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Effect of microbial growth precursor supplementation on chemical composition and fermentation characteristics of palm stem pith silage

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ABSTRACT. This study investigated the impact of precursor supplementation and incubation time on the quality of palm stem pith silage (nutrient content, *in vitro* digestibility, and rumen fermentability). A factorial complete randomised design was applied, and the first factor included precursor supplements (D1 – empty bunch ash filtrate, D2 – chicken faeces + urea, D3 – effective microorganism + molasses + urea), while the second factor was incubation periods (B1 – 7 days, B2 – 14 days, B3 – 21 days). Each treatment was replicated 3 times, resulting in 27 experimental units. The results revealed significant main effects of precursor supplementation, incubation time and their interaction on various silage parameters. In particular, a clear interaction ($P < 0.01$) was observed between precursor supplementation and duration of incubation, indicating significant synergistic effects on nutrient content (dry matter, organic matter, crude protein, crude fibre, extract ether and nitrogen-free extract), digestibility (organic matter and dry matter) and rumen fermentability (NH_3 concentration). Specifically, the combination of effective microorganism + molasses + urea supplementation, with a 14-day incubation period, emerged as the most effective strategy for enhancing silage quality. These findings highlight the crucial role of considering treatment interaction factors in optimising silage production processes.

Introduction

The process of replanting oil palm plants produces approximately 1.5 tonnes of palm stem pith waste per stem, amounting to 80–195 tonnes per hectare (Bakar et al., 2017). Approximately 73% of this waste consists of palm oil stem pith, which can be used as a source of carbohydrates, as it contains 35.20% cellulose and 17.36% haemicellulose. However, its high crude fibre and lignin content, reaching 36.54% and 17.20%, respectively, limits its use as feed. Therefore, to increase the nutrient content, digestibility and palatability of palm kernel pith, processing such as ensiling is carried out. Silage is an anaerobic feed processing technique that involves the activity of microorganisms (Wang et al., 2019a), which is influenced by cultivation time (Gupta et al., 2010) and substrate availability supporting microbial growth and activity (Febrina et al., 2020; Kim et al., 2021).

The availability of precursor supplementation during the silage process is a strategic factor to ensure its success, as it provides essential energy and protein required for microbial growth and development. Molasses contains water-soluble carbohydrate (WCS) as a source of glucose for microbial growth (Panigrahi et al., 2019). Urea provides nitrogen in the ammoniation process, which facilitate breaking lignocellulose and lignohemicellulose bonds (Lunsin et al., 2018). Ash filtrate of empty oil palm bunches is an alkaline compound similar to urea (Salleh et al., 2018). Effective microorganisms (EM) are a mixture of microbials, including *Lactobacillus*, Actinomycetes fungi, photosynthetic bacteria and yeast (Franczuk et al., 2019). Optimal microbial growth and activity in the silage process, supported with complete precursor supplementation, significantly affect the resulting silage products These impacts include reducing dry matter loss (McDonald et al., 2002), improving lactic acid synthesis, producing silage with pleasant aroma (Eş et al., 2018), lowering the fibre fraction proportion and cellulose crystallinity, as well as improving palatability, digestibility and fermentation products (Febrina et al., 2020). Additionally, precursor supplementation reduces feed costs (Gonçalves et al., 2015), lowers pH, and improves NH_3 concentration and fermentation quality (Amanullah et al., 2014). Digestibility is closely related to feed quality (Zhen et al., 2020) and describes the nutrients that livestock can utilise to meet their needs (Mertens and Grant, 2020). Higher digestibility signifies better feed quality, as more of the feed can be utilised (Zewdie, 2018).

Numerous studies have been carried out to improve the quality of palm stem pith, but the results have not been optimised, as they typically involved only one factor or precursor. For example, the shredding process reduces particle size (Nudri et al., 2020) but also results in lower glucose content. Moreover, the white-rot fungus Trametes versicolor (Singh et al., 2013) and urea ammoniation (Noersidiq et al., 2018b) have been applied to lower lignin content and improve digestibility. Noersidieq et al. (2020) reported that ammoniation of palm stem pith with 6% urea (1 precursor) increased the protein content from 3.22% to 8.60% and crude protein digestibility from 54.19% to 61.52%. This result was further confirmed by Chuchai et al. (2023), who found that the addition of a more complete precursors – namely 5% urea + 3% molasses (2 precursors) – to palm stem pith silage resulted in a higher crude protein content, increasing from 2.09% to 14.65%.

Therefore, efforts to improve the quality of palm stem pith must involve a holistic approach. In the ensiling process, the availability of supplement precursors such as glucose, nitrogen, potassium minerals, photosynthetic bacteria and urease enzymes, combined with the appropriate incubation time, greatly affects microbial growth and population. When all precursor supplements are available at optimal concentrations and applied at the right time, maximum microbial growth can be achieved. Therefore, this study aimed to investigate the interaction between precursor supplementation and incubation time on improving the quality of palm stem pith silage. By assessing the synergistic effects of these variables, our objective was to improve silage production techniques and promote sustainable agricultural practices. Through a comprehensive understanding of the complex dynamics of the process under study, we strived to provide practical insights into the improved utilisation of palm stem pith and increased livestock feed efficiency.

Material and methods

Raw materials preparation

The pith of the palm trunk was shredded into 3–5 cm pieces using a leaf chopper. Empty oil palm bunches were burned to ashes, and 200 g of these ashes was soaked in 1 l of water (w/v) for 24 h. After soaking, the mixture was filtered, resulting in the filtrate (Adli et al., 2022). Chicken faeces were dried to a moisture content of less than 14% and finely ground (Febrina et al., 2022). The supplement precursors used in the present study included urea, molasses and EM, each at 5%, as well as 10% of empty bunch ash filtrate and chicken faeces (Febrina et al., 2020). Molasses serves as a source of glucose for microbial growth, while urea provides nitrogen in the ammoniation process, which helps break lignocellulose and lignohemicellulose bonds. Oil palm empty bunch ash filtrate is an alkaline compound similar to urea, and EM are a mixture of microorganisms containing *Lactobacillus*, Actinomycetes fungi, photosynthetic bacteria, and yeast. The specific EM used contained *Lactobacillus casei*, *Saccharomyces cerevisiae* and *Rhodopseudomonas palustri* (PT Songgolangit Persada, Bali, Indonesia). The pith of the chopped palm trunk was weighed and then supplement precursor was added according to the treatment specifications.

