



Improving the Nutritional Value and Rumen Fermentation Profile of Oil Palm Fronds by Autoclaving and Ammoniation with Different Levels of Urea

SAITUL FAKHRI^{1*}, HENI SURYANI², TEJA KASWARI¹, MUNAWWAROH PANE¹, NENENG HARIYATI¹, DODI ROLIS LIMBONG¹, ANURAGA JAYANEGARA³, DANIEL KOMWIHANGILO⁴

¹Department of Nutrition and Feed Science, Faculty of Animal Husbandry, Jambi University, Jambi, Indonesia;

²Department of Animal Science, Feed Technology Study Program, Politeknik Negeri Lampung, Lampung, Indonesia;

³Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia;

⁴Tanzania Livestock Research Institute (TALIRI), Dodoma, Tanzania.

Abstract | This study was conducted to determine the optimal urea levels for ammoniated steamed oil palm fronds (OPF). Four treatments were evaluated, i.e., T1: ammoniated steamed OPF (control); T2, T1 + 1% urea; T3, T1 + 3% urea; and T4, T1 + 5% urea. Fresh OPF was steamed in an autoclave at 121°C and 1.4 atm pressure for 30 min. Steamed OPF was ammoniated with 1, 3, and 5% urea according to the treatments. After 10 days, the samples were dried, ground to a size of 1 mm, and analyzed for proximate parameters and fiber fractions. One gram of the sample from each treatment group was incubated with buffered rumen fluid at 39°C for 72 h. Gas production was measured after incubation for 2, 4, 6, 8, 12, 16, 24, 36, 48, 62, and 72 h. pH, NH₃, organic matter degradation (OMD) and microbial yield (MBO) were measured at the end of the incubation period. The gas data were fitted using an exponential model. Results revealed that the steam decreased ($P < 0.05$) CP, CF, NDF, and ADF and increased ($P < 0.05$) NFE but did not affect the hemicellulose content of OPF. The urea level in ammoniated steamed OPF resulted in a quadratic decrease in the NDF and ADF contents of OPF, while hemicellulose increased quadratically. A quadratic relationship was observed between the urea levels and either nutrient degradation or gas production in the rumen. Increasing the urea level in steamed OPF resulted in a linear increase in rumen NH₃, MBO, and MN. The rate and total gas production in T3 were the highest, whereas those in T4 were the lowest. T1 and T2 had comparable rates and total gas production for a 72h incubation period. It was concluded that the optimal level of urea used in ammoniated steamed OPF was approximately 3.2 - 3.6%.

Keywords | Oil palm fronds, Physicochemical, Rumen, Degradation, Microbial biomass

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***Correspondence** | Saitul Fakhri, Department of Nutrition and Feed Science, Faculty of Animal Husbandry, Jambi University, Jambi, Indonesia; **Email:** sfakhri@unja.ac.id

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The extensive development of palm oil plantations results in copious amounts of waste, including oil palm fronds (OPF), which could serve as an alternative to conventional fiber sources for ruminants. The oil palm tree generates between 40 and 50 fronds every year in Indonesia (Biyatmoko, 2013), with an average frond biomass production of 6.3 tonnes per hectare (Laksono and Ibrahim, 2020). Among the biomass produced by the oil palm industry, OPF accounts for more than half of the total (Ooi *et al.*, 2017). The quantity of OPF generated is not proportional to its nutritional value. Its crude protein (CP) content is low at approximately 2.23% (Suryani *et al.*, 2015), while its neutral detergent fibre (NDF) content is high at 76.09% (Suryani *et al.*, 2015), 74.7% (Harahap *et al.*, 2018), and 69.5–78.05% (Saminathan *et al.*, 2022a). Another limitation is the presence of lignocellulosic bonds, which hinder the digestibility of rumen microbes. Previous studies have shown that ester bonds connecting lignin, hemicellulose, and cellulose impede the degradation of OPF (Rasli *et al.*, 2017), leading to low degradation in the rumen (21.3%) (Bengaly *et al.*, 2004). Therefore, pretreatment is necessary before using OPF as ruminant feed.

Different pretreatments have been applied to OPF, including physical, chemical, biological, and, most recently, a combination of physicochemical and physico-biological treatments has gained popularity. The most effective physical treatments applied recently are steam using an autoclave and fiber cracking technology (FCT), both of which apply high pressure and temperature. This treatment hydrolyzes glycosidic bonds, reduces cellulose crystallinity, and expands the surface area of OPF (Jayanegara *et al.*, 2017). The application of either FCT (Harahap *et al.*, 2018) or autoclave treatment (Lestari *et al.*, 2023) resulted in a reduction in the proportion of fibers in OPF. Nevertheless, OM degradation and fermentation in the rumen did not increase. This is likely due to the fact that high-temperature physical treatments (FCT and autoclaving) generate Maillard products with low CP digestibility, as indicated by an increase in the nitrogen (N) content of acid-detergent fiber (ADIN) (Van Soest and Mason, 1991). Based on this, researchers have added urea to OPF before autoclaving, which also could not increase degradation and fermentation in the rumen (Lestari *et al.*, 2023). Application of 3% urea followed by autoclaving also failed to enhance the *in vitro* dry matter degradability (DMD) and organic matter degradability (OMD) of rice straw (Muthia *et al.*, 2021). This was largely due to the presence of urea, which has the potential to stimulate the Maillard reaction, as previously reported by Chen *et al.* (2000). Upon heating, urea releases ammonia, which competes with hydrogen sulfide to react with the Maillard reaction precursors, resulting in the production of nitrogen-containing compounds such as alkylpyrazines.

Mendowski *et al.* (2019) stated that extrusion temperatures of 140°C and 160°C appeared to protect dietary proteins from ruminal degradability; however, proteins seemed to be overprotected at 160°C. To circumvent the Maillard reaction, urea was introduced following autoclaving of the OPF. The question is how much urea should be added to provide sufficient ammonia for rumen microbial development without the toxic effects of ammonia in the rumen.

Saminathan *et al.* (2022b) treated OPF with 1–5% urea, resulting in a reduction in dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), and acid detergent lignin (ADL) content compared to the control. However, the CP content of treated OPF exhibited a quadratic increase with increasing levels of urea (1–5%). Additionally, Saminathan *et al.* (2022b) observed that the inclusion of 4 and 5% urea in OPF resulted in a higher *in vitro* VFA and ammonia-N production, as well as DM and OM degradability in goats, compared to the 1–3% urea levels. In a separate study, (Noersidiq *et al.*, 2020) demonstrated that the application of urea at concentrations of up to 6% on oil palm trunks could enhance the digestibility of dry matter (DM) and crude protein (CP) by 4.5 and 7.3 units, respectively. However, utilisation of urea in excess of 6% led to a reduction in nutrient digestibility, attributed to elevated rumen pH levels exceeding 7, even when urea was employed at a dosage of only 4%.

