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Effect of dietary palm stearin supplementation on rumen degradability and fermentation characteristics in ruminants

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ABSTRACT

The present study was conducted to evaluate the ruminal degradability of palm stearin and its effects on rumen fermentation in fistulated Murrah buffaloes. Three animals were allotted to a 3×3 Latin Square Design and fed concentrate diets containing 0% (T_0), 8% palm stearin (T_1), and 8% palm stearin with 1% sodium bicarbonate (T_2). Palm stearin degradability was assessed using the nylon bag technique, while rumen fermentation parameters were measured at the end of each feeding period. Results showed that palm stearin exhibited high rumen bypass characteristics with more than 75% of the fat remaining undegraded even after 48 h of incubation. Supplementation of palm stearin did not affect rumen pH, ammonia nitrogen, lactic acid or volatile fatty acid concentrations indicating stable rumen fermentation across treatments. Inclusion of sodium bicarbonate had no adverse effect on EE degradability or fermentation patterns. Hence, the study demonstrated that palm stearin can be used as a natural bypass fat source to enhance dietary energy without disturbing rumen function in buffaloes.

Keywords: Buffaloes, Fermentation, Fistulated animals, *In sacco* studies, Palm stearin

Ruminants play a major role in global food and nutrition security. They support the livelihoods of millions of farmers and contribute greatly to the livestock-based food supply chain. According to FAO (2025), more than half of the total protein provided by the livestock sector comes from ruminants, mainly through milk and meat. With the growing demand for dairy and meat products, there is increasing concern about how to feed animals efficiently and sustainably. In developing countries, cattle depend mostly on natural pastures and crop residues. These feeds are usually of poor quality, low in energy, and high in fibre (Sarwar *et al.* 2002). Such diets often result in poor growth of animals (Jabbar *et al.* 2006), delayed puberty, low calving rates and overall weak reproductive performance (Qureshi *et al.* 2002). In this regard, adding fat to the diet is a common method used to increase the energy density of the ration. This is especially helpful during periods when animals need more energy, such as early lactation (Grummer, 1993). When used properly, fat supplements can increase dietary energy without harming rumen function or fibre digestion (Palmquist and Jenkins, 1980).

Among different fat sources, palm stearin has attracted considerable interest as an energy supplement for ruminants because of its high content of saturated fatty acids, particularly palmitic acid (C16:0), and its high melting point. These characteristics are believed to reduce ruminal lipolysis and biohydrogenation, thereby increasing the level of fatty acids that escape rumen degradation and become available for intestinal absorption. Recent studies have emphasized that the physical form, degree of saturation, and fatty acid profile of lipid supplements greatly influence their digestibility and utilization in dairy animals (Gunstone, 2011, Rico *et al.* 2014, Dos Santos Neto *et al.* 2021). Such bypass fats are useful in high-producing animals because they provide extra energy directly to the animal without disturbing rumen microbes. It has also been reported that the saturated fatty acids like palmitic and stearic acids have fewer negative effects on rumen microorganisms compared to unsaturated fats which may inhibit fibre-digesting bacteria (Sears *et al.* 2023). However, despite the increasing use of palm-derived fat supplements in dairy nutrition, there is limited information on the actual ruminal degradability and bypass characteristics of palm stearin itself under practical feeding conditions. Most available studies have focused on calcium salts of palm fatty acids or purified fatty acid supplements rather than palm stearin as a distinct fat source. This lack of direct evidence creates uncertainty regarding its true rumen escape potential and utilization efficiency in lactating cows. In addition, the utilization of palm stearin may be enhanced by dietary buffers. Sodium bicarbonate can improve lipid emulsification and micelle formation in the small intestine, potentially increasing fat digestion and absorption (Vinarov *et al.* 2012). Therefore, the combined use of palm stearin and buffering agents may represent a promising nutritional

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strategy to improve energy utilization in high-producing dairy animals, although experimental evidence remains limited. Therefore, the present study was designed to: (i) determine the ruminal degradability of palm stearin using the nylon bag technique, and (ii) evaluate the rumen fermentation response to diets containing palm stearin along with sodium bicarbonate.

MATERIALS AND METHODS

This study was carried out to assess the effect of dietary supplementation of palm stearin, a by-product obtained during palm oil refining on rumen fermentation parameters in the rumen fistulated Murrah buffaloes. The experiment was conducted at the Experimental Animal Shed of the Animal Nutrition Division, ICAR–Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India. The institute is located at 28° 23′ 18.98″ N latitude and 79° 25′ 18.47″ E longitude, at an elevation of about 240 m above sea level.