Subsequently, it was placed into a laboratory-scale silo bottle with a capacity of 1.5 kg, which was tightly closed to establish anaerobic conditions. The precursors used included: empty bunch ash filtrate from oil palm plantations in Bukit Gajah Village, Ukui District, Pelalawan Regency, Riau Province, Indonesia; chicken faeces obtained from laying hen farms in Pekanbaru City, Riau Province, Indonesia; urea produced by PT Pupuk Indonesia (Persero) Group; EM produced by PT Songgolangit Persada, Bali, Indonesia, and molasses produced by PT PG Rajawali, Surabaya, Indonesia. Silo bottles were stored at room temperature (29 °C) and incubated according to treatment (7, 14 and 21 days) (Febrina et al., 2022). After the incubation period, the silo bottles were opened, silage products removed and dried (to a moisture content below 14%), finely ground and subsequently analysed for nutrient content, digestibility, and rumen fermentability. The flow of research is presented in Figure 1.

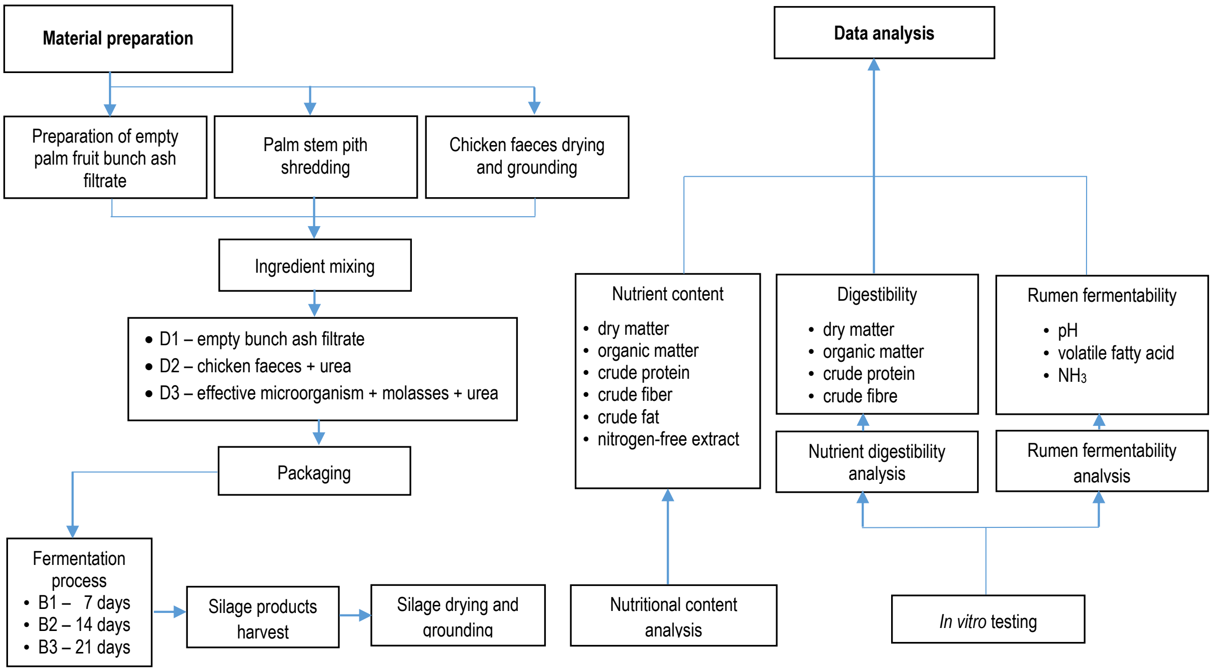

Figure 1. Experimental design

Rumen fermentability test *in vitro*

This experiment followed research ethics guidelines pertaining to livestock, in accordance with the Animal Science and Health regulations outlined in Government Law No. 41/2014 issued by the Republic of Indonesia. The *in vitro* procedure was performed according to the method described by Tilley and Terry (1963). This technique involved fermenting palm stem pith inoculated with various agents using cow rumen fluid. Initially, cow rumen fluid was filtered using sterile filter cloth to separate the supernatant and the precipitate. The supernatant underwent pH analysis along with measurements of NH_3, and volatile fatty acid (VFA) contents. The precipitate was used to analyse the digestibility of dry matter and organic matter. In each fermenter tube containing 500 mg sample, 10 ml of rumen fluid and 40 ml of McDougall solution were added. The fermenter tubes were shaken with flowing CO_2 gas for 30 s (pH 6.5–6.9). The fermentation tube was supplied with CO2 gas, then shaken for 30 s (pH 6.5–6.9) and closed with a vented rubber cap and sealed with a ventilated rubber cap. Subsequently, the tubes were placed in a water bath shaker at 39 °C. Fermentation continued for 4 h to analyse pH, NH_3, and total VFA, and for 48 h to assess dry matter and organic matter digestibility. The fermentation process was terminated by opening the ventilated rubber cap and adding two drops of $HgCl_2$ solution. Digestibility analysis included measurements of dry matter, organic matter, crude protein, and crude fibre.

Experimental design and data analysis

The study used a factorial complete randomised design. The first factor was precursor supplementation, i.e. D1 – empty bunch ash filtrate, D2 – chicken faeces + urea, D3 – EM + molasses + urea. The second factor was the length of incubation, i.e. B1 – 7 days, B2 – 14 days, and B3 – 21 days. Each treatment combination was replicated three times, resulting in a total of 27 experimental units. Detected outliers were removed from the data set when their Z-score was lower than −2 or higher than 2. Statistical analysis was performed using ANOVA at a significance level of 5%. Post-hoc comparisons between different treatments were conducted using Duncan's multiple range test, also at a significance level of 5%. Data analysis was conducted using R statistical software version 3.6.3, employing a combination of descriptive statistics, ANOVA, and post-hoc tests.

Results

The dry matter content in the silage was primarily influenced by incubation time and the interaction between the length of incubation and the addition of precursors, while supplementing the precursors alone did not affect the dry matter content (Figure 2A). Palm stem pith silage incubated for 14 days contained the highest dry matter content (93.97%), which was significantly different from other treatments. Increasing the incubation time from 14 to 21 days reduced the dry matter content. Additionally, the combination of chicken faeces and urea with 7-day incubation period resulted in similarly high dry matter content (94.61%), comparable to treatments D1B2, D2B2 and D3B2.