The provision of 1% urea from the evaluated substrate is analogous to the administration of 10 mg urea (calculated as 1% of the 1 g feed sample utilized in the *in vitro* experiment), which has the potential to yield 10 mg $\times$ 0.5667 $\text{NH}_3\text{-N}$ ammonia (equivalent to 5.667 mg ammonia). If the *in vitro* experiment employed 60 ml of an anaerobic solution, then the vessel bottle contained 5,667 mg ammonia per 60 ml, which is equivalent to 9.445 mg $\text{N-NH}_3/\text{dL}$ of anaerobic medium. The utilisation of 5% urea is equivalent to $(50 \text{ mg} \times 0.5667)/0.60\text{dL}$, which equates to 47.2 mg $\text{N-NH}_3/\text{dL}$ rumen fluid. Therefore, 1% urea is insufficient to provide the requisite ammonia for optimal microbial protein synthesis and NDF degradation in the rumen, which is 17.76–19.11 mg NH_3/dL rumen fluid (Neto *et al.*, 2019). Conversely, administration of 5% urea, which has the potential to provide $\text{NH}_3\text{-N}$ in excess of the requirements of rumen microbes, may result in an oversupply of ammonia within the rumen. If the excess is not fully absorbed by cattle through the villi of the rumen wall, it may prove toxic to rumen microbes (Patra, 2015). Furthermore, excess urea can be toxic to the host animal and may even result in environmental pollution if excreted in large quantities in the urine. However, if the supply of urea is balanced by the availability of energy sources, it is possible that excess ammonia can be used for rumen microbial synthesis. Several studies have demonstrated that the supplementation of ruminal fermentable carbohydrates results in a reduction in

NH₃ levels within the rumen, which can be attributed to an enhanced uptake of NH₃ for microbial protein synthesis (Hristov *et al.*, 2019). Autoclaving (steaming) is a technique that can be employed to provide sufficient nutrients that can be converted to energy in the rumen to meet the needs of rumen microbes. The objective of this study was to determine the optimal urea concentration for enhancing rumen nutrient degradation, fermentation, and microbial biomass yield in ammoniated steamed fresh OPF.

MATERIALS AND METHODS

SAMPLE PREPARATION AND TREATMENTS

Oil palm front (OPF) samples were collected from the PT. Perkebunan VI, Batanghari, Jambi Province. Fresh OPF was chopped into 2–3 cm lengths. Freshly chopped OPF was steamed in an autoclave at 121 °C and 1.4 atm pressure for 30 min. The steamed freshly chopped OPF was subjected to the following experimental treatments:

- T1: Ammoniation steamed OPF w/o urea
- T2: T1 + 1% urea
- T3: T1 + 3% urea
- T4: T1 + 5% urea

Subsequently, the steamed fresh OPF was subjected to DM analysis after autoclaving. Five plastic bottles with a capacity of approximately ±500 g were used as replicates for each treatment. For each replicate, 500 g of steamed fresh OPF with a dry matter (DM) content of 48.7% was prepared. The source of ammonia was urea fertiliser produced by PT PUSRI, with a nitrogen content of 48% and a water content of 0.5%. Granular urea was pulverised to a particle size of 1 mm using a mortar and pestle. The ground urea was then weighed according to the treatment (dry matter basis) and mixed thoroughly with steamed fresh OPF, which was then ammoniated in an airtight plastic bottle and stored at room temperature for 10 d. The ammoniated steamed fresh OPF was dried and ground using a hammer mill with a sieve size of 1 mm.

CHEMICAL COMPOSITION ANALYSIS

Samples from each treatment were analysed for DM, ash, CP, Ether extract (EE), and CF according to (AOAC, 2019) and for NDF and ADF (Van Soest *et al.*, 1991). Hemicellulose content was estimated by subtracting the ADF from the NDF. The chemical composition was determined in duplicate.

IN VITRO INCUBATION

Samples from each treatment were accurately weighed (1.0 g ± 0.0010 fresh weight in total) directly into a 120 ml serum bottle. Duplicate bottles for each treatment were prepared and incubated for varying periods that were terminated at 72h; one for true organic matter degradability (TOMD)

and the other for apparent OMD (AOMD). In addition, one blank was prepared for each parameter, resulting in a total of 12 incubation bottles for each run. This procedure was repeated on three separate occasions. Samples were incubated in 60 ml buffered rumen fluid, as described by (Mauricio *et al.*, 1999) at 39 °C for 72h. Rumen inoculum (from fluid and solid particles) was obtained from the fistulated Bali cattle. The diet fed to the Bali cattle was solely field grass. The Bali cattle were fed twice daily at 08:00 and 15:00 and had free access to feed and water. Ruminal contents were collected from the donor cattle through the rumen cannula before the morning feeding and then poured into a sterile bottle (1,000 mL), leaving no headspace in the bottle, which was taken to the laboratory within 15 min. The rumen liquor was squeezed through four layers of cheesecloth into a flask under a continuous flux of CO₂ in a water bath kept at 39°C until use. Gas production was measured at 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 60 and 72h post incubation. At the end of each incubation period, fermentation was stopped by the addition of 1.5 ml 1 M orthophosphoric acid. The residues were recovered for apparent and true OMD determination, and both were used to calculate MBO (Blümmel *et al.*, 1997b) and microbial nitrogen (MN) = MBO*0.09 (Demeyer, 1991). The cumulative gas volume (three duplicate runs) was fitted to the exponential equation $y = a + b(1 - e^{-ct})$ (Ørskov and McDonald, 1979).

STATISTICAL ANALYSIS

This experiment was conducted based on a completely randomised design (CRD), with four treatments and five replicates. Data were subjected to analysis of variance (ANOVA), and data showing significance between treatment means ($P \leq 0.05$) were separated using orthogonal polynomial contrasts (SAS, 2012). The contrasts used were linear (L) or quadratic (Q) or deviated from both L and Q (Dev). Statistical significance was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

CHEMICAL COMPOSITION

The results showed that the steaming process reduced the CF but increased the CP and NFE of OPF (Table 1). Increasing the urea level in the ammoniation process slightly decreased CF and NFE but increased CP. Fresh OPF had a higher CF content than OPF after fiber steam processing.

The steam process significantly decreased the NDF and ADF contents of OPF ($P < 0.01$) but had no significant effect on hemicellulose (Table 2). Increasing the urea level in the ammoniation process resulted in quadratic ($P < 0.05$) changes in the NDF and ADF content of steamed OPF (Figure 1). The addition of urea to steamed OPF is expected to hydrolyse the complex carbohydrate bonds into simple carbohydrates. This, in turn, resulted in an increase in NFE and hemicellulose.

Table 1: Nutrients contents (%) of fresh, steamed and ammoniated steamed OPF.

Items/ treatments	DM	Ash	CP	EE	CF	NFE
	% DM					
Fresh OPF	40.79	8.63	3.93	3.44	45.20	38.80
T1	42.46	8.45	6.50	3.46	35.42	46.17
T2	42.42	7.83	9.30	3.34	34.55	44.98
T3	42.21	7.63	10.60	3.35	33.88	44.54
T4	41.32	8.16	12.80	3.38	33.05	42.61
S.E.M.	0.238	0.162	1.177	0.024	0.450	0.662
P value	ns	ns	Linier (P<0.05)	ns	Linier (P<0.05)	Linier (P<0.05)

T1: ammoniated-steamed OPF without urea; **T2:** T1 + 1% urea; **T3:** T1 + 3% urea; **T4:** T1 + 5% urea; **OPF:** oil palm fronds; **DM:** dry matter; **CP:** crude protein; **EE:** ether extract; **CF:** crude fiber; **NFE:** nitrogen-free extract.

Table 2: Cell wall contents of fresh OPF, steamed OPF and ammoniated steamed OPF with varies level of urea (% DM).

