4000–500 cm^{-1} wavelength range. Peak positions were determined using Shimadzu IR Solution 1.50 software (Shimadzu).

Determination of ADF (Acid Detergent Fiber), NDF (Neutral Detergent Fiber) and hemicellulose composition

All treated samples were analysed for fibre component content (ADF, NDF, lignin, cellulose, and hemicellulose) according to Van Soest et al. (1991).

Table 1. Formula of slow-release urea with autoclaved oil palm empty fruit bunch (OPEFB)

Materials	Units	Treatments					
		SRU 100	SRU 98	SRU 96	SRU 94	SRU 92	SRU 90
Urea	g	100	100	100	100	100	100
OPEFB autoclaved	g	0	2	4	6	8	10
Manihot starch	g	7.5	7.5	7.5	7.5	7.5	7.5
Binder	g/mL	2.5/25	2.5/25	2.5/25	2.5/25	2.5/25	2.5/25
Lactose	g	65	65	65	65	65	65

Notes: SRU = slow-release urea, OPEFB = autoclaved oil palm empty fruit bunch, Binder= Manihot starch: distilled water

The neutral detergent solution (NDS) was prepared by measuring 4.56 g of Na_2HPO_4, 18.61 g of Na-EDTA, 6.81 g of $Na_2B_4O_7{\cdot}10H_2O$, and 30 g of SLS. These solids were placed in a 1000 mL Erlenmeyer flask, followed by the addition of 1000 mL of distilled water. The solution was stirred until homogeneous.

A 1-gram sample was measured and placed in a 100 mL beaker, to which 100 mL of NDF solution was added. The mixture was then boiled for one hour, with precautions taken to prevent foaming and colour changes from clear to yellow or yellow-brown. The sample solution was filtered using filter paper (b), and the residue was rinsed with 300 mL of hot water, followed by two washes with 25 mL of acetone each.

The residue was placed in a Petri dish and air-dried at room temperature until fully desiccated. It was then oven-dried at 105°C for 8 hours, after which it was transferred to a desiccator for 15 minutes before being weighed (c) using an analytical balance (Anam et al., 2012). The %NDF content was calculated using following formula:

$$\text{NDF}\ (\%) = \frac{\text{c}-\text{b}}{\text{a}} \text{x}\ 100\%$$

The formulation of the ADS (acid detergent solution) for ADF analysis required the measurement of 20 g of CTAB. The solids were placed in a 1000 mL Erlenmeyer flask, to which 28.05 mL of 1 N NaOH solution was then added, followed by the addition of distilled water. The mixture was stirred until homogeneous (Sitorus and Lumbantoruan, 2020).

A 1-gram specimen was measured and placed in a 100 mL beaker. Next, 100 mL of ADS solution was added, and the mixture was boiled for one hour, ensuring no foaming occurred. The sample solution was filtered using filter paper (b), and the resultant residue was rinsed with 300 mL of hot water, followed by two washes with 25 mL of acetone each.The residue was placed in a Petri dish and air-dried at room temperature until it exhibited minor whitening. It was then heated at 105°C for 8 hours in an oven. The dried sample was subsequently transferred to a desiccator for 15 minutes and weighed (c) using an analytical balance. The %ADF content was calculated using the following formula (Anam et al. , 2012):

$$\text{ADF}(\%) = \frac{\text{c} - \text{b}}{\text{a}} x100\%$$

Hemicellulose content was determined as:

$$\text{Hemicellulose}\ (\%) = \text{NDF}(\%) - \text{ADF}(\%)$$

Determination of Lignin and Cellulose Composition

The lignin and cellulose composition were determined by soaking the ADF residue in 10 mL of 72% sulphuric acid (H_2SO_4) for 3 hours. The solution was then filtered and washed with hot water and acetone. The residue was dried in an oven at 105°C for 4 hours (d). Next, the dried residue was incinerated in a furnace at 600°C, and the resulting ash was weighed (e) using an analytical balance (Jayanegara et al. , 2018). The following calculations were used (Bina et al. , 2023):

$$\text{Cellulose}\ (\%) = \frac{\text{c}-\text{d}}{\text{a}}\ \text{x}\ 100\%$$

$$\text{Lignin}\ \ (\%) = \frac{\text{d} - \text{e}}{\text{a}}\ \text{x}\ 100\%$$

Experiment 2

Formulation of slow-release urea (SRU)

