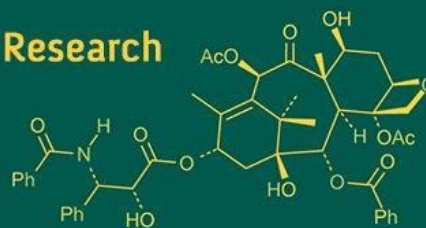


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Effects of palm stearin enriched diets on rumen fermentation in growing buffaloes

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Abstract

The present study aimed to assess the effects of dietary supplementation of palm stearin (PS), a byproduct derived from the palm oil refining industry, on the rumen fermentation parameters in growing buffaloes. Palm stearin, due to its high energy content and availability as a byproduct, offers potential as a low-cost dietary fat source in livestock nutrition. A total of eighteen clinically healthy buffaloes, all under the age of 18 months, were selected for the trial and randomly allocated into three dietary treatment groups: T₀ (control), T₁ (3% PS), and T₂ (6% PS), with six animals in each group. The control group (T₀) received a basal diet without any PS inclusion, whereas the T₁ and T₂ groups were fed concentrate mixtures containing 3% and 6% Palm Stearin, respectively. The feeding trial was conducted for 165 days. All animals were offered a diet consisting of a 50:50 ratio of concentrate to wheat straw, formulated in accordance with ICAR (2013) feeding standard. The study focused on rumen fermentation attributes, which are important indicators of digestive efficiency and microbial health in ruminants. At the end of the feeding period, rumen fluid samples were collected and analysed for key fermentation parameters including pH, ammonia nitrogen (NH₃-N, mg/dL), total volatile fatty acids (mM/dL), and the molar proportions of acetate, propionate, and butyrate, as well as the acetate-to-propionate (A:P) ratio. Results indicated that palm stearin supplementation had no significant ($p>0.05$) effect on any of the rumen fermentation parameters across the groups. These findings suggest that incorporating up to 6% palm stearin in the diet does not negatively influence rumen fermentation in growing buffaloes.

Keywords: Palm Stearin (PS), buffaloes, rumen fermentation, microbes & volatile fatty acid

Introduction

In ruminant nutrition, dietary energy is a critical component influencing growth performance, feed efficiency and overall animal productivity. Traditionally, cereal grains and other carbohydrate rich feedstuffs have served as primary energy sources; however, rising costs and competition with the human food supply have prompted the exploration of alternative, cost-effective energy supplements. One such alternative is the use of lipid based byproducts (Behan *et al.*, 2019) ^[5], which can enhance the energy density of ruminant diets without adversely affecting rumen function when used appropriately (Jenkins, 1993) ^[13].

Palm stearin (PS), a byproduct of the Palm oil refining industry, is a solid fraction obtained during the fractionation of palm oil and represents a promising lipid supplement due to its high energy content (Tomkins & Drackley, 2010) ^[21]. The primary constituents of palm stearin are long-chain saturated fatty acids, with palmitic acid being the most abundant (Norliza *et al.*, 2016) ^[15]. As a byproduct of the palm oil refining industry, PS offers a sustainable approach to utilising agro-industrial waste while potentially improving dietary energy efficiency in livestock production systems. However, the inclusion of dietary fats in ruminant diets must be approached with caution, as excessive unsaturated fats or inappropriate dosages of fat supplementation can disrupt rumen microbial activity (Jenkins, 1993) ^[13] and thereby alter the fermentation patterns and impair fibre digestion in ruminants (Allen, 2000) ^[2].

Rumen fermentation is a complex microbial-driven process that produces volatile fatty acids (VFAs), ammonia nitrogen (NH₃-N) and other metabolites essential for the host animal's energy supply and protein synthesis. Monitoring parameters such as rumen pH, NH₃-N concentration, total VFA levels, and the molar proportions of acetate, propionate, and

butyrate provides valuable insights into the effects of dietary interventions on rumen health and functionality.