Items	NDF	ADF	Hemicellulose
Fresh OPF	74.71	58.32	16.39
T1	63.70	47.36	16.34
T2	65.82	51.63	14.19
T3	64.13	47.44	16.69
T4	60.98	42.25	18.73
S.E.M.	0.61	0.69	1.28
P value	quadratic (P<0.05)	quadratic (P<0.05)	ns

T1: ammoniated-steamed OPF without urea; **T2:** T1 + 1% urea; **T3:** T1 + 3% urea; **T4:** T1 + 5% urea; **ADF:** acid detergent fiber; **NDF:** neutral detergent fiber; **OPF:** oil palm fronds.

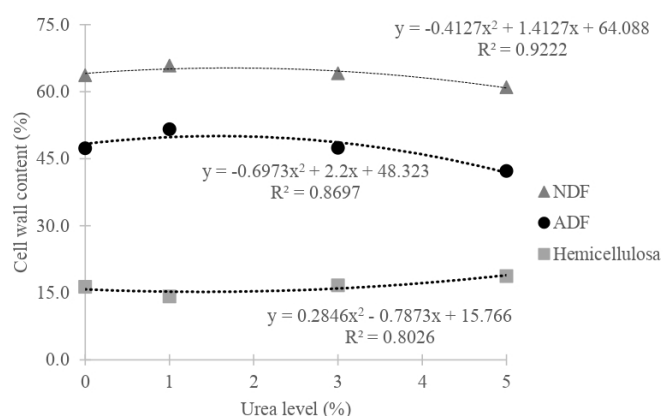


Figure 1: Cell wall compositions of ammoniated-steamed OPF with varies of urea (% DM).

The application of steam treatment utilizing an autoclave in conjunction with the addition of urea resulted in a reduction in the NDF and ADF contents of OPF, as evidenced by the findings presented in Table 2. Similar

results were obtained by Suyitman et al. (2018), who found that steam treatment of OPF reduced the NDF and ADF by 7.58% and 3.8%, respectively. The modification of fibers through extrusion and steam pressure can alter the fiber and nutritional properties of pineapple peels (Ishak et al., 2021). Steam treatment of flex seeds can reduce the crude fiber content (Damayanti and Sjoftan, 2022). The process by which urea increases crude protein and lowers crude fibre during ammoniation is dependent on the conversion of urea to ammonia, which then reacts with the feed material. The addition of urea to high-moisture feed results in hydrolysis, whereby urea is transformed into ammonia (NH₃) and carbon dioxide (CO₂). The ammoniation process, facilitated by the addition of urea, results in the hydrolysis of urea to ammonia, which increases the crude protein content of the feed (Sousa-Alves et al., 2020). Furthermore, the ammonia released during the ammoniation process also helps to break down lignocellulosic bonds within the feed fibre structure. This process softens the feed structure, making it more digestible and reducing the crude fibre content. The reduction in fibre content is attributed to the partial solubilisation of hemicellulose and the alteration of lignin, which are major components of the fibre fraction (Fariani et al., 2021); Sousa-Alves et al., 2020).

DEGRADATION AND MICROBIAL YIELD

The results of the analysis of variance demonstrated that the urea level in ammoniated steam OPF had a significant effect ($P < 0.05$) on DMD, AOMD, TOMD, MBO, and MN in the rumen (Table 3). The orthogonal polynomial test yielded a quadratic relationship ($P < 0.05$) between the urea level (X , %) and DMD (Y , %) using the equation $y = -0.3751x^2 + 2.547x + 29.357$ and an R^2 value of 0.866. A similar trend was observed between urea level and AOMD, with a quadratic equation ($P < 0.01$) as follows: $y = -0.3409x^2 + 2.0976x + 39.811$, $R^2 = 0.9429$ (Figure 2).

Table 3: DMD, AOMD, TOMD, MBO and MN of ensiled steamed OPF with various level of urea.

Parameters	Treatments				SEM	Contrast		
	T1	T2	T3	T4		L	Q	Dev.
DMD (mg g ⁻¹)	298.6	305.8	342.5	325.3	6.79	ns	*	ns
AOMD (mg g ⁻¹)	395.8	419.9	427.5	418.6	3.35	ns	**	ns
TOMD (mg g ⁻¹)	456.4	481.1	492.5	489.1	3.03	ns	*	ns
MBO (mg g ⁻¹)	60.6	61.2	65.0	70.5	2.28	ns	*	ns
MN (mg g ⁻¹)	5.45	5.51	5.85	6.34	0.20	ns	*	ns

T1: Ammoniated-steamed OPF without urea; **T2:** T1 + 1% urea; **T3:** T1 + 3% urea; **T4:** T1 + 5% urea; **OPF:** oil palm fronds; **DMD:** dry matter degradability; **AOMD:** apparent organic matter degradability; **TOMD:** true organic matter degradability; **MBO:** microbial biomass; **MN:** microbial nitrogen; * ($P < 0.05$); ** ($P < 0.01$).

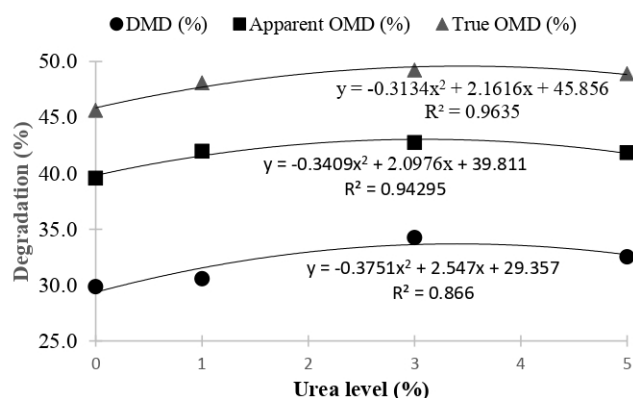


Figure 2: Relationship between urea level in ammoniated-steamed OPF and rumen degradation at 72 h post-incubation.

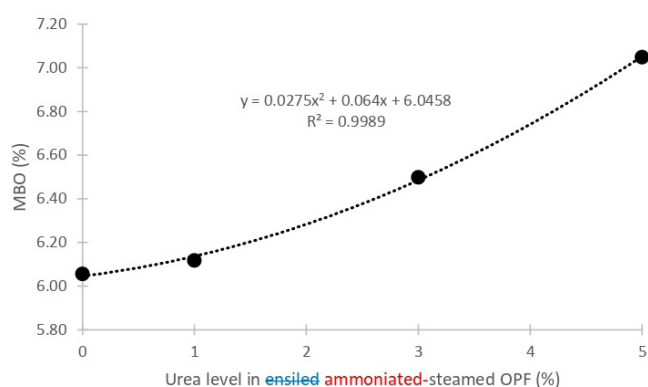


Figure 3: Relationship between urea level in ammoniated-steamed OPF and MBO at 72 h post-incubation.

Increasing the urea concentration in the ammoniated steamed OPF (X, %) led to a quadratic increase in the microbial biomass MBO (Y, %) in the rumen, as demonstrated by the exponential equation $y = 0.0275x^2 + 0.064x + 6.0458$, with an R-squared value of 0.9989 (Figure 3). This equation indicates that the addition of urea to steamed OPF prior to ammoniation has a non-additive effect on MBO.