This experiment aimed to formulate slow-release urea (SRU) using autoclaved oil palm

empty fruit bunch (OPEFB) as an additive, bound through the wet granulation method. Six different formulations were prepared, varying in the concentration of autoclaved OPEFB. The formulations included SRU 100 (0% autoclaved OPEFB as a control), SRU 98 (2 g autoclaved OPEFB), SRU 96 (4 g autoclaved OPEFB), SRU 94 (6 g autoclaved OPEFB), SRU 92 (8 g autoclaved OPEFB), and SRU 90 (10 g autoclaved OPEFB). The detailed composition of each formulation is presented in Table 1. Additionally, the ammonia release rate and pH values of the formulations were tested using the *in vitro* rumen incubation method based on Theodorou and Brooks (1990).

Procedure for Rumen Fluid Collection, Incubation, and Analysis of pH and NH_3 Concentration

Rumen fluid was collected from three freshly slaughtered cattle at a slaughterhouse in Bogor, West Java, Indonesia. Approximately 1 litre of fluid was stored in a thermos to maintain temperature and microbial viability. For the experiment, 75 mg of the treatment substrate was placed into a 125 mL bottle, followed by the addition of 50 mL of rumen fluid and 75 mL of a pre-saturated rumen buffer solution as the incubation medium.

The buffer solution, pre-saturated with CO_2, was prepared with the following composition per 1000 mL: 241 mL bicarbonate buffer solution, 121 mL macromineral solution, 0.061 mL micromineral solution, 0.61 mL resazurin, 362 mL distilled water, 23 mL reducing agent, and 253 mL rumen fluid. Once the substrate and buffer solution were combined, the bottles were sealed tightly with rubber stoppers to maintain airtight conditions and incubated in a water bath at 39°C for 24 hours. During the first 4 hours of incubation, the bottles were manually shaken every hour, followed by shaking every 2 hours until the 12-hour mark.

At 3, 6, 9, and 12 hours of incubation, rumen fluid samples were collected. The solid and supernatant fractions in the bottles were separated by centrifugation, and the supernatant was transferred to plastic bottles for further analysis of NH_3 concentration and pH. The NH_3 concentration was determined using the Conway microdiffusion method, following General Laboratory Procedures, (1966).

For this procedure, the rim and lid of a Conway dish were coated with petroleum jelly to ensure an airtight seal. One millilitre of supernatant was placed on one side of the dish, while 1 mL of saturated Na_2CO_3 solution was placed on the opposite side, ensuring the two liquids did not mix. In the centre of the dish, 1 mL of boric acid solution with an indicator was added. The dish was then sealed tightly, and the Na_2CO_3 solution was mixed with the supernatant. The mixture was left to react for 24 hours at room temperature.

After this period, the solution in the Conway dish was titrated with 0.005 N H_2SO_4 until the boric acid solution changed colour from blue to red, and the titration results were recorded. Additionally, the pH of the supernatant from each sample was measured using a pH meter to monitor the incubation conditions and evaluate the treatment effects. Each treatment was carried out in duplicate.

Data analysis

The data obtained were analysed descriptively to describe the distribution and variation of fibre component values in autoclaved and non-autoclaved oil palm empty fruit bunches. Additionally, the SRU formulation analysis evaluated the effectiveness of ammonia release rate and pH values observed during the in vitro rumen incubation process.

The experiment was conducted in triplicate, and analysis of variance (ANOVA) was performed on the collected data at a significance level of 0.05. Comparisons between different treatments were conducted using Duncan's multiple range test at the same significance level (0.05). Data analysis was carried out using IBM SPSS Statistics software, version 20.

Results and Discussion

The scanning electron microscopy (SEM) images at 2000× magnification reveal significant differences between autoclaved and non-autoclaved oil palm empty fruit bunch (OPEFB) samples (Figure 1). The non-autoclaved OPEFB exhibits an intact surface with clearly defined fibres and a relatively rough texture, indicating minimal structural alterations. In contrast, the autoclaved OPEFB demonstrates increased porosity, surface cracking, and a more fragmented texture, suggesting that exposure to temperature and pressure has modified its surface characteristics. These changes highlight the physical effects of autoclaving process on OPEFB.

