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RESEARCH ARTICLE

Potential of oil palm by-products supplemented with sago bagasse and solid decanter as basal diet for beef cattle feeding: Evidence from in vitro evaluation

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ABSTRACT

By-products from oil palm (*Elaeis guineensis* Jacq.) industry are abundantly available year-round and potential to be used as a sustainable alternative feed for beef cattle raised in the oil palm plantation. The objective of the study was to evaluate in vitro digestibility, gas and methane production, and rumen fermentation of oil palm industry by-product as basal diet for beef cattle supplemented with sago (*Metroxylon sagu* Rottb.) bagasse. Four formulated diets were (i) mixture of oil palm frond and palm kernel cake and urea as control diet (CO); (ii) CO supplemented by sago bagasse (COSB); (iii) CO supplemented by solid decanter (COSD); and (iv) CO supplemented by sago bagasse and solid decanter (COSDSB). These diets were evaluated using in vitro gas production. Results show that in vitro true DM degradability (IVTDMD) and methane production were similar among the four-diet treatments with the average 57.73% and 2.8108 mL g^{-1}, respectively. This indicates their potential feed suitability for beef cattle. In contrast, gas production is significantly different among treatments with the lowest value for CO (119.31 mL g^{-1}) and highest for COSB treatment (135.26 mL g^{-1}), suggesting less microbial activities without supplementation to frond palm. Supplementation of sago bagasse and solid decanter to the byproducts (COSB, COSD and COSDSB treatments) significantly increased protozoa population and fermentation thereby increase microbial biomass (MCP), total volatile fatty acids production and propionic acid production. It can be concluded that oil palm frond and palm kernel cake basal diet are potential to be used for beef cattle diet by supplementing with sago bagasse and solid decanter to enhance their digestibility and fermentation in the rumen.

Key words: *Elaeis guineensis*, in vitro gas production, oil palm frond, palm kernel cake, sago bagasse, solid decanter.

INTRODUCTION

Agricultural byproducts, generated from oil palm industry, are abundantly available year-round in Indonesia since Indonesia is the major global palm oil producer. The main challenge is to leverage these byproducts of oil palm industry as alternative source diets for animals. In tropical countries such as in Indonesia, beef cattle production is constrained by the limitation of forage availability, notably during the dry season. The scarcity of animal forage can be mitigated using innovative technology to process agricultural byproducts.

Byproducts derived from oil palm (*Elaeis guineensis* Jacq.) industries include kernel shell, empty fruit bunch, frond and trunk, leaves, mesocarp fibre, palm oil mill effluent, palm kernel cake (Diyanilla et al., 2020) and decanter cake (Lim et al., 2022). Oil palm frond (Santoso et al., 2024), palm kernel cake (Vargas and Mezzomo, 2023), and palm decanter cake (Abubakr et al., 2014) have gained particular attention as feed ingredients due to their availability, affordability, sustainability, and nutrition content.

Oil palm frond (OPF) represents the largest biomass from oil palm production and is available year-round offering potential as sustainable feed resource for ruminants in tropical regions (Chanjula et al., 2017; Lunsin et al., 2021). With its high fibre content (neutral detergent fibre [NDF] 70.1%) and low protein levels (crude protein [CP] 2.0%) (Chanjula et al., 2017), OPF serves valuable fibre source. It is typically chopped and mixed with other feed ingredients or processed into pellets. These pellets can replace up to 50% concentrate feed for lactating dairy cows without compromising feed digestibility and milk production (Lunsin et al., 2021). Additionally, ensiling OPF with urea and calcium hydroxide improves nutrient digestibility, energy intake and N balance when fed to goat (Chanjula et al., 2021).

Palm kernel cake (PKC) supports ruminal microbial activity due to its nutrients content (Ribeiro et al., 2018). The PKC addition at 36% in concentrate meat goat diets improve carcass quality, modifies fatty acid profile in carcass and improves consumer acceptance of the meat (da Silva et al., 2021). It also serves as an effective supplement for grazing animals such as buffalo, when included at 1% of body weight fed in grass-based diets (Amaral-Júnior et al., 2023). Furthermore, replacing tofu-dreg concentrates with 20% PKC-based concentrates in dairy goat diets does not negatively affect milk production or quality (Arief et al., 2020).

Palm decanter cake (PDC) produced during the oil extraction process from palm fruit, contains lignocellulosic material and residual oil (Abubakr et al., 2014). Rich in fibre, protein and energy, PDC is good feed for ruminant diets. Addition of PDC at 20% in concentrate diet for lactating dairy cow has shown no adverse effects on feed digestibility, milk production and milk quality (Lunsin, 2018). Additionally, incorporating PDC at 20% into total mixed ration (TMRs) with OPF as fibre source, increase digestibility and total volatile fatty acids (VFA) production which is crucial energy supply in ruminants (Chanjula et al., 2022).