The highest dry matter degradation (DMD) of 33.7% was obtained when urea was applied at a level of 3.2 - 3.6%; increased up to 3.2% urea, and then decreased when urea was applied above 3.6%. A similar trend was observed in AOMD, with AOMD increasing in proportion to the urea level until reaching a maximum of 43% at the 2.6-3.6% urea level. AOMD then decreased when urea was applied at concentrations above 3.6%. These findings are similar to those of Khattab et al. (2013) but lower than those reported by Gürsoy et al. (2023).

RUMEN FERMENTATION CHARACTERISTICS

Increasing the urea concentration in the ammoniated steamed OPF increased quadratically ($P < 0.05$) the pool gas (a + b) and total gas production from the fermentation

of fraction B but had no significant effect ($P > 0.05$) on the rate of gas production (Table 4 and Figure 4). The rate and total gas production in T3 were the highest, whereas those in T4 were the lowest. T1 and T2 had comparable rates and total gas production for a 72h incubation period (Figure 5).

Table 4: Rumen fermentation characteristics of oil palm fronds steamed and ammoniated steamed with various level of urea.

Parameters	Treatments				SEM	Contrast		
	T1	T2	T3	T4		L	Q	Dev.
a + b (ml g ⁻¹ OM)	103.6	104.6	108.8	102.5	1.04	ns	*	*
b (ml g ⁻¹ OM)	103.2	101.2	108.6	102.1	1.18	ns	*	*
c (ml h ⁻¹)	0.0475	0.0468	0.0493	0.0468	0.0013	ns	ns	ns
NH ₃ (mM)	10.33	12.83	14.17	16.83	1.112	*	ns	ns
pH	6.52	6.34	6.10	6.22	0.086	ns	ns	ns

T1: ammoniated-steamed OPF without urea; **T2:** T1 + 1% urea; **T3:** T1 + 3% urea; **T4:** T1 + 5% urea; **a:** gas production from the fermentation of the soluble fraction; **b:** gas production from the fermentation of the insoluble fraction, but potentially degraded; **c:** fermentation rate of the fraction; **NH₃:** ammonia; **SEM:** standard error of mean.

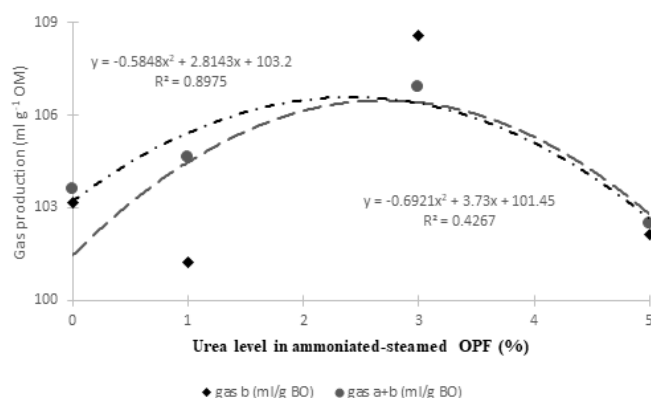


Figure 4: Graphic representation of gas production profile of b and a+b as a function of urea level.

The gas produced in the rumen is the result of enzymatic fermentation of carbohydrates and very little from fat and protein (Getachew et al., 1998). Among these dietary substances, some carbohydrates are soluble and easily fermented, are considered the soluble fraction, and belong to fraction A according to the model of Ørskov and McDonald (1979). This soluble matter consists of simple sugars, which are generally part of the NFE. The NFE content of each treatment was relatively the same (42.61 - 46.17%), so it is natural that the total gas produced from fermentation fraction A was not significantly different.

The orthogonal polynomial test demonstrated that an increase in urea level in ammoniated steamed OPF (X, %) was associated with an enhancement in rumen NH₃ value

(Y), with the equation $Y = 1.871X + 8.4727$ and coefficient of determination (R^2) = 0.7923 (Figure 6). However, the treatment had no significant effect on the rumen pH.

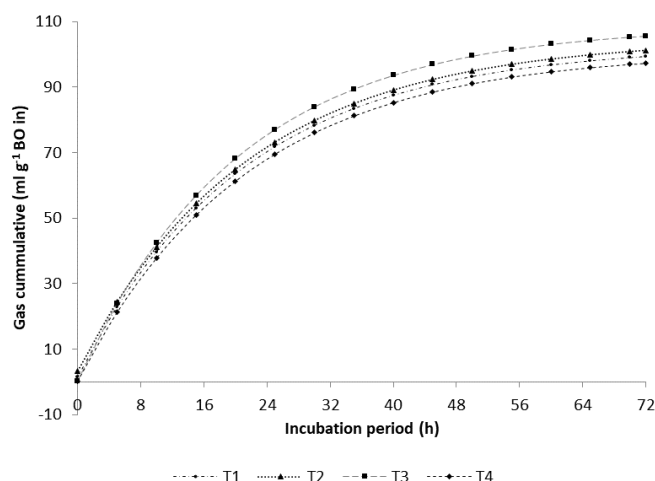


Figure 5: Gas production profile of each treatment during the 72-hour incubation period.

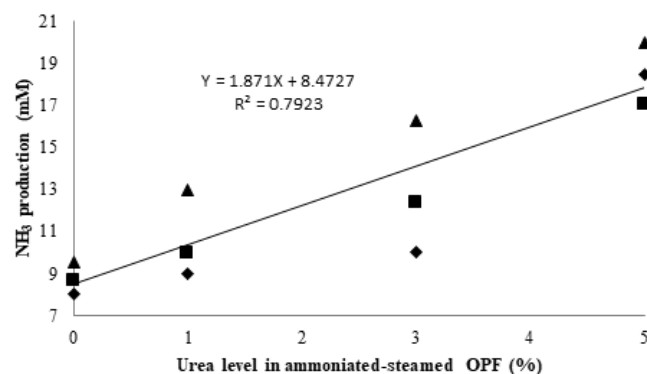


Figure 6: Relationship between urea levels in ammoniated-steam OPF and rumen NH_3 concentration after incubation with buffered rumen fluid for 72h.

The equation in Figure 2 indicates that the highest DMD of 33.7% was obtained when urea was applied at a level of 3.2-3.6%. DMD increased up to a urea level of 3.2-3.6% and then decreased when urea was applied above 3.6%. A similar trend was observed in AOMD, with AOMD increasing as the urea level increased until it reached a peak of 43% at the level of 2.6 - 3.6% urea. AOMD then decreased when the urea level exceeded 3.6%. The observed increase in DMD was likely due to an increase in nitrogen supply, which has been demonstrated to stretch the bonds between lignocellulose and lignocellulose, facilitating the degradation of cellulose and hemicellulose by rumen microbes. This ultimately leads to an increase in the DMD of ammoniated steamed OPF. Urea plays a role in the breakdown of lignin from lignocellulose and lignan compounds into cellulose and hemicellulose, thereby facilitating digestion of fibrous feed by rumen microbes (Jayanegara *et al.*, 2017). However, when the urea level was increased above 3.6%, a decrease

in DMD was observed. These findings are corroborated by the results of Noersidiq *et al.* (2020), who demonstrated that ammoniation of palm trunks using 6% urea increased the digestibility of DM, OM, and CP by 10.8%, 11.5%, and 13.5%, respectively. Utilisation of urea in excess of 6% (8% and 10%) has been observed to result in a reduction in nutrient digestibility attributable to a decline in rumen microbial activity (Shen *et al.*, 2023) with respect to the digestion of feed ingredients, which may be attributed to elevated pH and an acid-base imbalance within the rumen. Previous in vivo studies have also demonstrated that the infusion of urea into the rumen of Dorper crossbred sheep above 1.5% results in a reduction in dry matter intake (DMI) (Wang *et al.*, 2016). It is hypothesised that the addition of urea in excess of the maximum concentration that inhibits the rumen microbiome can lead to a decline in animal performance and, in extreme cases, may result in poisoning (Shen *et al.*, 2023).