Experimental animals, design, and housing: The animal were randomly allotted to three dietary treatments: T_0 (control), T_1, and T_2. The experiment followed a 3×3 Latin Square Design. All buffaloes had an average body weight of 450 kg. The animals were fed according to ICAR (2013) feeding standards to meet their nutrient requirements. The feeding trial consisted of three experimental periods, each lasting 21 days, followed by a 2-day sample collection period and a washout period of 10 days after which the animals were switched over.

The feeding trial lasted for 90 days. Throughout the study, the animals were housed in well-ventilated, clean, and hygienic conditions to support their wellbeing and minimize external stress factors that could influence the study's results. These preliminary steps were taken to ensure the animals remained in good health throughout the experimental period, thereby, enabling a reliable evaluation of the effects of the dietary treatments.

Feeding management: The rumen fistulated buffaloes in the control group (T_0) were fed a basal ration made up of concentrate and wheat straw. In the treatment groups (T_1 and T_2), palm stearin was added to the concentrate mixture at levels of 8% in T_1 and T_2 with additional 1% sodium bicarbonate in T_2. This was done by partly replacing maize and wheat bran in the concentrate, while keeping all diets iso-nitrogenous so that the protein level remained the same across treatments. The ingredients of the concentrate mixture included maize, deoiled soybean meal (DSBM), wheat bran, mineral mixture, salt and the respective inclusion of palm stearin (Table 1). Clean and fresh drinking water was provided to the animals *ad libitum***,** and water was routinely offered twice daily at 10:00 AM and 3:00 PM throughout the study period.

Table 1. Physical composition of concentrate mixture

Feed ingredients (%)	Dietary treatments		
	T_0	T_1	T_2
Maize	41	28	30.5
DSBM	19	21	22
Wheat bran	37	40	35.5
Palm stearin	0	8	8
Sodium bicarbonate	0	0	1
Mineral mixture	2	2	2
Common salt	1	1	1
Total	100	100	100
CP	18	18	18

Degradability and degree of inertness of palm stearin: The degradability of palm stearin in the rumen was assessed using the nylon bag (*in sacco*) technique at the Experimental Animal Nutrition Shed. The study followed three dietary treatments and three experimental periods of 21 d each, followed by a collection period. Nylon bags with pore size approximately 50µm containing 10 g of palm stearin were prepared and incubated in triplicate in the rumen for 0, 6, 12, 24, and 48 h after the animals had been on their respective diets for 15 d. After each incubation period, the bags were removed, rinsed thoroughly under running tap water until the wash water became clear, dried at 60°C for 48 h, and weighed. The ruminal degradability of fat was calculated following the method of Mehrez and Ørskov (1977).

The percentage of bypass fat (or undegraded fat) was calculated using the formula:

$$\% \text{ By pass Fat} = \frac{(W1-W) - (W2-W)}{(W1-W)} \times 100$$

W= weight of empty bag

W_1= bag + feed sample (palm stearin) before incubation

W_2= bag + palm stearin residue in bag after incubation

The degree of inertness of palm stearin was interpreted as the proportion of fat remaining undegraded in the rumen, with higher values indicating greater rumen bypass potential.

Rumen liquor collection and analysis: Rumen liquor samples were collected from each fistulated buffalo at the end of the 21-day feeding period during all three experimental phases. Immediately after collection, the samples were brought to the laboratory, and a portion was stored at –20°C to allow later analysis without any changes in composition. The pH of the rumen liquor was recorded on the spot using a pre-calibrated digital pH meter to ensure accurate measurement before any fermentation activity could alter the value. For the estimation of ammonia nitrogen (NH_3-N), a measured portion of each rumen sample was preserved by adding two drops of 20% H_2SO_4. The NH_3-N concentration was then determined according to the colorimetric method described by Weatherburn (1967). The volatile fatty acids (VFAs) were analyzed using a Nucon-5765 gas chromatograph based on the method developed by Cottyn and Boucque (1968).

Statistical analysis: Statistical analyses were performed using SPSS software (Version 26.0) following the

Table 2. Chemical composition of palm stearin (PS)

Attributes	Palm Stearin (%) / value
Chemical composition	
Dry matter	99.97
Organic matter	99.00
Crude protein	0.01
Ether extract	99.9
Total ash	1.00
Gross energy (GE, kcal/g)	9.11
Physicochemical properties	
Melting point (°C)	55
Iodine value (I_2/100 g)	34.62
Palmitic acid (C16:0)	77.55
Stearic acid (C18:0)	4.42
Oleic acid (C18:1)	12.96

procedures described by Snedecor and Cochran (1994). Data were analyzed using a repeated-measures mixed model appropriate for a 3 × 3 Latin Square Design. The model included treatment, period, time, and treatment × time interaction as fixed effects, while animal was considered as the subject effect. Repeated measurements obtained from the same animal over time were accounted for using the repeated-measures procedure.