The problems for using OPF, PKC and PDC for animal diets are low carbohydrate content and high neutral detergent fibre (NDF). The NDF content of OPF, PKC, and PDC were 79.2% (Santoso et al., 2024), 71.7% (Abdeltawab and Khattab, 2018), 45.6% (Abubakr et al., 2014), respectively. The low carbohydrate content could be overcome by adding sago (*Metroxylon sagu* Rottb.) pulp. Sago pulp, a by-product of sago flour processing, contains 49.5% starch (Husin et al., 2018), making it a valuable carbohydrate source for feed ensiling. Carbohydrate supplementation during ensiling enhances the fermentation process by providing substrate for lactic acid bacteria, which produce lactic acid and reduce pH to preserve the forage. Ensiling process particularly effective for preserving high moisture feed materials such as OPF, PDC and sago pulp to prevent undesirable bacterial and fungus growth during storage. This method of preservation is also more applicable for small scale farmer for feed storage. The use of sago pulp as animal feed can also mitigate negative environment impact, as most of sago wastes is often discarded in open areas or in nearby river (Husin et al., 2018).

Given the lignocellulosic nature of OPF, its digestibility enhancement through proper processing. The PKC serves as valuable protein source, while PDC contributes energy with its residual fat content, though its low DM content (23.54%) (Lim et al., 2022). These by-products, when incorporated into rations, offer economical and environmentally sustainable alternatives to conventional feedstuffs. However, their nutritional values and combined effects in feed formulations require further evaluation to ensure optimal animal performance. While previous studies have examined OPF, PKC or PDC individually or in pairs, no research has explored the use of all three in TMR or ensiled forms. In addition, no report study on the use of sago bagasse as supplement to oil palm by-products basal diet. This study aims to evaluate the in vitro digestibility, gas production and rumen fermentation of ensiled TMR based on OPF, PKC or PDC supplemented by sago bagasse for beef cattle, providing insight into their potential as alternative feed resources.

MATERIALS AND METHODS

Experimental design and dietary treatments

The study was conducted under ethical clearance No. 444/KE.01/SK/07/2023. The dietary treatment basal diets used oil palm (*Elaeis guineensis* Jacq.) frond (OPF), palm kernel cake (PKC) and urea as a control treatment (CO), basal diet supplemented with either sago (*Metroxylon sagu* Rottb.) bagasse (COSB), solid decanter (COSD), or both sago bagasse and solid decanter (COSDSB) (Table 1). The experiment was arranged in a completely randomized design (CRD) with five replicates. All diet treatments were formulated to contain 15% CP by adding urea as a source of non-protein N (NPN). Palm frond, PKC and palm solid decanter were obtained from private palm oil industry in Riau Province. The sago bagasse was obtained from smallholder sago starch processing. The palm frond was chopped into 2 cm length and mixed with sago bagasse and solid decanter as feed. The proportion of chopped palm frond was fixed at 40% DM in the diet and mixed with either sago bagasse, solid decanter or sago bagasse and solid decanter to form total mixed ration as given in Table 1. The feed samples were analysed for DM, ash and crude protein (CP) using the procedures of AOAC (2012) neutral detergent fibre (NDF) and acid detergent fibre (ADF) according to Van Soest et al. (1991) using Ankom fibre analysis (Table 2). Total digestible nutrient was calculated according to Hartadi et al. (1980). The ration was formulated in iso protein to contain crude protein 15% by adding urea in the ration to adjust CP content. The mixture was made in 1 kg each treatment. The diet was then ensiled by putting in silo of plastic bag and tightly tied to ensure the anaerobic condition and kept under ambient temperature for 3 wk (21 d). After 3 wk the silage was open and dried in the oven at 50-60 °C for 48 h or until constant weight. The dried diets were then ground and was used for in vitro digestibility evaluation.

Table 1. Composition of diet treatments (%). CO: Control consisted of palm frond, kernel cake and urea; COSB: control + sago bagasse; COSD: control + solid decanter; COSDSB: control + solid decanter + sago bagasse.