The addition of precursors alone did not affect the organic matter content of palm stem pith silage. However, incubation time and the interaction between precursor addition and incubation period had an effect on the organic matter content (Figure 2B). Incubation for 14 days resulted in the highest organic matter content (93.21%), which was significantly higher compared to incubation periods of 7 and 21 days. Specifically, the highest organic matter content was recorded for the incubation period of 14 days in combination with EM + molasses + urea (93.62%), but this result was not significantly different from treatment D2B1, D1B2, D2B2, and D1B3.

The duration of incubation, the addition of precursors and the interaction between incubation time and the addition of precursors strongly affected the crude protein content of palm stem pith silage (Figure 2C). The highest crude protein content was observed after 14 days of incubation with the addition of EM + molasses + urea (15.67%), and it was markedly higher compared to other treatments.

The crude fibre content of palm stem pith silage was influenced by the addition of precursors and the interaction between incubation time and the addition of precursors, but not incubation time alone (Figure 2D). The addition of complete precursors (EM + molasses + urea) resulted in the lowest crude fibre content (27.02%), and this result was significantly lower compared to the addition of one precursor (empty bunch ash filtrate) and 2 precursors (chicken faeces + urea). Palm trunk pith silage with EM + molasses + urea, incubated for **7** days contained the lowest crude fibre content (25.77%), but did not differ from treatments D3B2, D1B3, and D2B3.

The duration of incubation did not affect the crude fat content of palm stem pith silage, but the addition of precursors and the interaction between incubation time and precursor supplementation significantly affected the crude fat content (Figure 2E). The addition of three precursors (EM + molasses + urea) resulted in the highest fat content (1.43%), which was not significantly different from silage treated with one precursor (empty bunch ash filtrate), but markedly higher than in the treatments involving two precursors (chicken faeces + urea). Conversely, the lowest crude fat content was observed with a 21-day incubation period in combination with chicken faeces + urea (1.01%), but it did not differ significantly from treatments D2B1, D3B1, D2B2, D3B2, and D1B3.

Incubation time and the addition of precursors did not affect the nitrogen-free extract (NFE) content of palm stem pith silage. However, the interaction between incubation period and the addition of precursors significantly influenced the nitrogen free extract (Figure 2F). The highest content of nitrogen free extract was determined for an incubation period of 21 days in combination with chicken faeces + urea addition (52.51%), but it did not differ from treatments D1B3, D3B1, and D3B2.

The digestibility of dry matter of palm stem pith silage was influenced by the addition of precursors, incubation time, and their interaction (Figure 3A). Pith silage of palm trunks incubated for 14 days was characterised by the highest digestibility of dry matter (62.5%), and this outcome was significantly different from the 7-day incubation period, while showing no significant difference compared to the 21-day incubation. Increasing the incubation time from 14 to 21 days reduced the dry matter digestibility of silage. Palm stem pith silage with the addition of one precursor (empty bunch ash filtrate) showed the lowest dry matter digestibility (59.2%), which was markedly different from other treatments. The addition of three precursors (EM + molasses + urea) resulted in the highest dry matter digestibility (64%). Specifically, combining the addition of these three precursors with a 14-day incubation period with yielded the highest digestibility of dry matter