RESULTS AND DISCUSSION

Chemical composition of palm stearin: The chemical composition of palm stearin is presented in Table 2. Palm stearin contained fat (99.9% EE), though extremely low in CP and ash. It contained high level of palmitic acid (77%). High melting point (55°C) and saturation level make it highly resistant to ruminal breakdown. The iodine value of palm stearin was found to be 34.62. Similar observations were reported earlier by Khaskheli and Chou, 2021 who reported I_2 value of 38.7g I_2/100g, temp 50.8˚C and high oleic acid content 32% can serve as a fat source for animals. Norzila *et al. (*2016) reported palmitic acid content ranging from 54.36- 79.7%.

Bypass percent of palm stearin: The degree of inertness of palm stearin was studied using *in-sacco* technique. Palm stearin exhibited consistently high rumen bypass percentage across all experimental diets (Table 3) with undegraded residue decreasing gradually over time but remaining above 75%, confirming its low ruminal degradability due to high saturation and melting point. The control (T_0) and palm stearin diet (T_1) maintained significantly higher bypass compared to T_2, where, sodium bicarbonate slightly increased rumen degradability, most likely to be caused by enhancing microbial activity. Saturated fats such as palm stearin are inherently resistant to rumen lipolysis and biohydrogenation. Soren *et al.* (2002) reported 57% of the undegraded fat portion from the bypass fat produced from rice bran oil. The fat content of bypass fat obtained in the present study was lower than the findings of the earlier workers, which were 85% (Garg and Mehta 1998), 84% (NRC 2001), 86.1% (Alexander *et al.*2002), 82 to 84.5% (Anonymous 2002, Naik *et al.* 2007) and 70.6% of (Sanz Sampelayo *et al.* 2004) but higher than 40%, reported by Haland *et al.* (1999).

Rumen pH: Rumen pH was in normal physiological limits (6.66–6.86) throughout the treatments, with no significant effect of palm stearin (Table 4). The non-significant effect may be attributed to the highly saturated nature of palm stearin, which has limited interaction with rumen microorganisms and fermentation processes. Unlike unsaturated fats, saturated fatty acids such as palmitic acid are less inhibitory to cellulolytic bacteria and therefore have minimal impact on ruminal pH. This is in accordance to the findings of Ahmed *et al*. (2025). Similar results were obtained by Vandoni *et al.* (2010) who found no significant effect on rumen fermentation parameters by supplementing protected fats. The similarity in results might be due to the rumen-inert characteristics of these fat sources, which minimize their effects on rumen fermentation.

Rumen ammonia-N: Rumen ammonia-N values ranged from 8.90 to 10.52 mg/dL with no significant difference among treatments (P > 0.05). This suggested that palm

Table 3. Effect of palm stearin supplementation with and without sodium bicarbonate on degree of inertness of palm stearin

Time (h)	T_0	T_1	T_2	Period mean	SEM	T	P	T*P
0	96.20	96.10	95.33	95.87^{z}	0.948	0.002	<0.001	0.897
3	92.59	92.99	90.88	92.15^{y}				
6	92.60	89.77	87.77	90.05^{y}				
12	90.04	85.32	82.22	85.86^{x}				
24	84.94	82.10	77.99	81.68^{w}				
48	82.11	79.44	76.33	79.29^{w}				
Mean	89.74^{b}	87.62^{b}	85.08^{a}					

T_0 = Control diet (0% palm stearin); T_1 = 8% palm stearin; T_2 = 8% palm stearin + 1% sodium bicarbonate, fed along with wheat straw.a,b,c: Means with different superscripts within the Treatment Mean row differ significantly ($P< 0.05$). w,x,y,z: Means with different superscripts within a column over time differ significantly ($P< 0.05$). SEM = Standard error of mean, T = Treatment effectP = Period effect and T×P = Treatment × Period interaction

Table 4. Effect of palm stearin supplementation with and without sodium bicarbonate on rumen fermentation parameters

Time (h)	T_0	T_1	T_2	Treatment mean	SEM	T	P	T*P
				pH				
2 h	6.86	6.73	6.66	6.75	0.075	0.881	0.754	0.896
4 h	6.65	6.83	6.86	6.85				
Mean	6.86	6.78	6.76					
				Ammonia nitrogen (mg/dL)				
2 h	9.37	10.43	8.90	9.57	0.264	0.221	0.229	0.728
4 h	10.45	10.52	9.78	10.23				
Mean	9.91	10.48	9.30					
				Lactic acid				
2 h	11.21	10.76	7.59	9.85	0.435	0.174	0.246	0.113
4 h	9.46	7.51	10.42	9.13				
Mean	10.34	9.13	9.01					