Ingredients (%)	Treatments			
	CO	COSB	COSD	COSDSB
Oil palm frond	40.00	40.00	40.00	40.00
Solid decanter	0.00	0.00	18.50	8.00
Palm kernel cake	58.65	45.50	40.00	40.00
Sago bagasse	0.00	12.50	0.00	10.00
Urea	1.35	2.00	1.50	2.00
Nutrient content of the diet treatment (%DM)				
Organic matter	95.07	93.98	93.80	93.59
Crude protein	15.00	15.06	15.41	15.11
Ether extract	7.16	5.83	8.65	6.74
Crude fibre	39.63	37.10	39.16	37.38
Nitrogen free extract	33.28	35.99	30.58	34.36
Neutral detergent fibre	65.00	59.39	62.74	59.49
Acid detergent fibre	53.32	49.27	53.39	50.08
Acid detergent lignin	14.75	13.51	18.47	15.36
Hemicellulose	11.68	10.12	9.35	9.41
Cellulose	38.57	35.76	34.92	34.72
Total digestible nutrient	66.04	62.82	73.01	65.41

Table 2. Chemical composition of feed ingredients used in the study.

Chemical composition (% DM)	Oil palm frond and leaves	Palm kernel cake	Solid decanter	Sago bagasse
Organic matter	95.83	96.74	90.66	93.09
Crude protein	6.77	14.49	14.52	1.06
Ether extract	2.77	10.32	18.49	0.24
Crude fibre	43.44	37.95	35.71	19.65
Neutral detergent fibre	62.55	68.18	56.52	26.77
Acid detergent fibre	55.53	53.05	53.84	23.38
Acid detergent lignin	16.84	13.67	33.88	4.46
Gross energy, kcal kg^{-1}	4943.30	4628.02	3906.47	3951.22

In vitro experiment

Two methods were used for the in vitro study: (i) Theodoru method to measure fermentation characteristics, total gas and methane gas production, and (ii) Daysi method to measure feed digestibility.

Samples of the ration were weighed (0.5 g) and transferred into 50 mL serum bottles. Each treatment diet incubation was performed in five replicates with four blanks. Rumen fluid was taken before the morning feed from rumen-cannulated, male cross bred Ongole beef cattle (about 500 kg body weight) receiving fresh native grass *ad libitum* and supplemented with commercial concentrate at 1% BW. Rumen fluid was collected into a pre-warmed insulated bottle, homogenized in a laboratory blender, strained using nylon cloth with a pore size of 100 µm and then filtered using a glass wool. All handling was carried out with continuous flushing by CO_2. The well-mixed and CO_2-flushed rumen fluid was added to the buffered mineral solution in a proportion 2:1 (mL/mL), which was maintained in a water bath at 39 °C, and mixed. Buffered rumen fluid (40 mL) was dispensed into each serum bottle containing ration samples. Bottles were sealed with rubber stoppers and aluminium caps. The bottles were immediately placed in a water bath at 39 °C for 48 h for in vitro gas test. Thirty minutes after starting the incubation, the bottles were gently mixed and repeated every 2 h. Four bottles only containing 40 mL rumen inoculum served as blanks also included. The blank values were subtracted from each measured value to give the net gas production. Gas production was recorded at 24 and 48 h incubation. Gas production was read manually using glass syringes. Then gas was transferred into glass vial for methane gas analysis. After terminating the incubation at 48 h, the pH of each bottle was measured using portable pH meter (HANNA Instruments HI 8424 microcomputer, Kallang, Singapore), then the supernatant from each bottle was taken and divided into two portions. The first portion was acidified with one drop of sulfuric acid to stop fermentation process of microbe's activity and kept in the freezer, pending for volatile fatty acids (VFA) fermentation parameters and NH_3 concentration analysis. The total VFA and molar proportion of acetic, propionic, butyric, valeric, iso butyric and iso-valeric acids were determined using gas chromatography (Model G1540N, Agilent Technologies, USA). The NH_3 concentration was analysed using Conway micro-diffusion technique (Conway, 1962). The second portion of supernatant was preserved with 10% formalin solution at a 1:9 (v/v) ratio and stored at 4 °C for measuring protozoal population. The total counts of protozoa were done based on the use of a haemocytometer.

In vitro true DM digestibility was measured using Daisy in vitro method. A truly degradable fermented substrates (in vitro true DM degradability, IVTDMD) were incubated for 48 h. In this incubation, 500 mg sample was weight into incubation bag, and place in bottle containing rumen buffer medium. The medium was prepared according to Makkar et al. (1995). After terminating the incubation at 48 h, the bag was transferred in Ancom fibre analysis. The procedure of Van Soest et al. (1991) was applied by refluxing the incubation residue in the bag in NDF solution for 1.0 h and washing undigested matter with hot water, and dried in the oven overnight at temperature 100 °C. The IVTDMD was calculated as the difference between incubated DM (DM) and remaining non-degraded DM.