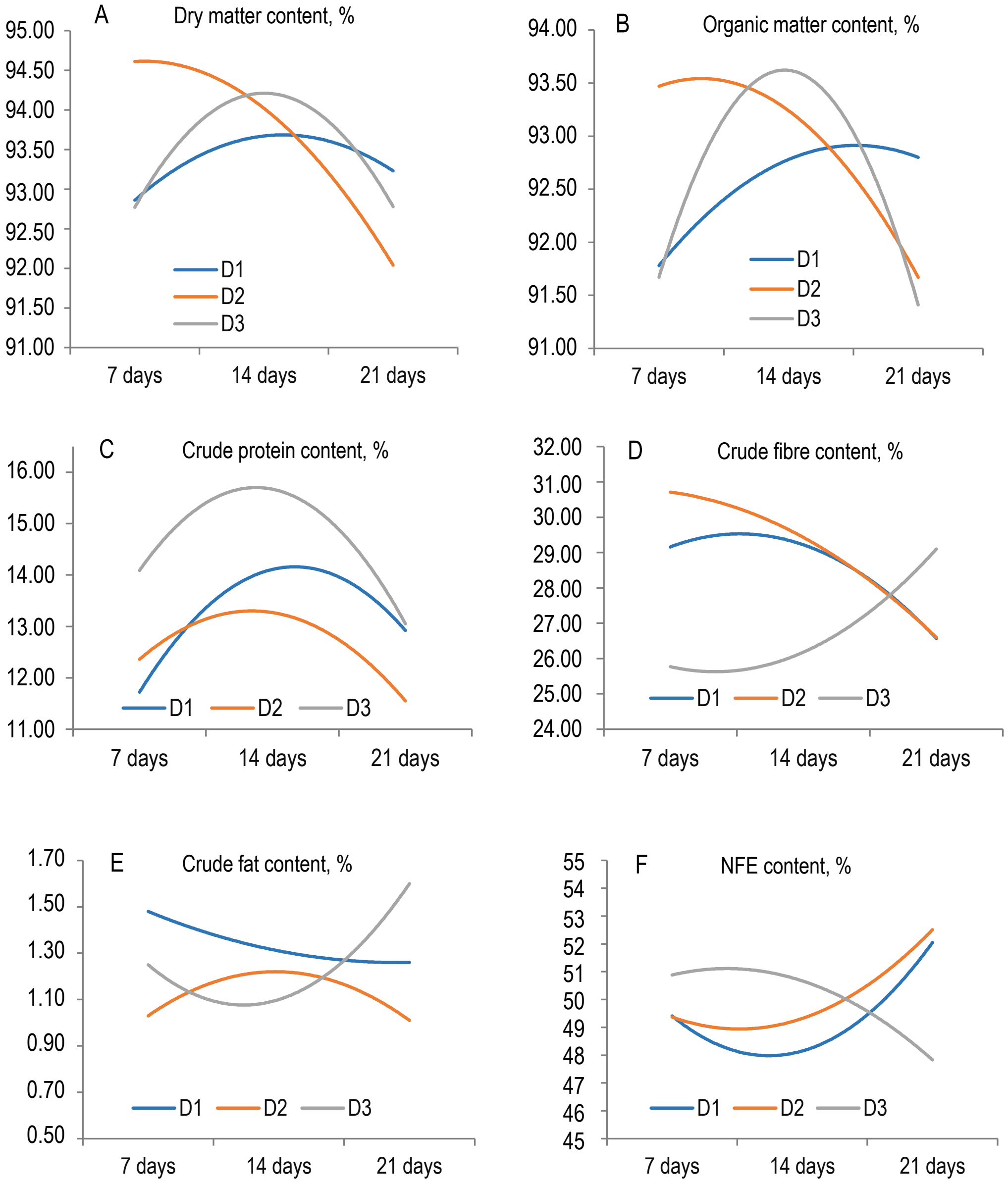

Figure 2. Nutrient content of palm stem pith silage with precursor supplementation and different incubation periods
D1 – empty bunch ash filtrate, D2 – chicken faeces + urea, D3 – effective microorganism + molasses + urea, NFE – nitrogen-free extract

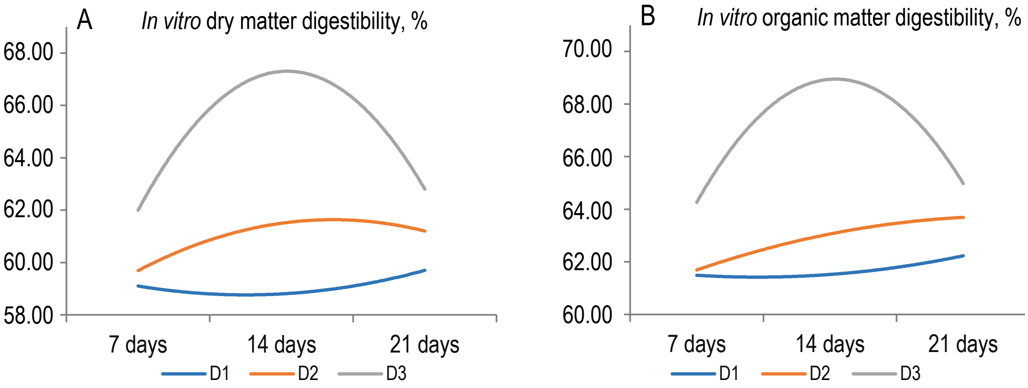

Figure 3. Nutrient digestibility of palm stem pith silage with precursor supplementation and different incubation period
D1 – empty bunch ash filtrate, D2 – chicken faeces + urea, D3 – effective microorganism + molasses + urea

(67.30%), significantly surpassing all other treatments (Figure 3A).

Figure 3B illustrates how the length of incubation, precursor addition and the interaction between incubation time and the addition of precursors affected the digestibility of silage organic matter. The 14-day incubation period resulted in the highest digestibility of organic matter (64.51%), which was significantly higher than the 7-day incubation, showing no significant difference compared to the 21-day treatment. The addition of EM + molasses + urea precursors yielded the highest digestibility of organic matter (66.06%), which was markedly higher compared to the addition of one or two precursors. Pith of palm stems with three precursors (EM + molasses + urea) ensiled for 14 days resulted in the highest digestibility of organic matter (68.94%), and this outcome was significantly different compared to other treatments.

The highest crude protein digestibility was obtained after 14 days of incubation (81.36%), and this result was significantly higher than outcomes obtained for 7- and 21-day incubations. Extending the incubation time from 14 to 21 days markedly decreased the digestibility of crude protein. Moreover, the addition of three precursors to palm trunk pith silage led to the highest crude protein digestibility (81.69%), significantly surpassing the digestibility achieved with the addition of one or two precursors (Table 1).

The digestibility of crude fibre of palm stem pith silage was influenced by the addition of precursors, but not by incubation time or the interaction between these two factors. The addition of all precursors (EM + molasses + urea) resulted in the highest crude fibre digestibility (60.97%), significantly higher than in the case of treatments with one and two precursors (Table 1).

Table 1. Nutritional digestibility of palm stem pith silage with different precursors for microbial growth and incubation times

Parameters	Factor B (incubation time)	Factor D			Average
		D1	D2	D3	
IVCPD	B1	76.47 ± 3.26	79.95 ± 1.72	80.82 ± 3.48	79.08^{a}± 0.96
	B2	80.09 ± 0.68	80.24 ± 0.53	83.74 ± 1.22	81.36^{b} ± 0.36
	B3	79.20 ± 2.46	77.61 ± 1.28	80.51 ± 1.52	79.85^{a} ± 0.39
	Average	78.59^{a}± 1.32	79.27^{a}± 0.60	81.69^{b}± 1.23	
IVCFD	B1	58.74 ± 1.07	57.61 ± 1.84	58.93 ± 1.02	58.43 ± 0.46
	B2	56.35 ± 0.73	59.79 ± 1.68	63.37 ± 2.96	59.84 ± 1.12
	B3	58.34 ± 2.67	59.32 ± 2.21	60.62 ± 0.94	59.43 ± 0.90
	Average	57.81^{a} ± 1.04	58.91^{a} ± 0.27	60.97^{b} ± 1.15	

IVCPD – *in vitro* crude protein digestibility, IVCFD – *in vitro* crude fibre digestibility, B1 – 7 days, B2 – 14 days, B3 – 21 days, D1 – empty bunch ash filtrate, D2 – chicken faeces + urea, |D3 – effective microorganism + molasses + urea; data are presented as mean value ± SEM; ^{ab} – means within a column with different superscripts are significantly different at $P < 0.05$

The concentration of NH_3 in palm stem pith silage was affected by the supplementation of precursors, the duration of incubation and their interaction (Figure 4). The addition of all precursors (EM + molasses + urea) resulted in the highest concentration of NH_3 (15.61 mM) and was significantly higher compared to the addition of a single precursor (9.56 mM) or two precursors (9.56 mM). The 14-day incubation period led to the highest NH_3 level (11.89 mM), which was significantly greater than the 7-day treatment (11.39 mM). Extending the incubation time from 14 to 21 days lowered the NH_3 concentration from 11.89 mM to 11.44 mM.