Table 3 illustrates the average DMD of ensiled steamed OPF, which ranged from 29.86 to 32.53%. This is comparable to the results of Puastuti *et al.* (2015), who reported an average DMD ranging from 24.56 to 35.72%. The quadratic increase in DMD of ensiled steamed OPF with increasing urea levels is likely due to an increase in the ammonia concentration, which further stimulates rumen microbial activity. Therefore, the higher the urea content in ensiled steamed OPF, the more significant the rumen microbial activity was until it reached 3.2%, after which it decreased when the urea content exceeded 3.6%. This can be attributed to the fact that urea, as non-protein nitrogen (NPN), does not provide sufficient carbohydrates for fermentation and microbial protein synthesis in an in vitro bath culture. The results confirmed that the decrease in OMD at urea levels above 3.6% was due to the higher ammonia concentration in the in vitro batch culture owing to the absence of absorption. This, in turn, led to an increase in MBO at urea levels above 3.6% and peaked at 5% urea, as MBO represents residual OM that is soluble in neutral detergent solutions (Blümmel *et al.*, 1997a). Estimate microbial nitrogen supply (EMNS) per apparently degraded organic matter in rumen (DOMR) range from 0.83 to 2.55 g N/kg DOMR where increasing ratio of concentrate:roughage, the EMNS/DOMR is also enhanced (Hanim *et al.*, 2019).

Ammonia-N is vital for microbial growth. The rumen microbiota requires 5-11 mmol/L ammonia equivalent to 8.5-18.7 mg ammonia per decilitre (dL) of rumen fluid in order to achieve the greatest possible synthesis of microbial protein (Schwab and Broderick, 2017). Neto *et al.* (2019) state the optimal concentration for microbial protein synthesis efficiency was found to be 19.11 mg/dL NH_3 -N in the rumen. The current study found that ammonia concentrations were 10.33, 12.83, 14.17 and 16.83 mM for treat-

ments with 0, 1, 3, and 5% urea levels, respectively.

It is established that the degradation and fermentation profiles within the rumen is contingent upon the microbial activity that occurs within the rumen. Rumen microbes are capable of producing enzymes that facilitate the digestion of crude fibres that are not digestible by the host itself, resulting in the production of short-chain fatty acids (primarily acetate, propionate, and butyrate) (Weimer, 2015). In addition, these microbes synthesise microbial proteins from ammonia, which is derived from the deamination of amino acids and hydrolysis of non-protein nitrogen (NPN) such as urea. This provides primary protein synthesis precursors for the host (Schwab and Broderick, 2017). It is essential to maintain an optimal internal environment within the rumen, including pH and ammonia concentration, to facilitate the efficient degradation of crude fibres and microbial protein synthesis (Russell and Rychlik, 2001). To achieve optimal rumen microbial growth, the rumen microbiota must be provided with a concentration of ammonia within the range of 5–11 mmol/L (Schwab *et al.*, 2005), which is equivalent to 15–33 mg urea/dL rumen liquor.

The gas produced from fermentation of potentially degradable food substances is represented by the B value. As the urea level increased, the gas production from fraction B also increased until the urea level reached 3.5%, after which gas production decreased. This increase may be due to the availability of sufficient energy and nitrogen for rumen microbial growth so that rumen microbes develop quite well. This was in line with the production of microbial biomass, which increased with the level of urea treatment (Figure 3). The decrease in gas production when the urea level was greater than 3.5% may be due to the unsynchronised supply of surplus nitrogen, which was not balanced by the supply of energy obtained from nutrient degradation, particularly carbohydrates (both simple and complex CHO). The inefficient utilisation of surplus nitrogen in relation to the energy derived from nutrient degradation, particularly carbohydrates, may result in metabolic imbalances and inefficiencies. This is due to the closely intertwined nature of nitrogen and energy metabolism, with energy derived from carbohydrates being a crucial factor for the efficient utilisation of nitrogen in the biosynthesis of microbial proteins. The availability of N sources must be synchronised with the availability of energy for efficient microbial growth and optimal microbial protein synthesis. Otherwise, N is wasted in the form of ammonia excretion, ultimately resulting in environmental pollution (Kebreab *et al.*, 2002). The ratio of rumen degradable protein (RDP) to readily available carbohydrates (RAC) influences the degradation, fermentation, and microbial yield in the rumen, with a ratio of 2.30, is the best (Yunilas *et al.*, 2023). The steam treatment in this study broke down the cell wall

of the OPF, which was reflected by the decrease in NDF content and the increase in hemicellulose (Table 2). However, CHO was fermented completely before 65 h of incubation, after which its availability became limited until the 72-hour incubation period. The findings of this study indicate that the utilisation of urea in ensiled steamed OPF at a level exceeding 3.5% resulted in a reduction in microbial activity, thereby limiting the degradation and fermentation of nutrients and ultimately leading to lower total gas production at T4, where a 5% urea level was employed.

The reduction in gas production resulting from utilisation of urea above 3.6% in steamed OPF is indicative of a decline in rumen microbial activity. The low activity of rumen microbes is not related to the concentration of NH₃, as the concentration produced in this study is still within the normal range of 10.33 to 16.83 mM, in accordance with the recommendations set forth by Dewhurst and Newbold (2022). These recommendations state that the normal concentration of ammonia is 6–21 mM. Consequently, the reduced microbial activity observed in the rumen may be attributed to urea, which serves as a source of non-protein nitrogen (NPN) and lacks organic matter in the form of fermentable carbohydrates. This limits the availability of substrates for microbial protein synthesis in rumen. The lower the microbial activity and availability of fermentable organic matter in the rumen, the lower is the total gas production. These results are consistent with those of Zain *et al.* (2024), who reported that rumen microbial fermentation and protein synthesis are based on carbohydrate availability. Gas production is derived from nutrients fermented in the rumen, which, in turn, reflects nutrient degradation.