Despite the potential advantages of Palm Stearin as a dietary fat source, limited information is available regarding its impact on rumen fermentation characteristics, particularly in buffaloes, a species with growing importance in global meat and milk production. Therefore, the present study was undertaken to evaluate the effects of dietary palm stearin supplementation on rumen fermentation profile in growing buffaloes. This investigation aims to determine whether palm stearin can be safely incorporated into ruminant diets without compromising rumen microbial activity and fermentation efficiency.

Materials and Methods

The current study was conducted to evaluate the impact of dietary supplementation with Palm Stearin, which is a byproduct derived from the palm oil refining process, on the rumen fermentation parameters of growing buffaloes. The experimental trial was performed at the Experimental Animal Shed of the Animal Nutrition Division, ICAR-Indian Veterinary Research Institute (IVRI), located in Izatnagar, Bareilly, Uttar Pradesh. The institute is positioned at a geographical coordinate of 28° 23' 18.98" N latitude and 79° 25' 18.47" E longitude, with an altitude of 240 meters above mean sea level.

Experimental animal, designs and housing

A total of 18 growing buffalo calves were enrolled in the experiment and randomly assigned to one of three dietary treatment groups: T₀ (control), T₁, and T₂, with six calves in each group. To ensure unbiased allocation and balanced distribution among groups, the animals were assigned using randomised block design (RBD). The study was carried out at the Experimental Animal Shed, Animal Nutrition Division, ICAR-Indian Veterinary Research Institute (ICAR-IVRI), located in Izatnagar.

The feeding trial lasted for 165 days. Before the trial began, all calves underwent a routine deworming regimen to eliminate internal parasitic infections. 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 buffalo calves in the control group (T₀) were provided with a standard diet comprising a 50:50 ratio of concentrate and wheat straw, formulated to meet the nutritional requirements recommended by ICAR (2013) feeding standards. In the treatment groups, T₁ and T₂, palm stearin was included in the concentrate mixtures at levels of 3.0% and 6.0%, respectively. This was achieved by partially substituting maize and wheat bran, while maintaining the diets as iso-nitrogenous to ensure a consistent protein content across all groups. The composition of the concentrate mixtures, detailed in Table 1, consisted of maize, deoiled soybean meal (DSBM), wheat bran, mineral mixture, salt, and the respective levels of palm stearin. To support vitamin A intake, green fodder was supplied twice a week. Clean and fresh drinking water was made available ad

libitum, with routine distribution occurring twice daily at 10:00 AM and 3:00 PM throughout the duration of the study.

Table 1: Composition of the concentrate mixture of experimental feed

Ingredients (%)	Concentrate Mixture		
	T ₀	T ₁	T ₂
Maize	33	30	27
DSBM	19	20	21
Wheat Bran	45	44	43
Mineral Mixture	2	2	2
Salt	1	1	1
Palm stearin	0	3	6

Rumen liquor collection and analysis

Rumen liquor samples were collected from the experimental buffaloes on the last day of the feeding trial using the stomach tube technique, and the samples were preserved at -20°C for subsequent analysis. Rumen pH was measured immediately after sample collection using a precalibrated digital pH meter. A portion of each rumen liquor sample was preserved with two drops of 20% sulfuric acid (H₂SO₄) for the determination of ammonia nitrogen (NH₃-N), following the method outlined by Weatherburn (1967) [23]. The concentration of volatile fatty acids (VFAs) in the rumen liquor was analysed using a Nucon-5765 gas chromatograph fitted with a dual flame ionization detector and a glass column packed with Chromosorb 101, as per the protocol described by Cottyn and Boucque (1968) [8].

Statistical analyses

Statistical analyses were performed in accordance with the standard procedures described by Snedecor and Cochran (2004), utilising SPSS software (version 26.0). All tests were evaluated at a significance threshold of $p < 0.05$.

Results

pH and ammonia nitrogen

The data on pH and ammonia nitrogen (NH₃-N) levels in relation to the effects of PS on rumen fermentation are presented in Table 2. The pH values were similar across the dietary groups, with recorded levels of 6.42 in T₀, 6.40 in T₁, and 6.38 in T₂. Statistical analysis indicated no significant differences ($p > 0.05$) in pH values among the treatment groups compared to the control. Similarly, NH₃-N concentrations (mg/dL) in T₀, T₁, and T₂ were 12.38, 12.31, and 12.24, respectively. The statistical evaluation confirmed that NH₃-N levels were comparable across all dietary groups, with no significant differences ($p > 0.05$) observed in comparison to the control group.