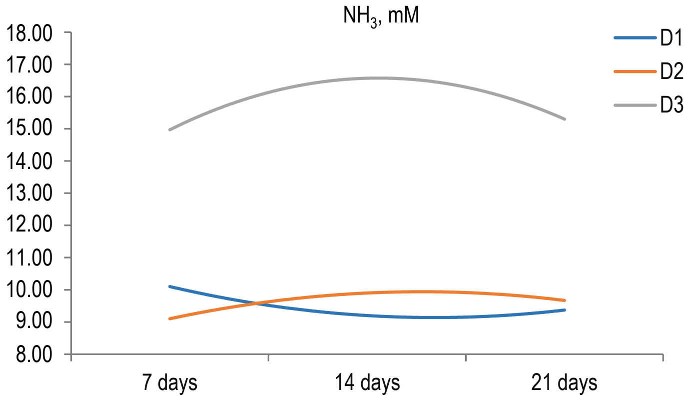

Figure 4. NH_3 silage concentration in palm stem pith silage with microbial growth precursor supplementation and different incubation periods D1 –empty bunch ash filtrate, D2 – chicken faeces + urea, D3 – effective microorganism + molasses + urea

Palm stem pith silage incubated for 14 days with the addition of EM, molasses and urea resulted in an NH_3 concentration of 16.57 mM, significantly exceeding all other treatments.

The total VFA content of palm stem pith silage was not affected by incubation time or the interaction between the length of the treatment and the addition of precursors; however, this parameter was influenced by precursor addition alone. Palm stem pith silage supplemented with complete precursors (EM + molasses + urea) yielded the highest total VFA levels (136.89 mM), and this value was significantly higher compared to the addition of a single precursor (128.67 mM) or two precursors (133.78 mM) (Table 2).

Table 2. Rumen fermentability of palm stem pith silage with different precursors for microbial growth and incubation times

Parameters	Factor B (incubation time)	Factor D D1	 D2	 D3	 Average
VFA, mM	B1	125.00 ± 5.00	133.33 ± 1.53	135.00 ± 5.00	131.11 ± 2.00
	B2	129.33 ± 1.15	132.67 ± 2.52	139.33 ± 1.15	133.78 ± 0.79
	B3	131.67 ± 2.89	135.33 ± 4.16	136.33 ± 1.53	134.44 ± 1.32
	Average	128.67^{a} ± 1.93	133.78^{b} ± 1.33	136.89^{c} ± 2.12	
pH	B1	6.78 ± 0.02	6.80 ± 0.03	6.80 ± 0.03	6.79 ± 0.00
	B2	6.82 ± 0.03	6.76 ± 0.07	6.82 ± 0.05	6.80 ± 0.02
	B3	6.80 ± 0.06	6.80 ± 0.05	6.82 ± 0.04	6.81 ± 0.01
	Average	6.80 ± 0.02	6.79 ± 0.02	6.81 ± 0.01	

VFA – volatile fat acids, B1 – 7 days, B2 – 14 days, B3 – 21 days, D1 – empty bunch ash filtrate, D2 – chicken faeces + urea, D3 – effective microorganism + molasses + urea; data are presented as mean value ± SEM; ^{abc} – means within a column with different superscripts are significantly different at $P < 0.05$

The duration of incubation had no effect on rumen fluid pH value, precursor addition or the interaction between incubation time and precursor supplementation. The rumen pH obtained in this study was in the range of 6.786.82, which was a normal value for rumen microbial growth and development.

Discussion

Nutrient content of palm stem pith silage

The highest dry matter content was determined in treatment D2B1 (94.61%), but the differences compared to treatments D1B2, D2B2, and D3B2 were not statistically significant. The high dry matter content in this treatment was attributed to the availability of precursors that supported microbial growth, such as molasses, a source of dissolved carbohydrates, urea, providing non-protein nitrogen (NPN), and EM, containing photosynthetic bacteria. This study have confirmed that the availability of complete precursors supports microbial growth and activity, thereby increasing dry matter content compared to the addition of only one precursor, such as dissolved carbohydrates (Nishino and Hattori, 2007), urea (Lazarus and Lawa, 2022) or lactic acid bacteria (Jatkauskas and Vrotniakienë, 2009). Ensiling stimulates the growth of lactic acid bacteria and thus increases the dry matter content. Wang et al. (2019b) reported that adding precursors accelerated this process, lowered pH, inhibited the growth of pathogenic microbes, increased dry matter content, and reduced silage moisture.

The highest organic matter content (93.62%) was obtained in treatment D3B2, which involved palm stem pith silage with the addition of three precursors and an incubation duration of 14 days. This result was not significantly different from those obtained in treatments D2B1, D1B2, D2B2, and D1B3. Molasses is a source of easily soluble carbohydrates with a high organic matter content (92.10%); it is utilised by microbes to form lactic acid, thereby increasing the content of organic matter. Microorganisms convert these carbohydrates into organic acids such as lactic acid, acetic, propionic and butyric, affecting the silage organic matter content (Kim et al., 2021). The composition of organic matter correlates with the content of dry matter, as the former constitutes the largest portion of dry matter. Therefore, an increase in dry matter content results in a corresponding elevation in organic matter.

Treatment D2F3, namely palm stem pith silage fermented with three precursors for 14 days, resulted in the highest crude protein content (15.67%). This outcome demonstrated that an optimal incubation time with the availability of complete precursors – molasses as an energy source, urea as an NPN source, and EM as a source of photosynthetic bacteria – stimulated optimal microbial growth and activity. Consequently, these microbes produced proteolytic enzymes that degraded the substrate, leading to the highest crude protein content. Adequate nutrient availability for microbial growth and development increase both the content and digestibility of nutrients (Olafadehan and Adebayo, 2016; Ahmad et al., 2018; Hartati et al., 2022). This study has confirmed that supplementing precursors that provide all necessary nutrients result in higher protein levels acquired. For instance, palm oil frond silage supplemented with one precursor (urea) yielded a crude protein content of 8.60% (Noersidiq et al., 2020), while supplementation with two precursors (chicken faeces + urea) resulted in a protein content of 12.43% (Febrina et al., 2022). The high N content of urea also increases the crude protein content of silage, as it contains 45–46% N (Cantarella et al., 2018), which corresponds to 262–281% crude protein (Mayulu, 2014). Furthermore, the addition of urea has been shown to increase dry matter, crude protein and TDN content (Al-Arif et al., 2017), while incorporating EM was shown to enhance the nutrient profile of fruit waste silage (Mirwandono et al., 2018).