Microbial crude protein (MCP; mg g^{-1} DM) was calculated using the equations proposed by Menke and Steingass (1988) and Blümmel et al. (1997):

$$MCP = DMD - (GP_{24} \times 2.2)$$

where GP_{24} is gas production (mL 200 mg^{-1} DM) at 24 h fermentation, CP is crude protein (%, DM basis), DMD is DM degradability (mg g^{-1} DM), 2.2 is stoichiometric factor expressing the specific C, hydrogen, and oxygen requirements (mg) needed for the production of 1.0 mL gas.

Microbial synthesis efficiency was evaluated using the partitioning factor (PF; mg DMD mL^{-1} gas), which was obtained by dividing the DM degradability (mg) by the volume (mL) of gas production (Blümmel et al., 1997).

Statistical analysis

Data collected were analysed using a randomized complete block design with general linear model (GLM) procedure of SAS package version 9.0 (2002; SAS Institute, Cary, North Carolina, USA), following statistical model:

$$Y_{ij} = \mu + s_i + e_{ij}$$

where Y_{ij} is the dependent variable, μ is the general mean, s_i is the effect of the treatment and e_{ijk} is the experimental error. Means were compared using Duncan's multiple range test. Significance was declared at $p < 0.05$. Correlation and principal component analysis between parameters was analysed using R studio software.

RESULTS

In vitro digestibility

The in vitro true DM degradability (IVTDMD), total gas and methane production of four diet treatments are presented in Table 3. The IVTDMD was not significantly different among diet treatments, varying from 57.30% to 58.22% with the average digestibility was of 57.73%. The in vitro neutral detergent fibre (NDF) digestibility of control diet (oil palm frond, palm kernel cake and urea, CO) was significantly higher ($P < 0.05$) than solid decanter (SD) and sago bagasse (SB) (COSDSB) and COSB treatments but was not significantly different ($P > 0.05$) to COSD diet. The total gas production was significantly highest ($P < 0.05$) in COSB diet, while other diets were not significantly different. The methane (CH_4) production varied from 2.46 to 3.17 mL g^{-1} and was not significantly ($P > 0.05$) different among diet treatments.

Table 3. In vitro true DM digestibility (IVTDMD), total gas and methane production of diet treatments. CO: Control; COSB: control + sago bagasse; COSD: control + solid decanter; COSDSB: control + solid decanter + sago bagasse; IVNDFD: in vitro true neutral detergent fibre degradability; PF: partitioning factor; MCP: microbial crude protein. Different superscripts in a similar row are significantly different ($P < 0.05$).

	Treatments					
Parameters	CO	COSB	COSD	COSDSB	SEM	*P* values
IVTDMD, %	57.93^a	57.30^a	57.47^a	58.22^a	0.6738	0.7588
IVNDFD, %	35.27^a	28.09^c	32.22^{ab}	29.78^{bc}	0.6738	0.0017
Total gas production, mL g^{-1} sample	119.31^b	135.26^a	115.26^b	120.03^b	1.6146	0.0028
PF, mg DMD mL^{-1} gas	6.61^a	5.93^b	6.95^a	6.95^a	0.0765	0.0019
CH_4 production, mL g^{-1} sample	3.1654^a	2.4593^a	2.8309^a	2.7878^a	0.1930	0.4314
Proportion CH_4 production, %	1.3398^a	0.9222^a	1.2415^a	1.1777^a	0.1208	0.1588
MCP, mg g^{-1} DM	386.23^a	$392,82^a$	359.72^b	396.84^a	2.5921	0.0015
Protozoa population, $\times 10^5$ cell mL^{-1}	41.08^d	86.70^b	65.70^c	152.24^a	5.5643	< 0.0001

Rumen fermentation

The effect of diet treatments on rumen fermentation characteristics is given in Table 4. The pH of the supernatant medium varied between 6.77 and 6.80 for all diets. It was significantly lower ($P < 0.05$) in COSB compared to other diet treatments, which in turn were not significantly different ($P > 0.05$) among them.

Table 4. Effect of diet treatments on rumen fermentation characteristics. CO: Control; COSB: control + sago bagasse; COSD: control + solid decanter; COSDSB: control + solid decanter + sago bagasse; VFA: volatile fatty acids. Different superscripts in a similar row are significantly different ($P < 0.05$).