Table 2: Effect of feeding PS on the pH and NH₃-N in Murrah buffaloes

Attributes	T ₀	T ₁	T ₂	SEM	P Value
pH	6.42±0.10	6.40±0.13	6.38±0.09	0.06	0.977
NH ₃ -N (mg/dL)	12.38±0.19	12.31±0.27	12.24±0.30	0.14	0.932

Control (T₀), Treatment (T₁) & Treatment (T₂): Dietary groups having concentrate containing (0, 3 & 6% PS) along with wheat straw in the diet.

NH₃-N-ammonia nitrogen, mg/dL-milligrams per deciliter.

Total volatile fatty acid and its fractions

Table 3 presents data on total volatile fatty acids (TVFA) and their fractions. The mean TVFA concentrations (mM/dL) were comparable across the treatment groups, with values of 9.58 mM/dL in T₀, 9.49 mM/dL in T₁, and 9.45 mM/dL in T₂. Statistical analysis revealed no significant differences ($p>0.05$) among the dietary treatments. Similarly, acetate percentages were recorded as 72.40% in T₀, 71.88% in T₁, and 71.21% in T₂, with no statistically significant differences ($p>0.05$) observed between groups. The propionate proportions were 17.46% in T₀, 17.69% in T₁, and 18.10% in T₂, with statistical analysis confirming no significant variation ($p>0.05$) across treatments. Additionally, butyrate levels were measured at 6.37% in T₀, 6.31% in T₁, and 6.30% in T₂, showing no significant differences ($p>0.05$) among the groups. Furthermore, the acetate-to-propionate (A:P) ratio was assessed and remained statistically similar across treatments. No significant differences ($p>0.05$) were detected in the A:P ratio, indicating that dietary supplementation with PS did not significantly ($p>0.05$) influence this parameter.

Table 3: Effect of PS on the Total volatile fatty acid and its fractions

Attributes	T ₀	T ₁	T ₂	SEMP	P Value
TVFA (mM/dL)	9.58±0.12	9.49±0.12	9.45±0.11	0.06	0.717
Acetate (%)	72.40±0.63	71.88±0.48	71.21±0.79	0.37	0.446
Propionate (%)	17.46±0.65	17.69±0.55	18.10±0.83	0.38	0.798
Butyrate (%)	6.37±0.40	6.31±0.23	6.30±0.37	0.19	0.987
A:P ratio	4.18±0.19	4.09±0.16	3.98±0.22	0.11	0.767

Control (T₀), Treatment (T₁) & Treatment (T₂): Dietary groups having concentrate containing (0, 3 & 6% PS) along with wheat straw in the diet.

TVFA-Total volatile fatty acid, mM/dL-millimoles per decilitre, A:P ratio-Acetate to Propionate ratio.

Discussions

pH and Ammonia nitrogen (NH₃-N)

In the present study, dietary supplementation with PS did not significantly affect rumen pH levels, as the values remained comparable across all treatment groups. Similarly, the ammonia nitrogen (NH₃-N) concentrations were consistent across treatments, with no significant variations ($p>0.05$) observed. These findings align with previous studies that have investigated the effects of various fat supplements on rumen fermentation parameters.

Teh *et al.* (1994) [20] reported no significant ($p>0.05$) alterations in ruminal pH or NH₃-N levels when bypass fat was incorporated into the diet, suggesting that such supplementation does not interfere with microbial fermentation processes. Likewise, Vandoni *et al.* (2010) [22] found that supplementing beef cattle diets with three distinct bypass fat sources—calcium salts (CaS), hydrogenated fatty acids (HF), and triglycerides did not lead to any significant differences in rumen fermentation characteristics or pH levels. Similar findings were reported by Demirel *et al.* (2004) [9], who observed no discernible changes in rumen pH when animals were fed three different types of rumen-protected fat (RPF).