After 14 days, microbial growth passed the lag/adjustment phase and entered the log/exponential phase, characterised by rapid microbial proliferation and increased enzyme production, resulting in the highest crude protein content. This was an improved outcome compared to previous studies, according to which optimal results were obtained after 21 days (Gupta et al., 2010; Noersidiq et al., 2020; Kim et al., 2021). Our findings are consistent with studies of Wang et al. (2019a) and Febrina et al. (2020), who reported that bacteria were in the log phase on day 14, i.e. a period of rapid, exponential growth where both the bacterial population and enzyme production increased significantly. Thus, the present study demonstrates that the availability of comprehensive precursor supplementation (sources of glucose, nitrogen and bacteria) optimises microbial growth and activity, thereby shortening the ensiling process to just 14 days.

Palm stem pith silage treated with three precursors and incubated for 7 days (D3B1) had the lowest crude fibre content (25.77%), but did not differ significantly from treatments D3B2, D1B3, and D2B3. This reduction in crude fibre content was associated with the availability of precursors, particularly molasses, which serves as an energy source stimulating the growth of lactic acid bacteria. This process lowers pH levels, inhibits pathogenic bacteria growth, and minimises nutrient loss during fermentation. Ensiling proceeds aerobically, the ensilage process occurs anaerobically causing the conversion of glucose to lactic acid and the generation of heat, which facilitates the breaking of lignocellulose and lignohemicellulose bonds.

Increasing the number of precursors (three precursors) in palm stem pith silage markedly decreased the crude fibre content (Figure 2D) compared to silage supplemented with one precursor (10% urea), which yielded 43.53% crude fibre (Noersidiq et al., 2020), and silage supplemented with two precursors (chicken faeces + urea), resulting in 37.81% crude fibre (Febrina et al., 2022). This illustrates that optimal microbial activity with complete precursors significantly reduces crude fibre content. Under anaerobic conditions, microbes release cellulolytic enzymes that break down cell wall carbohydrates into cellulose and hemicellulose (Salama and Mariod, 2022).

The inclusion of urea softens the texture of palm stem pith silage. Urea breaks down into ammonia, which loosens the ester bond between lignin and cellulose, expands cellulose fibres, and softens the material (Li et al., 2019). This process facilitates the penetration of cellulase enzymes (Baharin et al., 2018), enabling microbes to utilise cellulose and hemicellulose as energy sources. EM have the ability to degrade crude fibre because they produce lactase and peroxidase enzymes that can depolymerase and dissolve lignin (Zhao et al., 2016; Falade et al., 2017). The release of lignin from cellulose provides additional carbon sources, especially cellulose, fostering microbial growth and enhancing degradation processes. Since lignin and cellulose are components of crude fibre, increased microbial lignin degradation ultimately reduces crude fibre content. For instance, Rahmatullah et al. (2020) reported a decrease in crude fibre content with the addition of EM to banana peel silage.

The lowest crude fat content (1.01%) was observed in treatment D2B3, where palm stem pith silage was supplemented with chicken faeces + urea and incubated for 21 days; however, this result was not statistically different from the treatments D1B3, D2B1, D2B2, D2B3, D3B1, and D3B2. The low crude fat content is associated with high lipase activity facilitated by the addition of precursors resulting in increased fat degradation to fatty acids and glycerol. Urea is a source of non-protein nitrogen (NPN) and promotes microbial protein formation and serves as an energy source for microbes. Consequently, the addition of urea to the silage mixture increases lipase enzyme activity and reduces crude fat content. The present findings demonstrated that incorporating complete precursors to the silage resulted in lower crude fat content (1.01–1.48%) compared to pith silage fermented with a single precursor (urea 2–10%) where the crude fat content ranged from 1.96 to 3.09% (Noersidiq et al., 2020).

Nitrogen free extract (NFE) is a component of organic matter that provides soluble carbohydrates for lactic acid-producing bacteria, thereby lowering pH. The highest NFE content (52.51%) was determined in treatment D2B3, i.e. palm stem pith silage with chicken faeces and urea incubated for 14 days; however, this result was not significantly different from those obtained in treatments D3B2, D3B1, and D1B3. The addition of molasses as a source of soluble carbohydrates provides energy for microbes, thereby increasing lactic acid production, which affects the NFE content.

In vitro digestibility of palm stem pith silage

Improved nutrient quality of palm stem pith silage through precursor supplementation and varying incubation periods (Figure 2) correlated with increased nutrient digestibility (Figure 3 and Table 1). This could be observed in treatment D3B2, involving interaction between 14 days of incubation and three precursors (EM + molasses + urea), and resulting in the significantly highest digestibility of dry matter (67.3%) compared to other treatments. Our result is consistent with prior studies by Ni et al. (2017), Nascimento et al. (2019), and Febrina et al. (2022), who also observed a similar trend where 14-day incubation produced better results in terms of nutrient digestibility than shorter or longer incubation periods.

The high dry matter digestibility in treatment D3B2 is attributed to the supplementation of complete precursors (three precursors), particularly ammonia derived from urea. Ammonia facilitates the breakdown of lignocellulose and lignohemicellulose bonds, making cellulose and hemicellulose available as energy sources for microbes. Urea also serves as a source of protein, which can increase microbial growth, activity and population. Molasses, on the other hand, contains carbohydrates that can be utilised by microorganisms as an energy source. Growing microbial population, stimulated by these precursors, results in increased enzyme production. These enzymes effectively degrade feed components, thereby enhancing the overall digestibility of dry matter (Desta et al., 2021). The higher dry matter digestibility with a 14-day fermentation period compared to 7 days underscores the benefit of extended incubation, allowing microbes to more effectively ferment and degrade feed components. This prolonged period improves nutrient conversion efficiency and results in higher quality fermented products. In addition, during a longer incubation period, there was an increase in the activity of microbial enzymes responsible for feed ingredient degradation, which directly contributes to nutrient digestibility (Pazla et al., 2023). Conversely, increasing the incubation time from 14 to 21 days reduced dry matter digestibility. This decline could be attributed to microbes entering the stationary phase, where growth rate equals the mortality, thereby reducing their overall activity (Barer, 2012). Darwish et al. (2012) reported a similar trend in maize stalk silage, where prolonged incubation decreased both dry matter content and organic matter digestibility.