Figure 6 shows that the higher ammonia concentration in the rumen with a higher urea level in steamed OPF silage is due to the nitrogen (N) content in steamed OPF, which increases with increasing urea level in the treatment. In the rumen, non-protein nitrogen (urea) added to ensiled steamed OPF is hydrolysed and deaminated into ammonia and carbon dioxide by rumen microbes (Zhong *et al.*, 2022), thus increasing ammonia production in the rumen (Muthia *et al.*, 2021). Ammonia is then synthesised into rumen microbial proteins, thereby increasing microbial biomass (Lima *et al.*, 2023). It was evident from this study that increasing urea levels in ensiled steamed OPF quadratically increased the MBO and MN (Table 3). When 3.6% urea was used, the ammonia yield was 14.46 mM. This indicates that the concentration of ammonia produced was high, suggesting that rumen microbes hydrolyse ammonia very quickly. Furthermore, the synthesis of microbial proteins requires ammonia, which is derived from the deamination of amino acids and hydrolysis of non-protein nitrogen (NPN) such as urea. It is the main precursor of protein synthesis in the host (Schwab and Broderick, 2017). Ammonia derived from urea in the rumen is uti-

lised for microbial protein synthesis, whereas feed proteins are degraded into peptides and amino acids by proteolytic enzymes. Microbial proteins and rumen undegraded proteins constitute the primary protein sources for ruminants. In a study conducted by Wang *et al.* (2016), it was observed that the inclusion of 2.5% urea in the total mixed ration resulted in a reduction in dry matter intake (DMI) by approximately 10% compared to a control group that did not receive any urea supplementation.

pH: The pH of the in vitro batch culture decreased until the urea level in ensiled steamed OPF was approximately 3%, after which it increased again with 5% urea. More acidic conditions cause an increase in the population of fibre-digesting microbes in the rumen, leading to a high fermentation process, which is reflected in increased gas production and possibly also increased production of other fermentation products, such as ammonia and VFA. The observed increase in pH when the urea level in ensiled steamed OPF exceeded 3.6% was likely due to the build-up of ammonia as a result of urea breakdown by the rumen microbes. Ammonia is used for microbial protein synthesis in the rumen and excreted through urine when its concentration exceeds the required level (Yanuartono *et al.*, 2018). In this in vitro study, there was no urine production, resulting in ammonia accumulation in the fermenter bottle and a subsequent increase in urea concentration, which elevated the pH value. Ammonia can be absorbed by the rumen mucosa in two ways: by diffusion as ammonia molecules (i.e. NH_3) or via cation channels as ammonium ions (that is, NH_4^+). Their uptake depends on several rumen conditions, especially pH (Rosendahl *et al.*, 2016). At near-neutral pH, this amount can reach considerable levels. Urea is typically non-toxic, whereas ammonia is toxic to all mammals (Patra, 2015). The inability of the liver to convert excessively absorbed ammonia from the rumen into nontoxic urea results in increased ammonia concentrations in the blood, which can lead to ammonia poisoning. High ammonia levels in in vitro batch cultures are toxic to rumen microorganisms, leading to a reduction in the population and processes of degradation and fermentation in the rumen. This was reflected in the decrease in gas production for T4 in this study. This is in line with Shen *et al.* (2023), who demonstrated a robust inverse relationship between free ammonia nitrogen (FAN) and microbial populations (total bacteria, protozoa, fungi, and methanogens) and in vitro rumen fermentation profiles (gas production, dry matter digestibility, total volatile fatty acid, acetate, and propionate). This has led to the development of slow-release urea products, which have not been shown to negatively affect digestibility, rumen parameters, milk production, or livestock performance (Niazifar *et al.*, 2024).

Therefore, it is crucial to determine the optimal level of urea utilisation in OPF pretreatment. The results of this

study indicated that the optimal urea concentration range was 3.2–3.6%, which exhibited the highest rates of nutrient degradation and gas production. It can be reasonably deduced that at urea levels below 3.2%, available ammonia is insufficient for microbial protein synthesis in the rumen. Consequently, microbial biomass production is also low, which in turn has an impact on nutrient degradation and gas production. At urea levels above 3.6%, it is probable that ammonia production exceeds the requirements for microbial synthesis in the rumen, resulting in an increase in the rumen pH. However, in the present study, the pH decreased. This is likely due to the availability of sufficient substrate in the form of hemicellulose resulting from the autoclaving process. The substrate is then degraded and fermentation produces VFA, resulting in a decrease in pH.

Ammonia is a substrate used by a multitude of rumen microbes for the synthesis of microbial proteins. To reduce production costs, NPN (primarily urea) is employed as a substitute for a portion of high-quality protein sources (such as soybean meal) provided to ruminants (Patra, 2015; Schwab and Broderick, 2017). This is due to the fact that the cost of protein derived from urea is less expensive than that derived from feeds. However, Alizadeh *et al.* (2020) recommended the use of slow-release urea instead of urea, as it is more effective in supplying ammonia at an appropriate rate.

CONCLUSIONS AND RECOMMENDATIONS

The addition of urea at a concentration of 1–5% following steamed treatment has been demonstrated to enhance the chemical composition, nutrient degradation, microbial yield, and rumen characteristics of OPF without exerting any deleterious effects in vitro. The findings of this study indicate that the optimal urea level for achieving the aforementioned benefits is within the range of 3.2–3.6%.

Further research is required to evaluate the effects of long-term urea application, as in this study, on the profile of degradation, fermentation, and microbial yield in the rumen. This should employ techniques such as Rusitec, as well as the direct utilisation of livestock.

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The improvement in the quality of low-quality feed, such as OPF, is typically achieved through autoclaving or FCT, which involves the application of high pressure and temperature. However, this approach is not effective because of the potential for Millard reaction, which can result in protein inactivation. This study introduces a novel approach to address this challenge by replacing unavailable proteins with urea. This approach allows the identification of the optimal urea level, which can be used to improve the quality of low-quality feed (OPF).

AUTHOR'S CONTRIBUTIONS

Saitul Fakhri: Designed and conceived the experiments, analyzed the data, and drafted the manuscript.

Munawwaroh Pane, Neneng Hariyati and Dodi Rolis Limbong: Performed the experiments and carried out the laboratory analysis.

Heni Suryani: Supervised the experiments and revised the manuscript.

Teja Kaswari, Anuraga Jayanegara, and Daniel Komwihangilo reviewed and edited the manuscript.

All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

- Alizadeh Z, Yansari AT, Chashnidel Y, Kazemifard M, Azarpajouh S (2020). Effect of soybean meal replacement by slow-release urea on ruminal parameter, blood metabolites, and microbial protein synthesis in *zel ram*. *Acta Scientiarum. Anim. Sci.*, 43(1): e48684. <https://doi.org/10.4025/actascianimsci.v43i1.48684>
- AOAC (2019). Official Methods of Analysis of AOAC INTERNATIONAL. 22nd Edition, AOAC International, Washington DC.
- Bengaly K, Liang JB, Jalan ZA, Ho YW, Ong HK (2004). Optimization of steam treatment as a method to increase in situ degradability of oil palm (*Elaeis guineensis*) frond in Malaysia. *Livestock Res. Rural Dev.*, 16(3): 45–53.
- Biyatmoko D (2013). Respons peningkatan nutrisi pelepah sawit fermentasi yang diinokulasi dengan inokulum yang berbeda. *Jurnal Zira'ah*, 36(1): 20–24.
- Blümmel M, Makkar HPS, Becker K (1997a). In vitro gas production: A technique revisited. *J. Anim. Physiol. Anim. Nutr.*, 77(1): 24–34. <https://doi.org/10.1111/j.1439-0396.1997.tb00734.x>
- Blümmel M, Steingäß H, Becker K (1997b). The relationship between in vitro gas production, in vitro microbial biomass yield and 15 N incorporation and its implications for the prediction of voluntary feed intake of roughages. *Brit. J. Nutr.*, 77(6):911–921. <https://doi.org/10.1079/BJN19970089>