	Treatments					
Parameters	CO	COSB	COSD	COSDSB	SEM	*P* values
Ph	6.80^a	6.77^b	6.80^a	6.80^a	0.0054	0.0024
NH_3, mg dL^{-1}	10.61^a	10.20^a	10.20^a	11.70^a	0.5080	0.7639
Total VFA, mmol	58.01^b	75.78^a	67.12^{ab}	62.29^{ab}	5.5339	0.0400
Proportion of VFA partial, %						
Acetate	78.62^{ab}	77.68^c	79.01^a	77.91^{bc}	0.2382	0.0064
Propionate	14.68^b	15.83^a	14.53^b	15.69^a	0.1859	0.0003
Butyrate	5.08^a	5.00^a	5.00^a	5.04^a	0.0532	0.6002
Iso valerate	0.49^a	0.42^b	0.43^b	0.45^b	0.0095	0.0005
Iso butyrate	0.75^a	0.66^a	0.67^a	0.56^a	0.0733	0.3715
Valerate	0.36^a	0.41^a	0.36^a	0.34^a	0.0446	0.7372
Acetate/propionate ratio	5.36^a	4.91^b	5.44^a	4.97^b	0.0672	0.0004

The addition of either SB, SD or both to palm frond and palm kernel (CO) did not result in significant differences (*P* > 0.05) in total volatile fatty acids (VFA) production, with values varying between 62.29 and 75.78 mmol. However, the COSB treatment significantly increased (*P* < 0.05) total VFA compared to the control (75.78 *vs*. 58.01 mmol).

The proportion of acetic acid in COSD diet was significantly higher (79.01%) than COSDSB and COSB diets (77.68%-77.91%), but it was not significantly different (*P* > 0.05) to CO diet. Similar trend also occurred in the proportion of propionic acid. Ruminal NH_3 production and the proportion of butyric acid did not significantly differ (*P* > 0.05) among diet treatments. Protozoa population increased (*P* < 0.05) with SB or SD supplementation, while the lowest (P < 0.05) population was observed for the CO diet.

Correlation matrix was used to evaluate the relationship between parameters of diet treatments (Figure 1). There were strong positive correlations between microbial biomass (MCP), partial VFA and digestibility. The increased MCP production enhanced digestibility, which in turn raised VFA concentration. Likewise, MCP was positively correlated with IVTDMD ($r = 0.47$; $p < 0.05$) and propionate ($r = 0.57$; $p < 0.05$). In contrast, MCP showed a strong negative correlation with gas production ($r = -0.70$; $p < 0.001$) and a moderate negative correlation with acetate ($r = -0.53$; $p < 0.05$). In addition, there was a strong negative correlation between acetate and propionate ($r = -0.96$; $p < 0.001$), and the acetate and A:P ratio ($r = -0.97$; $p < 0.001$), indicating that the increase in propionic acid and ratio A:P reduced the proportion of acetic acid. Ammonia N (NH_3-N) was positively correlated with butyrate concentration ($r = 0.61$; $p < 0.01$). Total VFA showed a moderate positive correlation with propionate ($r = 0.49$; $p < 0.05$). Although propionate displayed a positive correlation with protozoa ($r = 0.60$), this relationship was nonsignificant ($p > 0.05$). Furthermore, in vitro NDF degradability (IVNDFD) showed a moderate, but nonsignificant, correlation with CH_4 ($r = 0.46$; $p > 0.05$).

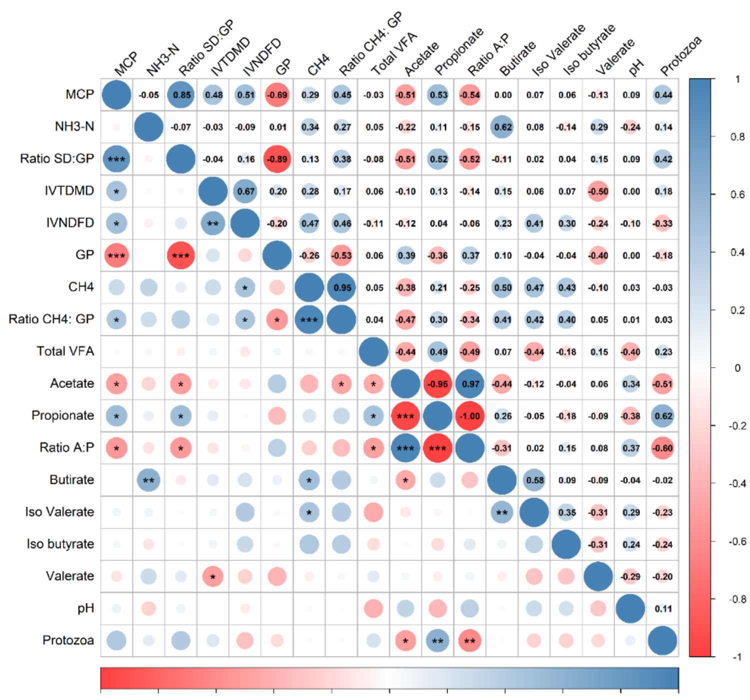

Figure 1. Correlation matrix of each parameter of diet treatment evaluated using in vitro study. Positive and negative correlation coefficients are displayed in blue and red scale, respectively. Significant levels: Very highly significant ($^{***}p < 0.001$), highly significant ($^{**}p < 0.01$), and significant ($^{*}p < 0.05$). MCP: Microbial crude protein; SD:GP ratio: ratio of substrate fermented to gas production; IVTDMD: in vitro true DM degradability; IVTNDFD: in vitro true neutral detergent fibre degradability; total VFA: total volatile fatty acids; ratio A:P: ratio of acetate to propionate.