The present study demonstrates that the availability of complete precursors supporting microbial growth results in higher dry matter digestibility (62.00–67.30%) compared to using only one or two precursors. Noersidiq et al. (2020) reported that the addition of 2–10% urea to ammoniated palm stem pith yielded dry matter digestibility of 41.43–45.91%. Similarly, Hartutik et al. (2020) found that supplementing 6% EM to sugarcane over 0–21 days of incubation resulted in dry matter digestibility of 42.79 –47.07%. This indicates that after 14 days of incubation, there was an increase in lactic acid production, crucial for successful fermentation (Skonberg et al., 2021) . Lactic acid plays an important role in suppressing the growth of pathogenic microorganisms and increasing silage stability during storage. These findings are in line with other studies, e.g. by Ameen and Abid (2019), who reported no significant difference in dry matter digestibility when adding 2 precursors (urea and ruminant faeces) to straw silage (44.06%), or Lunsin et al. (2018), who found a dry matter digestibility of 37.3% with urea + molasses in sugarcane. This illustrates that integrating feed processing technology with comprehensive precursor supplementation optimises the quality and digestibility of palm stem pith silage. Nutrient supplementation supports microbial growth, thereby increasing digestibility (Wahyono et al., 2022).

The digestibility of organic matter follows the pattern of dry matter digestibility (Figure 3A, B). The highest digestibility of organic matter (68.94%) was achieved in treatment D3B2, with three precursors and incubation time of 14 days. This correlation indicates that improvements in dry matter digestibility also lead to higher organic matter digestibility, considering that organic matter constitutes a significant portion of dry matter. Furthermore, the study revealed that the administration of more complete precursors in terms of required nutrients also enhanced the digestibility of organic matter compared to using one or two precursors that did not provide all required nutritional sources. For instance, silage of palm stem pith supplemented with only urea resulted in organic matter digestibility of 41.54–46.33% (Noersidiq et al., 2020), whereas combining urea with ruminant faeces in straw silage achieved 46.82% organic matter digestibility (Ameen and Abid, 2019). The incubation period also affects the digestibility of organic matter, as it is related to the time the microorganisms have been active and able to degrade feed substances. An optimal incubation time provides microbes with the opportunity to decompose all feed substances, which ultimately increases the digestibility of organic matter (Pazla et al., 2021a). Dolci et al. (2011) found that a 14-day incubation period provided the most optimal results compared to other incubation periods tested. At this time, microbial activity and number in the silage reaches its peak, leading to the production of high-quality fermented products.

The administration of more complete precursors, specifically EM + molasses + urea, resulted in the highest crude protein digestibility (81.69%), surpassing that achieved with one precursor (78.59%) or two precursors (79.29%) (Table 2). In line with previous findings, the silage from fermented palm stems supplemented with 1 precursor, urea, exhibited crude protein digestibility ranging from 54.19% to 61.52% (Noersidiq et al., 2020); with the addition of Phanerochaete chrysosporium, it reached 75.91%, and with starbio, it was 78.26% (Noersidiq et al., 2018a). Pazla et al. (2023) reported that higher crude protein content increased its digestibility, which was also confirmed in our study (Figure 2C). Feed digestibility is closely related to its chemical composition (Sripo et al., 2016), and the activity of proteolytic bacteria that produce extracellular protease enzymes, breaking down proteins, and thus increasing crude protein digestibility (Xiao et al., 2022).

Palm stem pith silage supplemented with three precursors (EM + molasses + urea) was characterised by the highest digestibility of crude fibre (60.97%), which was significantly higher compared to the addition of either one (57.81%) or two precursors (58.91%). This enhancement can be attributed to the availability of essential nutrients for microbial growth: molasses as an energy source, urea as a nitrogen source, and EM that provide photosynthetic bacteria. These nutrients support microbial growth and activity, which leads to elevated digestibility of nutrients (Detmann et al., 2014). EM comprise various microorganisms, such as photosynthetic bacteria (Rhodopseudomonas sp.), *Actinomycetes* sp., *Streptomyces* sp., lactic acid bacteria, yeasts, and fungi (*Aspergillus* and *Penicillium*) (Górski and Kleiber, 2010), and their inclusion in palm stem pith accelerates the ensiling process and improves feed digestibility. The high crude fibre digestibility in treatment D3 was also associated with a low crude fibre content of 27.02% (Figure 2D), promoting optimal cellulase enzyme activity (Jamarun et al., 2017). Feed ingredients with low crude fibre content have been previously found to be more assimilable because microbes can more easily penetrate their thinner cell wall. Conversely, materials with high crude fibre content have thicker cell walls, making digestion more challenging. According to Khattab et al. 2020, feed digestibility depends on microbial activity in the rumen, which in turn is related to feed nutrient content (Soltan et al., 2018). Microorganisms transform feed compounds into smaller particles that differ from original substances they are derived from (Krehbiel, 2014).

Rumen fermentability

NH_3 concentration indicates the extent of crude protein degradation by rumen microbes (Bach et al., 2005) and reflects the fermentability of feed (Hassanat and Benchaar, 2013). Higher NH_3 concentrations correspond to greater fermentability of the ration (Bi et al., 2018). The interaction between the addition of three precursors (EM + molasses + urea) and a 14-day incubation period of palm stem pith silage (D3B2) resulted in the highest NH_3 concentration of 16.67 mM (Figure 4). This outcome was consistent with the high crude protein content of 15.67% (Figure 2C) and its digestibility amounting to 83.74% (Table 1). These results demonstrate that the comprehensive addition of precursors to palm kernel pith silage can stimulate the growth and activity of rumen microbes, resulting to higher NH_3 levels compared to the addition of a single precursor (e.g. empty fruit bunch ash filtrate) or two precursors (chicken faeces + urea). These results confirm previous research, where the addition of one precursor (6% urea) to palm stem pith silage resulted in NH_3 concentration of 9.02 mM (Noersidiq et al., 2020), while supplementing

2 precursors (urea 1% + molasses 2%) to Leucaena silage led to a NH_3 concentration of 22.8 mg/dl (Phesatcha and Wanapat, 2016).

The 14-day incubation period provided the most optimal results in terms of NH_3 levels compared to other time points, as it was during this period when the number, diversity and activity of microorganisms in the silage reached the optimal point for the production of high-quality fermented products. Tampoebolon et al. (2019) similarly found that fermenting maize husks for 14 days resulted in the highest NH_3 concentrations of the silage. Optimal nutrient digestibility and rumen fermentability occur when there is sufficient availability of energy and protein substrates (Millen et al., 2016). As nutrient digestibility increases, NH_3 concentration also rises, reflecting the enhanced digestibility of crude protein and organic matter in the rumen (Mezzomo et al., 2011; Jiang et al., 2019).