Elevated temperatures enhance molecular kinetic energy, facilitating the diffusion of acetylating agents into the fibre matrix. Onwuka et al. (2019) observed that higher temperatures improve compatibility of reaction components and increase the swellability of cellulosic fibres, which is crucial for efficient acetylation. Additionally, pressure application during acetylation enhances reaction efficiency by promoting deeper penetration of acetylating agents into the fibre structure. Quintana et al. (2018) emphasised the role of hydroxyl group accessibility in cellulose for effective acetylation. The combination of high temperature and pressure disrupts the fibre's dense cellular structure, leading to the formation of pores and modifications in physical properties, such as increased fibre diameter and surface roughness (Chen et al., 2022; Li et al., 2013).

Table 2 presents the wavenumber peaks and corresponding functional groups in non-autoclaved and autoclaved OPEFB, indicating various structural modifications. The increase in the stretching vibration of *C-H* bonds from 2922 cm^{-1} to 2924 cm^{-1} suggests changes in cellulose and hemicellulose structure, potentially due to increased crystallinity or alterations in hydrogen bonding networks (Chen et al., 2023; Rotaru et al., 2024). The shift in the bending vibration of water molecules associated with hemicellulose from 1635 cm^{-1} to 1647 cm^{-1} indicates changes in water-hemicellulose interactions, likely resulting from disrupted hydrogen bonds (Wei et al., 2024). Additionally, the increased vibration of *C-H* and *C-O-C* pyranose in cellulose (from 1159 cm^{-1} to 1161 cm^{-1}) and the stretching of *C-O-C* glycosidic bonds (from 1041 cm^{-1} to 1058 cm^{-1}) reflect structural modifications that may enhance the rigidity or stability of the cellulose matrix (Csóka et al., 2024; Wang et al., 2024). The substantial increase in *C-O-C* glycosidic bond vibrations from 667 cm^{-1} to 896 cm^{-1} suggests significant structural rearrangements, possibly leading to increased crystallinity or altered hydrogen bonding within the cellulose (Chen et al., 2023). These observed shifts in wavenumber indicate structural modifications in OPEFB components as a result of autoclaving.

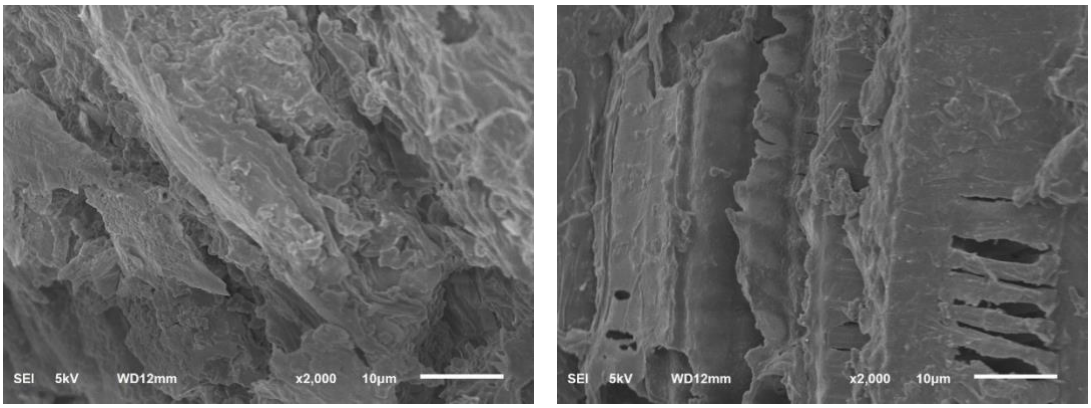

Figure 1. Scanning electron microscopy (SEM) of non-autoclaved oil palm empty fruit bunch (OPEFB) (left) and autoclaved OPEFB (right) at 200

Table 2. Wavenumber peaks and corresponding bonds in non-autoclaved and autoclaved oil palm empty fruit bunch (OPEFB)

Wavenumber (cm^{-1})		Description	Compound
Non-autoclaved OPEFB	Autoclaved OPEFB		
3421	3419	Stretching O-H hydrogen bond from intra-molecular cellulose[1]	Cellulose
2922	2924	Stretching C-H from methyl (CH_3) and methylene (CH_2) groups[1]	Cellulose and Hemicellulose
1741	1741	Stretching of C=O group from the acetyl and uronate ester groups of hemicellulose[1]	Hemicellulose
1635	1647	Bending of water molecules[2]	Hemicellulose
1543	1542	Stretching C=C from the aromatic ring[3]	Lignin
1462	1460	Water absorption by cellulose and hemicellulose[4]	Cellulose and Hemicellulose
1246	1246	Stretching C-O-C from alkyl ether[3]	Lignin
1159	1161	Vibration of C-H and C-O-C pyranose[5]	Cellulose
1041	1058	Stretching C-O-C glycosidic from pyranose ring[5]	Cellulose
667	896	C-O-C glycosidic bond[6]	Cellulose

Notes: [1] = Nazir et al. (2013), [2] = Kostryukov et al., (2023), [3] = Pangau et al., (2017), [4] = Fularz et al., (2021), [5] = Sá et al., (2015), [6] = Vârban et al., (2021).