- Chen Y, Xing J, Chin CK, Ho CT (2000). Effect of urea on volatile generation from Maillard reaction of cysteine and ribose. *J. Agric. Food Chem.*, 48(8):3512–3516. <https://doi.org/10.1021/jf991076l>
- Damayanti A, Sjoefan O (2022). Effect of Steaming on Nutrients Content of Flaxseed as a Feed Ingredients. *Indonesian J. Appl. Res. (IJAR)*, 3(2): 95–102. <https://doi.org/10.30997/ijar.v3i2.199>
- Demeyer DI (1991). Quantitative aspects of microbial metabolism in the rumen and hindgut. In: Jouany JP (Eds), *Rumen Microb. Metab. Ruminant Digestion*, pp. 217–237. INRA, Paris.
- Dewhurst RJ, Newbold JR (2022). Effect of ammonia concentration on rumen microbial protein production in vitro. *Brit. J. Nutr.*, 127(6): 847–849. <https://doi.org/10.1017/S000711452100458X>
- Fariani A, Muslim G, Pratama ANT, Warly L (2021). Evaluation of the digestibility of various types of swamp grass in south sumatra on pampangan local buffalo using the in sacco method. *American J. Anim. Vet. Sci.*, 16(3): 152–160. <https://doi.org/10.3844/ajavsp.2021.152.160>
- Getachew G, Blümmel M, Makkar HPS, Becker K (1998). In vitro gas measuring techniques for assessment of nutritional quality of feeds: A review. *Anim. Feed Sci. Technol.*, 72(3–4): 261–281. [https://doi.org/10.1016/S0377-8401\(97\)00189-2](https://doi.org/10.1016/S0377-8401(97)00189-2)
- Gürsoy E, Sezmiş G, Kaya A (2023). Effect of urea and molasses supplementation on in vitro digestibility, feed quality of mixed forage silages. *Czech J. Anim. Sci.*, 68(6): 266–276. <https://doi.org/10.17221/212/2022-CJAS>
- Hanim C, Yusiati LM, Muhlisin (2019). Evaluation of rumen microbial nitrogen supply in Batur sheep fed different ratio of concentrate-forage diets. *IOP Conference Series: Earth and Environmental Science. Inst. Phys. Publishing*, p. 012104. <https://doi.org/10.1088/1755-1315/387/1/012104>
- Harahap RP, Jayanegara A, Nahrowi, Fakhri S (2018). Evaluation of oil palm fronds using fiber cracking technology combined with *Indigofera* sp. in ruminant ration by Rusitec. *AIP Conf. Proc.* pp. 0500081–0500086. <https://doi.org/10.1063/1.5062758>
- Hristov AN, Bannink A, Crompton LA, Huhtanen P, Kreuzer M, McGee M, Nozière P, Reynolds CK, Bayat AR, Yáñez-Ruiz DR, Dijkstra J, Kebreab E, Schwarm A, Shingfield KJ, Yu Z (2019). *Invited review: Nitrogen in ruminant nutrition: A review of measurement techniques*. *J. Dairy Sci.* 102(7): 5811–5852. <https://doi.org/10.3168/jds.2018-15829>
- Ishak Z, Bakri NFM, Maarof S, Hassan H, Razali R, Hashim MF (2021). Effect of extrusion and steam pressure on fiber and nutritional properties of pineapple peels. *Asian Food Sci. J.*, 20(8):96–105. <https://doi.org/10.9734/afsj/2021/v20i830334>
- Jayanegara A, Ayinda RSK, Laconi EB (2017). Urea treatment of rice straw at elevated temperature and pressure: Effects on fiber content, rumen fermentation and digestibility. *J. Indones. Trop. Anim. Agric.*, 42(2): 81–87. <https://doi.org/10.14710/jitaa.42.2.81-87>
- Kebreab E, France J, Mills JAN, Allison R, Dijkstra J (2002). A dynamic model of N metabolism in the lactating dairy cow and an assessment of impact of N excretion on the environment. *J. Anim. Sci.*, 80(1): 248–259. <https://doi.org/10.2527/2002.801248x>
- Khattab IM, Salem AZM, Abdel-Wahed AM, Kewan KZ (2013). Effects of urea supplementation on nutrient digestibility, nitrogen utilisation and rumen fermentation in sheep fed