SEM = pooled standard error of mean; values apply to all treatment means within a parameter. T = treatment effect; P = sampling time effect; T×P = treatment × sampling time interaction.

stearin supplementation neither altered ruminal protein degradation nor affected the availability of nitrogen for microbial growth. Since palm stearin is almost entirely composed of fatty acids and contains negligible protein, its inclusion in the diet was not expected to contribute additional rumen-degradable nitrogen. Furthermore, the highly saturated nature of palm stearin limits its interaction with rumen microorganisms, thereby preserving normal microbial activity and ammonia production. This was in accordance to the findings of Ahmed *et al.* (2025) who found non-significant changes in rumen ammonia concentration in growing buffaloes supplemented with bypass fat. Teh *et al.* (1994) reported no significant ($p>0.05$) alterations in ruminal pH or NH_3-N levels when bypass fat was incorporated into the diet, suggesting that such supplementation does not interfere with microbial

Table 5. Effect of palm stearin supplementation with and without sodium bicarbonate on volatile fatty acid concentration of rumen

				Acetate (mmol/dL)				
Time (h)	T_0	T_1	T_2	Treatment mean	SEM	T	P	T*P
2 h	6.83	7.71	6.59	7.04	0.326	0.447	0.941	0.411
4 h	8.16	6.80	6.31	7.09				
Mean	7.49	7.26	6.45					
				Propionate (mmol/dL)				
2 h	1.47	1.89	1.75	1.70	0.059	0.223	0.969	0.437
4 h	1.69	1.80	1.62	1.65				
Mean	1.58	1.84	1.68	1.70				
				Butyrate (mmol/dL)				
2 h	0.86	0.68	0.56	0.70	0.034	0.091	0.688	0.247
4 h	0.73	0.61	0.69	0.68				
Mean	0.80	0.65	0.63	0.69				
				TVFA(mmol/dL)				
2 h	9.17	10.28	8.91	9.45	0.358	0.439	0.973	0.412
4 h	10.58	9.23	8.63	9.48				
Mean	9.88	9.76	8.77					
A:P Ratio								
2 h	4.66	4.07	3.84	4.19	0.201	0.181	0.972	0.874
4 h	4.87	3.77	3.88	4.18				
Mean	4.76	3.92	3.86					

fermentation processes. Likewise, Vandoni *et al.* 2010 found that supplementing beef cattle diets with three distinct bypass fat sources including calcium salts (CaS), hydrogenated fatty acids (HF), and triglycerides did not lead to any significant differences in rumen fermentation characteristics or pH levels. The similarity of the present findings with earlier studies indicated that rumen-protected or highly saturated fat source has a minimal effect on nitrogen turnover in the rumen and does not compromise the balance between ammonia production and microbial utilization. Demirel *et al.* (2004) observed no discernible changes in rumen pH when animals were fed three different types of rumen protected fat (RPF).

Rumen lactic acid: Lactic acid levels were not significantly affected (P>0.05). The stability of lactic acid reflects absence of rumen acidosis and indicated that palm stearin did not alter carbohydrate fermentation patterns. Similar findings were suggested by Lende *et al.* (2018) as who found that feeding concentrate mixture supplemented with bypass fat @ 2.5% DM intake separately and in combination had no adverse effect on the rumen fermentation pattern of local goats in terms of pH, ammonia nitrogen and volatile fatty acids.

Volatile fatty acids: The concentration of rumen volatile fatty acids was similar in all the treatments (Table 5). Acetate, propionate and butyrate values remained statistically comparable across treatments (P>0.05). Total VFA concentrations ranged from 8.77 to 9.88 mmol/100 mL across treatments, with no significant treatment or time interaction (P>0.05). The acetate-to-propionate ratio (A:P) remained stable across all groups, with period means between 3.87 and 4.77 and no significant variation due to palm stearin feeding. Ahmed *et al.* (2025) found similar non- significant effect on volatile fatty acid concentration in the rumen of growing buffaloes due to dietary supplementation of palm stearin at 6% level which may be attributed to the highly saturated and rumen-inert nature of palm stearin, which underwent limited ruminal fermentation and therefore had minimal influence on the activity of fermentative microorganisms responsible for VFA production. Since VFA concentrations are primarily determined by the fermentation of dietary carbohydrates, supplementation with palm stearin was unlikely to alter the pattern or extent of fermentation when the basal diet remains unchanged.

The findings indicated that palm stearin (8%) can be considered a practical and potentially cost-effective bypass fat source for dairy farmers and feed manufacturers, particularly for formulating energy-dense rations for high-producing and early-lactation animals. Its inclusion may help improve energy supply, without negatively affecting rumen fermentation, thereby supporting efficient nutritional management and productive performance.

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