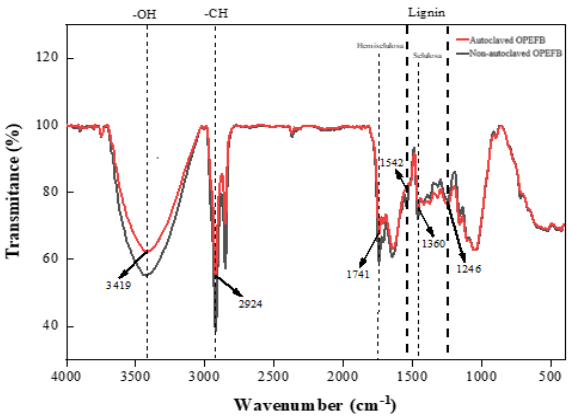

Figure 2. Fourier transform infrared spectroscopy (FTIR) of non-autoclaved oil palm empty fruit bunch (OPEFB) (grey colour) and autoclaved OPEFB (red colour)

In *Figure 2*, the peaks at wavenumbers 1543–1566 cm^{-1} and 1246–1265 cm^{-1} become broader or exhibit an increase in transmittance. This rise in transmittance suggests that lignin undergoes degradation during delignification process. Disappearance of peaks around 1800 cm^{-1} (carboxylic bonds) and 1200 cm^{-1} (carbonyl bonds) in materials such as rice straw and oil palm fronds further indicates lignocellulose degradation (Dewi et al., 2018). Moreover, a decrease in cellulose crystallinity, evidenced by increased transmittance in specific FTIR regions, suggests disruption of hydrogen bonds within the cellulose structure (Dewi et al., 2018).

Table 3 presents chemical composition data of OPEFB treated with non-autoclave and autoclave methods. The results show that autoclaving descriptively reduced NDF, ADF, cellulose, lignin, and hemicellulose levels. The most significant decrease occurred in hemicellulose, from 20.66% to 5.12%. This is because hemicellulose is the polysaccharide most easily hydrolysed under autoclave conditions (heating with high-pressure steam). Hemicellulose is the most easily hydrolysed component under autoclave conditions, as it is less crystalline and more amorphous compared to cellulose (Zhuang et al., 2012).

Table 3. The impact of autoclave treatment on the composition NDF, ADF, cellulose, lignin, and hemicellulose in OPEFB

Treatments	NDF (%)	ADF (%)	Cellulose (%)	Lignin (%)	Hemicellulose (%)
Non-autoclaved OPEFB	89.68	69.01	51.52	16.97	20.66
Autoclaved OPEFB	48.02	42.90	32.43	9.82	5.12

Notes: NDF = neutral detergent fibre, ADF = acid detergent fibre , OPEFB = oil palm empty fruit bunches

Table 4. pH value of slow-release urea additives using autoclaved oil palm empty fruit bunch and urea via wet granulation by in vitro fermentation during 24 h of incubation.

Treatments	Incubation durations (h)			
	3	6	9	24
SRU 90	8.05[a]	8.43	8.51	8.60
SRU 92	8.12[b]	8.44	8.51	8.60
SRU 94	8.14[b]	8.45	8.52	8.60
SRU 96	8.14[b]	8.46	8.53	8.61
SRU 98	8.17[c]	8.46	8.54	8.62
SRU 100	8.47[d]	8.49	8.54	8.65
SEM	0.03	0.01	0.01	0.01
p-value	<0.01	0.22	0.61	0.19

Notes: SRU 100 = 0% autoclaved OPEFB as a control, SRU 98 = 2 g autoclaved OPEFB, SRU 96 = 4 g autoclaved OPEFB, SRU 94 = 6 g autoclaved OPEFB, SRU 92 = 8 g autoclaved OPEFB, and SRU 90 = 10 g autoclaved OPEFB, SEM = standard error of the mean.