The factor loadings revealed distinct groupings of variables related to digestibility and fermentation traits among the four diet treatments (Figure 2). The first principal component (Dim1) accounted for 30% of the variation in the in vitro parameters, while the second principal component (Dim2) explained 18%, resulting in a cumulative variance of 48%. Treatments COSDSB and COSB were positioned on the right-hand side of the scatter plot, aligning with total VFA, propionate, and protozoa population. In contrast, CO and COSD appeared on the left side of the plot, closely associated with gas production, acetate and the acetate-to-propionate (A:P) ratio. Additionally, COSBD, COSD, and CO clustered in the upper region of the plot, indicating associations with higher methane production and improved DM and NDF degradability.

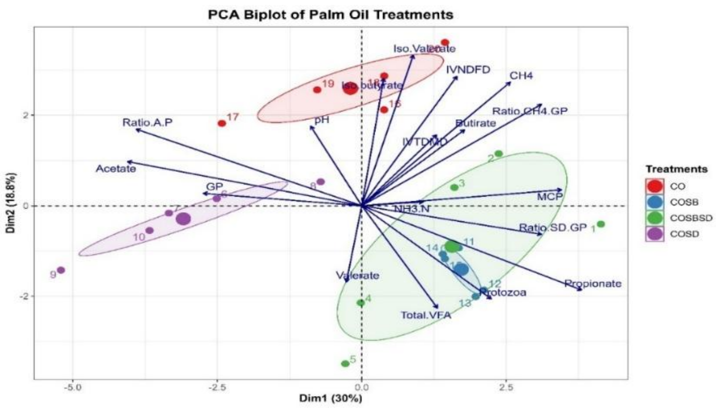

Figure 2. Principal component analysis of the diet treatments. CO: Control; COSB: control + sago bagasse; COSD: control + solid decanter; COSDSB: control + solid decanter + sago bagasse; MCP: microbial crude protein; SD:GP ratio: ratio of substrate fermented to gas production; IVTDMD: in vitro true DM degradability; IVTNDFD: in vitro true neutral detergent fibre degradability; total VFA: total volatile fatty acids; ratio A:P: ratio of acetate to propionate.

DISCUSSION

In vitro degradability

The in vitro gas production technique measured fermentation products resulting from substrate degradation, including microbial biomass, short-chain fatty acid (SCFAs), and gas volume. The in vitro true DM degradability (IVTDMD) showed nonsignificant differences among diet treatments, with an average value of 57.73%. This is attributed to the diets were formulated in iso protein resulting similar extent digestibility, irrespective of their differences in composition associated with different types of ingredient supplementation. However, among the formulated diets, control diet (CO) with sago bagasse (SB) (COSB) resulted in the highest gas production, suggesting that the addition of SB to the palm frond and palm kernel cake enhanced fermentative activity of rumen microbial. This high gas production is likely due to the high content of fermentable carbohydrate in SB, which provide an additional energy source to support greater microbial activity and fermentation, thereby boosting gas production. Previous studies showed that SB contained high starch up to 54.6% (Husin et al., 2018) and 58% (Sumiana et al., 2020).

Moreover, the fermentation of SB likely contributes to elevated levels of SCFAs, with generated gaseous by-products such as CO_2 and CH_4 (Blümmel et al., 1997). Furthermore, according to Blümmel et al. (1997), there is an inverse relationship between gas production and microbial biomass yield. This finding agrees well with the strong negative correlation observed between microbial crude protein (MCP) and gas production in the present study (Figure 1). The negative correlation indicated that a greater proportion of fermented diet was used for microbial protein synthesis rather than gas production. The fermentation products are beneficial due to MCP is used as protein source for ruminants. Both COSB and control diet, solid decanter (SD) and SB (COSDSB) diets showed similar in vitro digestibility and MCP production but the COSDSB diet resulted in lower gas production compared to COSB diet, which produce high gas production. This variability in gas production among different diets has also been observed previously by Blümmel et al. (1997).

The combination of SD and SB supplementation (COSDSB) diet is not significantly enhancing gas production could be due to the SD contained high fat as revealed by ether extract achieving 18.49% (Table 2). Lipids require hydrolysis by the enzyme lipase before microbial fermentation can occur, and this additional step may slow down the overall fermentation process (Mutungwazi et al., 2021). Additionally, substrates rich in structural carbohydrate tend to exhibit a strong correlation between gas volume and SCFA production in vitro studies. In the present study. the evaluated substrate was primarily composed of structural carbohydrates, as revealed by their high neutral detergent fibre (NDF) content ranging from 59.39% to 65.00% (Table 1).