The high NH_3 concentration in treatment D3B2 was due to the addition of urea which functions as a source of non-protein nitrogen (NPN). NPN in the rumen is utilised by microbes for protein synthesis, while protein is degraded into peptides and amino acids by proteolytic enzymes produced by these microorganisms. The breakdown of NPN produces ammonia, thereby increasing NH_3 concentration, while urea functions as an N source for microbial protein synthesis (Wahyono et al., 2022). NH_3-N concentration influences rumen microbial growth (Khattab et al., 2013), and its level rises linearly with increasing doses of NPN supplementation (Loosli and McDonald, 1968; Xu et al., 2019). Ammonia production depends on the solubility and amount of dietary protein, food retention time in the rumen and rumen pH (Wahyono et al., 2022). This study demonstrated that the addition of three precursors (EM + molasses + urea) contributed to a higher NH_3 concentration compared to the addition of one or two precursors. Supplementing one precursor (urea-treated ensiled threshed sorghum [UTST] 0-700 g/kg) to sorghum fermentation resulted in NH_3 concentrations of 89.9 95.2 mg/l (Olafadehan and Adebayo, 2016). Similarly, NH_3 levels after the addition of two precursors (a combination of NPN and slow-release urea [SRU] in low quality forage) ranged from 12.42 to 15.52 mg/dl (Gonçalves et al., 2015); these concentrations were within normal limits, i.e. 6–21 mM (Edwards, 1923; McDonald et al., 2002) and 5–25 mM (Wu, 2017), for optimised microbial protein synthesis (Hall and Mertens, 2017).

VFA are a product of carbohydrate and protein metabolism of rumen microbes (Krehbiel, 2014) and their total contents indicate the availability of energy for these microorganisms (Millen et al., 2016; Bhatia and Yang, 2017; Dryden, 2021; Kaplan-Shabtai et al., 2021). The highest VFA level was found in treatment D3, i.e. palm stem pith silage supplemented with three precursors (EM + molasses + urea), reaching 136.89 mM. This value was significantly higher than the level acquired when supplementing one (128.67 mM) or two precursors (133.78 mM). This shows that the high level of feed fermentability is due to the presence of all required nutrients provided with precursors (urea – protein source, molasses – energy source, and EM – a source of photosynthetic bacteria). All these elements support optimal microbial growth and activity, resulting in the highest VFA content. This outcome is consistent with previous studies, e.g. Xu et al. (2019) added urea as a single precursor (0–30 g/kg DM) to rations prepared from maize silage and obtained a total VFA content of 105.7 111.9 mM; Hartati et al. (2022) supplemented two precursors (150 mg ZnSO4/kg DM and 2% Zn-Cu isoleucine/kg DM ration) to sorghum silage and obtained a total VFA content of 121.25 131.67 mM. Microbial activity reaches its peak during an incubation period of 14 days, as assessed by the microbial population and diversity, resulting in optimal feed fermentability. For instance, fermentation of maize husks for 14 days yielded the highest VFA concentration (Tampoebolon et al., 2019).

High concentrations of rumen VFA indicate increased fermentation of carbohydrates in the rumen (Khattab et al., 2013). Elevated levels of VFA recorded in treatment D3 could be attributed to the availability of urea as a nitrogen source, molasses as an energy source, and EM as a source of photosynthetic bacteria for microbial growth and activity, leading to higher energy production. Additionally, the low crude fibre content (Figure 2D) and high crude fibre digestibility (Table 1) in treatment D3 contributed to the highest VFA levels recorded at 136.89 mM. This finding is supported by the study of Aschenbach et al. (2011), who also showed that high crude fibre digestibility increased total rumen VFA production. VFA concentrations in this study ranged from 125.00–139.33 mM which was suitable for microbial growth. The concentration of VFA required for rumen microbial growth ranges from 80 160 mM (Weimer and Moen, 2013; Phesatcha et al., 2021).

The high digestibility of organic matter in treatment D3B2 also contributed to the elevated total VFA levels. VFA concentration is related to the availability of fermentable carbohydrates in the rumen, which are crucial for microbial energy production. Carbohydrates significantly influence the digestibility of organic matter as they are the largest component in feed and serve as major energy producers (VFA) (Pazla et al., 2021a)

The duration of incubation, the addition of precursors and their interaction did not significantly affect the pH value of rumen fluid. In this study, the rumen pH ranged from 6.78 to 6.82, which were appropriate values for the development and activity of rumen microbes. This range reflects a balance between VFA and NH_3 production and the buffering action of solutions, maintaining stable rumen pH. These findings were consistent with a study of Kargar et al. (2023), who reported that rumen pH remained constant due to the equilibrium between NH_3 (alkaline) and VFA (acidic), aided by the buffering capacity of McDougall solution reflecting saliva composition. Similar results were also reported by Pazla et al. (2021b). The ideal rumen pH range facilitating effective digestion is 6.72–6.85. The consistency of the findings presented in this study demonstrates that the observed rumen pH values are conducive to optimal rumen digestion and development of cellulolytic and amylolytic microbes (Vargas et al., 2023).

The rumen pH values obtained in this study were lower than those reported by Noersidiq et al. (2020), where the addition of urea to palm stem pith fermentation produced a pH of 6.96–7.05. This higher pH can be attributed to the alkaline nature of urea. Moreover, the addition of starbio and Phanerochaete to palm stem pith silage by the latter authors yielded pH values of 6.94 and 6.96, respectively (Noersidiq et al., 2018a).

Conclusions

In vitro experiments concerning the complete supplementation of microbial growth precursors in the process of ensiling palm pith carried out in the current study demonstrated that supplementing a combination of effective microorganisms, molasses, and urea to a silage mixture incubated for 14 days has provided the best response in terms of nutrient content (dry matter, organic matter, crude protein, crude fibre, extract ether and nitrogen free extract) and digestibility (dry matter, organic matter), as well as NH_3 concentration. These findings suggest that this optimal treatment variant can be recommended for further *in vivo* research.

Conflict of interest

The Authors declare no conflict of interest.

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