According to Muthia et al., (2021), although lignin is relatively more resistant to hydrolysis, autoclaving still causes its degradation, leading to a reduction in its levels from 16.97%. Cellulose, as the main component of the cell wall and the most difficult to degrade, also experienced a decrease in levels from 51.52% to 32.43%. By significantly lowering the levels of NDF, ADF, cellulose, lignin, and hemicellulose, autoclaving can facilitate better digestibility and nutrient availability, making OPEFB a more viable feedstock for livestock and bioenergy production (Moura et al., 2020).

Table 4 and Table 5 indicate the pH value & NH_3 concentration (mM) of the rumen *in vitro* at different levels of OPEFB in the SRU formulation and varying incubation durations (hours). The pH value and NH_3 concentration of the rumen *in vitro* increased with extended incubation time, while elevated OPEFB levels in the SRU formula reduced ammonia release ($p<0.01$). Variations were observed in the pattern of NH_3 increase at different levels of OPEFB in SRU. In SRU 90, 92, 94, 96, 98, and 100, the NH_3 concentration gradually increased from 3 h to 24 h. However, SRU 90 exhibited the slowest ammonia release. Upward trend in NH_3 concentration was associated with an increase in pH, as ammonia exhibits alkaline properties (Sasongko et al., 2024).

The autoclave treatment of OPEFB decreases lignin & hemicellulose content while increasing the exposure of amorphous fibre regions, thereby enhancing microbial colonization and degradation. Structural changes in biomass can favour specific microbial populations that exhibit greater efficiency in degrading the modified fibre structure (Zhang et al., 2021).

Table 5. Ammonia release of slow-release urea additives using autoclaved oil palm empty fruit bunch and urea via wet granulation by in vitro fermentation during 24 h of incubation.

Treatments	Incubation durations (h)			
	3	6	9	24
SRU 90	136.90[a]	129.84[a]	140.71[a]	167.00[a]
SRU 92	144.87[b]	140.35[b]	146.30[b]	168.69[b]
SRU 94	144.90[b]	143.49[c]	146.82[b]	171.78[c]
SRU 96	146.34[c]	148.61[d]	147.65[c]	172.62[d]
SRU 98	148.57[d]	151.51[e]	152.35[d]	174.68[e]
SRU 100	159.60[e]	152.17[f]	155.46[e]	181.19[f]
SEM	1.67	1.87	1.14	1.11
p-value	<0.01	<0.01	<0.01	<0.01

Notes: SRU 100 = 0% autoclaved OPEFB as a control, SRU 98 = 2 g autoclaved OPEFB, SRU 96 = 4 g autoclaved OPEFB, SRU 94 = 6 g autoclaved OPEFB, SRU 92 = 8 g autoclaved OPEFB, and SRU 90 = 10 g autoclaved OPEFB, SEM = standard error of the mean.

In addition, Studies demonstrate that starch-based binders, including *Amilum manihot*, can effectively regulate the release rate of urea in the rumen. The starch matrix formed during the binding process may create a barrier that inhibits the hydrolysis of urea, resulting in a more controlled release of ammonia (NH_3) (de Medeiros et al., 2018).

The gradual release of nitrogen is essential for aligning nitrogen availability with microbial fermentation of carbohydrates in the rumen, which is critical for enhancing microbial protein synthesis (Pinos-Rodríguez et al., 2010). The application of *Amilum manihot* ensures a consistent nitrogen supply, thereby stabilising the ruminal environment and enhancing digestion and nutrient absorption (Pereira et al. , 2017).

Conclusions

In conclusion, autoclaving oil palm empty fruit bunches (OPEFB) enhances their suitability as a slow-release urea (SRU) additive for ruminant feed by improving their physical and chemical properties. The autoclaving process increases porosity, modifies fibre structure, and reduces the levels of neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, lignin, anhemicellulose—particularly hemicellulose—thereby improving digestibility. FTIR analysis confirmed structural alterations in cellulose and hemicellulose, facilitating enhanced microbial degradation. *In vitro* rumen incubation demonstrated that autoclaved OPEFB in SRU formulations effectively regulates ammonia release, optimising nitrogen utilisation in ruminants. The findings indicate that autoclaved OPEFB may be a viable alternative for regulating urea release and improving feed efficiency in ruminant nutrition.

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