Although the COSB diet produced the highest total gas volume (135.26 mL g^{-1}) and total VFA production, its total VFA was comparable to those observed in COSD and COSDSB diets. The volume of gas produced during in vitro incubation could reflect the degree of a substrate degradability and fermentation (Blümmel et al., 1997). The higher MCP (Table 3) and total VFA (Table 4) in COSB diet are beneficial due to microbial biomass and VFA are useful fermentation products derived from degradable dietary compound.

Total protozoa were higher in COSB attributed to the increase in energy source. A similar result was reported by Saeed et al. (2023), who observed the increase in protozoa number due to increase energy when corn replaced a palm kernel-based concentrate diet. Furthermore, the higher protozoa populations in COSDSB diets indicated the dominant role of carbohydrate availability from SB than from SD in providing energy to support protozoa growth. Protozoa play an important role in fibre digestion and in maintaining the microbial ecosystem in the rumen. In contrast to the current study, Saeed et al. (2023) reported a decrease in bacterial population associated with an increase in protozoa. This negative relationship is likely due to the protozoal predation on bacteria, which may vary depending on the type of protozoa present. Holotrich protozoa types have lower predatory activity compared to entodiniomorphids protozoa (Francisco et al., 2019). Microbial crude protein consisted of bacteria, protozoa and fungi. Since protozoa are larger in size than bacteria, an increase in protozoa population may not necessarily reduce MCP production, though some bacterial loss through predation (Francisco et al., 2019). Abubakr et al. (2013) reported a reduction in protozoa population in an in vivo study, where goats were fed a rice straw basal diet supplemented with a concentrate containing either palm decanter cake or palm kernel cake. The decrease was attributed to the relatively high fat content of these supplements (7.6% and 6.8%) which suppressed protozoa growth.

Although, protozoa population increased due to SB and SD supplementations, their methane (CH_4) production was not significantly different to the control. It seems that no correlation of methane production with protozoa population (Figure 1). Protozoa contribute to the process of methane production indirectly by producing hydrogen during the fermentation of carbohydrates and protein degradation. This hydrogen is then used by methanogens for methane production synthesis (Mutungwazi et al., 2021). Supplementation with SB or SD had no effect on methane production. However it supports protozoa growth, which may not significantly alter the activity of methanogenic archaea that is considered the primary producers of methane in the rumen (Siqueira et al., 2021).

Rumen fermentation

The ruminal pH of all diet treatments ranged from 6.77 to 6.80, which falls within the normal range (6.0-7.0) for rumen fermentation (Satter and Slyter, 1974; Kirwan et al., 2022; Saeed et al., 2023). This suggests that addition of SB and SD does not negatively affect the optimal rumen pH. Kirwan et al. (2022) reported similar pH values (6.46-6.65) when supplementing grass silage-based diets with various carbohydrate sources (rolled barley, maize meal or soya hulls). Likewise, Saeed et al. (2023) reported pH value of 6.46-6.80 in diets supplemented with corn in a palm kernel cake-based concentrate.

Ruminal NH_3-N concentration plays a crucial role in microbial protein production, particularly in vitro. The ruminal NH_3-N content was comparable among diet treatments, ranging from 10.20 to 11.7 mg dL^{-1}, suggesting a similar degradability of the different diets. This indicates that N efficiently utilized for microbial protein synthesis, preventing NH_3-N accumulation and that MCP was not affected by diet composition (Putri et al., 2021). When fermentable energy is available, the decline in NH_3-N concentration reflects more efficient N-utilization by rumen microbes (Saeed et al., 2023). In this study, the ruminal NH_3-N concentration of diet treatments ranged from 10.20 to 11.7 mg dL^{-1}, which exceeds the optimal range of 5-8 mg dL^{-1} suggested by

Satter and Slyter (1974). The higher NH_3-N production observed indicates that the N availability exceeds the amount required to meet energy from SB, resulting in excess NH_3-N accumulation in the medium. In contrast, ruminal NH_3-N production in this study (10.20-11.70 mg dL^{-1}) is much lower than those reported by Saeed et al. (2023), which ranged 32.79-36.79 mg dL^{-1}, and by Chanjula et al. (2022), which ranged 20.53-21.79 mg dL^{-1}. These differences suggest that the ruminal NH_3-N production depends on type of basal diet and the nature of supplement used. Saeed et al. (2023) used rice straw basal diet supplemented with palm kernel cake while Chanjula et al. (2022) evaluated oil palm frond basal diet in total mixed ration containing oil palm meal.