- diets containing dates. *Livestock Sci.*, 155(2–3): 223–229. <https://doi.org/10.1016/j.livsci.2013.05.024>
- Laksono J, Ibrahim W (2020). Pengaruh metode pengolahan dan waktu pemerahan terhadap kualitas nutrisi pelepah sawit sebagai bahan pakan ternak kerbau rawa (*Buffelus asiaticus*). *J. Ilmiah Pet. Terpadu*, 8(1): 27–32. <https://doi.org/10.1017/CBO9781107415324.004>
- Lestari WM, Kaswari T, Fakhri S, Suryani H, Hutabarat ALR (2023). Pengaruh penambahan urea dan perlakuan autoclaf terhadap degradasi nutrient pelepah sawit. *J. Pet. Indones.*, 25(3): 265–277. <https://doi.org/10.25077/jpi.25.3.265-277.2023>
- Lima J, Ingabire W, Roehe R, Dewhurst RJ (2023). Estimating Microbial Protein Synthesis in the Rumen—Can ‘Omics’ Methods Provide New Insights into a Long-Standing Question? *Vet. Sci.* 10(12): 679. <https://doi.org/10.3390/vetsci10120679>
- Mauricio RM, Mould FL, Dhanoa MS, Owen E, Channa KS, Theodorou MK (1999). A semi-automated in vitro gas production technique for ruminant feedstuff evaluation. *Anim. Feed Sci. Technol.*, 79(4): 321–330. [https://doi.org/10.1016/S0377-8401\(99\)00033-4](https://doi.org/10.1016/S0377-8401(99)00033-4)
- Mendowski S, Chapoutot P, Chesneau G, Ferlay A, Enjalbert F, Cantalapiedra-Hijar G, Germain A, Nozière P (2019). Effects of replacing soybean meal with raw or extruded blends containing faba bean or lupin seeds on nitrogen metabolism and performance of dairy cows. *J. Dairy Sci.*, 102(6): 5130–5147. <https://doi.org/10.3168/jds.2018-15416>
- Muthia D, Ridla M, Laconi EB, Ridwan R, Fidriyanto R, Abdelbagi M, Harahap RP, Jayanegara A (2021). Effects of Ensiling, Urea Treatment and Autoclaving on Nutritive Value and *In-vitro* Rumen Fermentation of Rice Straw. *Adv. Anim. Vet. Sci.*, 9(5): 655–661. <https://doi.org/10.17582/journal.aavs/2021/9.5.655.661>
- Neto JAS, Oliveira JS, Oliveira CJB, Santos EM, Costa ECB, Saraiva CAS, Pinho RMA (2019). Ammonia levels on *in vitro* degradation of fibrous carbohydrates from buffel grass. *S. Afr. J. Anim. Sci.*, 49(3): 585–597. <https://doi.org/10.4314/sajas.v49i3.19>
- Niazifar M, Besharati M, Jabbar M, Ghazanfar S, Asad M, Palangi V, Eseceli H, Lackner M (2024). Slow-release non-protein nitrogen sources in animal nutrition: A review. *Heliyon*, 10(13): e33752. <https://doi.org/10.1016/j.heliyon.2024.e33752>
- Noersidiq A, Marlida Y, Zain M, Kasim A, Agustin F, Huda N (2020). The effect of urea levels on in-vitro digestibility and rumen fermentation characteristic of ammoniated oil palm trunk. *Int. J. Adv. Sci. Eng. Inf. Technol.*, 10(3):1258–1262. <https://doi.org/10.18517/ijaseit.10.3.11574>
- Ooi ZX, Teoh YP, Kunasundari B, Shuit SH (2017). Oil palm frond as a sustainable and promising biomass source in Malaysia: A review. *Environ. Prog. Sustainable Energy*, 36(6): 1864–1874. <https://doi.org/10.1002/ep.12642>
- Ørskov ER, McDonald I (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.*, 92(2): 499–503. <https://doi.org/10.1017/S0021859600063048>
- Patra AK (2015). Urea/ammonia metabolism in the rumen and toxicity in ruminants. In: Puniya, A, Singh, R., and Kamra, D. (Editors), *Rumen Microbiology: From Evolution to Revolution*. pp. 329–341, Springer, New Delhi. https://doi.org/10.1007/978-81-322-2401-3_22
- Puastuti W, Haryati T, Yulishani D (2015). Kecernaan (*in vitro* dan *in sacco*) pelepah sawit yang diolah dengan penambahan urea dan enzim. *Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner*, 7–11 November 2015, Jakarta. pp. 106–114
- Rasli SRAM, Ahmad I, Lazim AM, Hamzah A (2017). Extraction and characterization of cellulose from agricultural residue - oil palm fronds. *Malaysian J. Analytic. Sci.*, 21(5): 1065–1073. <https://doi.org/10.17576/mjas-2017-2105-08>
- Rosendahl J, Braun HS, Schrapers KT, Martens H, Stumpff F (2016). Evidence for the functional involvement of members of the TRP channel family in the uptake of Na⁺ and NH₄⁺ by the ruminal epithelium. *Pflügers Archiv European J. Physiol.*, 468(8): 1333–1352. <https://doi.org/10.1007/s00424-016-1835-4>
- Russell JB, Rychlik JL (2001). Factors that alter rumen microbial ecology. *Science* 292(5519): 1119–1122. <https://doi.org/10.1126/science.1058830>
- Saminathan M, Mohamed WNW, Noh AMD, Ibrahim NA, Fuat MA, Ramiah SK, Chung ELT, Dian NLHM (2022a). Treated oil palm frond and its utilisation as an improved feedstuff for ruminants - an overview. *J. Oil Palm Res.*, 34(4): 591–607. <https://doi.org/10.21894/jopr.2021.0041>
- Saminathan M, Mohamed WNW, Noh ‘AM, Ibrahim NA, Fuat MA, Ramiah SK, Jusoh S, Dian NLHM (2022b). Effects of urea-treated oil palm frond on nutrient composition and in vitro rumen fermentation using goat rumen fluid. *J. Anim. Physiol. Anim. Nutr.*, 106(6): 1228–1237. <https://doi.org/10.1111/jpn.13670>
- SAS (2012). *Statistical Analysis System User’s Guide*. 9.6 (Version 9.1th ed), SAS. Inst. Inc. Cary. N.C. USA.
- Schwab CG, Broderick GA (2017). A 100-Year Review: Protein and amino acid nutrition in dairy cows. *J. Dairy Sci.*, 100(12): 10094–10112. <https://doi.org/10.3168/jds.2017-13320>
- Schwab CG, Huhtanen P, Hunt CW, Hvelplund T (2005). Nitrogen requirements of cattle. In: Pfeffer E, Hristov AN (Eds). *Nitrogen and Phosphorus Nutrition of Cattle: Reducing the Environmental Impact of Cattle Operations*. pp. 13–70. CABI Publishing, Wallingford. <https://doi.org/10.1079/9780851990132.0013>
- Shen J, Zheng W, Xu Y, Yu Z (2023). The inhibition of high ammonia to in vitro rumen fermentation is pH dependent. *Front. Vet. Sci.*, 10:1163021. <https://doi.org/10.3389/fvets.2023.1163021>
- Sousa-Alves W, Rigueira JPS, Almeida-Moura MM, De-Jesus DLS, Monção FP, Rocha-Júnior VR, Aspiázú I, De-Jesus NG, De-Melo JAR, Da-Silva MFP (2020). Fermentative characteristics and nutritional value of sugarcane silage added with two types of urea. *Revista Colombiana de Ciencias Pecuarias*, 33(3): 182–194. <https://doi.org/10.17533/udea.rccp.v33n3a02>
- Suryani H, Zain M, Ningrat RWS, Jamarun N (2015). Supplementation of Direct Fed Microbial (DFM) on in vitro Fermentability and Degradability of Ammoniated Palm Frond. *Pak. J. Nutr.*, 15(1): 89–94. <https://doi.org/10.3923/pjn.2016.89.94>
- Suyitman, Warly L, Evitayani (2018). In-vitro digestibility of palm leaf waste treated with different processing methods. *Pak. J. Nutr.*, 17(8):368–373. <https://doi.org/10.3923/pjn.2018.368.373>
- Van Soest PJ, Mason VC (1991). The influence of the Maillard reaction upon the nutritive value of fibrous feeds. *Anim. Feed Sci. Technol.*, 32(1–3): 45–53. [https://doi.org/10.1016/0377-8401\(91\)90008-G](https://doi.org/10.1016/0377-8401(91)90008-G)

- Van Soest PJ, Robertson JB, Lewis BA (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci.*, 74(10): 3583–3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Wang B, Ma T, Deng KD, Jiang CG, Diao QY (2016). Effect of urea supplementation on performance and safety in diets of Dorper crossbred sheep. *J. Anim. Physiol. Anim. Nutr.*, 100(5): 902–910. <https://doi.org/10.1111/jpn.12417>
- Weimer PJ (2015). Redundancy, resilience, and host specificity of the ruminal microbiota: Implications for engineering improved ruminal fermentations. *Front. Microbiol.*, 6:296. <https://doi.org/10.3389/fmicb.2015.00296>
- Yanuartono, Nururrozi A, Indarjulianto S, Purnamaningsih H, Rahardjo S (2018). Urea: Benefit on ruminant. *J. Ilmu-ilmu Pet.*, 28(1): 10–34. <https://doi.org/10.21776/ub.jiip.2018.028.01.02>
- Yunilas Y, Zain M, Tanuwiria UH, Syamsu JA (2023). The balance of rumen degradable protein and readily available carbohydrate in sheep rations on in vitro fermentability. *J. Adv. Vet. Anim. Res.*, 10(4): 738–743. <https://doi.org/10.5455/javar.2023.j729>
- Zain M, Tanuwiria UH, Syamsu JA, Yunilas Y, Pazla R, Putri EM, Makmur M, Amanah U, Shafura PO, Bagaskara B (2024). Nutrient digestibility, characteristics of rumen fermentation, and microbial protein synthesis from Pesisir cattle diet containing non-fiber carbohydrate to rumen degradable protein ratio and sulfur supplement. *Vet. World*, 17(3): 672–681. <https://doi.org/10.14202/vetworld.2024.672-681>
- Zhong C, Long R, Stewart GS (2022). The role of rumen epithelial urea transport proteins in urea nitrogen salvage: A review. *Anim. Nutr.*, 9: 304–313. <https://doi.org/10.1016/j.aninu.2022.01.008>