The COSB and COSDSB diets showed high MCP contents than COSD diets. This indicates that supplementation with SB and its combination with SD, which supply fermentable energy, did not significantly alter microbial N utilization. According to Russell and Rychlik (2001) the MCP is an essential source of protein for ruminants and its production is influenced by the balance between N availability and fermentable energy sources. The stable MCP across diet treatments imply efficient microbial N metabolism, likely supported by the presence of fermentable carbohydrate that enhance microbial proliferation.

The total VFA production was higher in COSB diet than CO, but was similar to COSD and COSDSB diets. In the COSB and COSDSB diets, the increased propionate production resulted in a reduction in acetic to propionic ratio (Table 4). Propionate is important for energy metabolism in ruminants (da Silva et al., 2021) and a major precursor for glucose production through hepatic gluconeogenesis in ruminants (Azzaz et al., 2019). This enhanced energy availability supports growth, milk production and overall metabolism.

Supplementation of SB in COSB and COSDSB resulted in higher propionate production owing to starch fermentation to promote higher glucose availability and support propionate synthesis (Dong et al., 2021).

The propionate and ratio acetic to propionic acid in COSB and COSDSB diets are lower than CO and COSD diets but they had similar methane production. This indicates the supplement type in any diets did not affect methane production and that propionic acid did not affect methane production (Figure 1). This outcome may be explaining hydrogen release during fibrous substrate fermentation which primarily lead to the production acetate and butyrate. The hydrogen is subsequently utilized by rumen microbia to synthesize propionate thereby limiting hydrogen availability for methanogenesis and preventing an increase in methane production (Prachumchai et al., 2024). However, Kim et al. (2012) reported the increased propionate production during feed fermentation reduced methane emissions in the rumen due to propionate fermentation utilizes hydrogen (does not liberate hydrogen), which is a key substrate for methane production. The low methane production improves feed efficiency and reducing environmental impact. The methane is primarily generated through the conversion of metabolic hydrogen (H_2) by ruminal methanogens during anaerobic fermentation processes.

Treatments COSDSB and COSB can improve energy efficiency, as indicated by the increased total VFA concentrations and a higher proportion of propionate (Figure 1). Notably, the COSB treatment demonstrated the most consistent and effective response on in vitro ruminal digestibility and fermentation. This is supported by the principal component analysis (PCA), where the COSB group occupied the smallest area on the plot, indicating low variability and a strong positive association with total VFA and propionate. In contrast, CO and COSD treatments were associated with the higher acetate production, gas production, and an increased acetate-to-propionate ratio, suggesting less efficient fermentation with greater energy loss. The negative correlations of MCP with acetate and gas production further support this pattern. Moreover, COSBD, COSD, and CO clustered with higher methane and fibre digestibility, suggesting that although fibre breakdown was improved, it may have enhanced methanogenesis.

CONCLUSIONS

Oil palm fronds and palm kernel cake with urea supplementation have potential to be used as beef cattle diet by improving their fermentation products (microbial biomass, volatile fatty acid, and propionic acid) by adding sago bagasse or a combination of sago bagasse and solid decanter in diet formulations.

In vitro gas production from diets containing oil palm fronds (OPF) and palm kernel cake (PKC) as the basal diet, supplemented with sago bagasse, solid decanter, or their combination, yielded similar concentrations of ammonia N (NH_3-N) and microbial crude protein (MCP), confirm good digestibility across all treatments.

Overall, these findings highlight sago bagasse, solid decanter and their combination as the potential supplements to increase carbohydrate content of palm frond and kernel cake as based diets. Supplementation of sago bagasse, solid decanter or their combination to OPF and PKC maintains N balance, ensures stable MCP synthesis, and supports efficient rumen fermentation without compromising microbial activity. Further in vivo studies are recommended to assess their long-term effects on animal performance and nutrient utilization.

Authors contribution

Conceptualization: D.Y., A.P., I.G.A.P.M., S.I.W.R. Methodology: D.Y., A.P., I.G.A.P.M., A.A., R.A.G., G.E.T., Y.Y. Data curation: D.Y., I.G.A.P.M., A.A. Formal analysis and investigation: D.Y., A.P., I.G.A.P.M., S.I.W.R. Visualization: G.E.T., F.S. Writing original draft preparation: D.Y., A.P., I.G.A.P.M., H.D., Y.Y., R.A.G., G.E.T., F.S. Writing review and editing: D.Y., A.P., A.A., S.I.W.R. Funding acquisition: A.P., I.G.A.P.M. Supervision: D.Y. All authors have read and agreed to the published version of the manuscript.

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