Behan *et al.* (2019) [5] also demonstrated that supplementation with prill fat had no significant ($p>0.05$) impact on ruminal pH or NH₃-N concentrations, further supporting the notion that fat-based dietary interventions do

not disrupt the ruminal fermentation environment. Szumacher-Strabel *et al.* (2001) [19] reported comparable results in rams supplemented with 6% dietary tallow and Bergafat (a fat source rich in palmitic acid (C16:0) and stearic acid (C18:0)), where ammonia-N levels remained unaffected. Similarly, Behan *et al.* (2019) [5] observed no significant changes in ammonia nitrogen concentrations when animals were fed 5% dietary prilled fat containing 72.98% palmitic acid.

Conversely, Bae *et al.* (2023) [3] investigated the effects of palmitic and stearic acid supplementation in lamb diets and found that while these fatty acids did not alter rumen pH, ammonia nitrogen concentrations significantly increased ($p<0.05$) in lambs fed 3% stearic acid or a mixture of palmitic and stearic acids. However, other studies, such as that by Wu *et al.* (2016) [24], reported no significant changes in rumen pH or ammonia nitrogen levels following bypass fat supplementation.

In addition to these studies, several other researchers have reported similar findings, highlighting the lack of significant changes in rumen fermentation parameters following protected fat supplementation. Research by Ngidi *et al.* (1990) [14], Bhatt *et al.* (2013) [6], Schauff *et al.* (1992) [18], Hightshoe *et al.* (1991) [11], Grummer *et al.* (1988) [10], and Bhatt *et al.* (2011) [7] further corroborates that ruminal pH remains stable across different dietary treatments, reinforcing the consensus that fat supplementation in recommended levels does not adversely affect rumen fermentation dynamics.

These collective findings suggest that fat supplementation at recommended levels, including PS, does not significantly influence ruminal pH or ammonia nitrogen concentrations, thus maintaining normal fermentation processes in ruminants.

Volatile fatty acid and its fractions (VFA)

The findings of the present study indicate that dietary supplementation with PS did not lead to any statistically significant ($p>0.05$) alterations in total volatile fatty acid (TVFA) concentrations or their individual fractions. The mean TVFA levels remained consistent across all treatment groups, demonstrating that the dietary intervention had no substantial impact on ruminal fermentation. Furthermore, the proportions of acetate, propionate and butyrate were stable among the treatment groups, with no significant differences ($p>0.05$) detected. Similarly, the acetate-to-propionate (A:P) ratio was comparable to that of the control group, with statistical analysis confirming the absence of significant ($p>0.05$) variations.

These results align with previous research that has examined the impact of fat supplementation on ruminal fermentation. Behan *et al.* (2019) [5] reported that TVFA concentrations were uniform across all treatment groups, consistent with the earlier findings of Hightshoe *et al.* (1991) [11]. Hightshoe *et al.* (1991) [11] also demonstrated that neither the source nor the level of rumen-protected fat (RPF) significantly influenced TVFA concentrations or the A:P ratio in steers fed calcium soaps of fatty acids and hydrogenated animal fats. Similarly, Demirel *et al.* (2004) [9] observed no significant differences in ruminal pH and VFA concentrations when animals were supplemented with three different types of rumen-protected fats (RPF).

Patra and Yu (2012) [16] further corroborated these findings by demonstrating that increasing dietary fat levels did not

significantly ($p>0.05$) affect TVFA concentrations or acetate proportions. In a subsequent study, Patra (2013) [17] confirmed that neither TVFA levels nor acetate percentages in ruminal fluid were significantly influenced ($p>0.10$) by dietary fat supplementation. However, a linear increase in propionate levels ($P=0.03$) was observed with higher fat inclusion. Additionally, dietary fats exhibited a tendency to reduce butyrate proportions ($P=0.10$) and the A:P ratio ($P=0.07$), though these effects were relatively weak.

More recently, Bae *et al.* (2023) [3] supplemented lamb diets with palmitic and stearic acids and found that TVFA concentrations remained stable across treatment groups. Similarly, Wu *et al.* (2016) observed no significant changes in acetate, propionate or butyrate levels following bypass fat supplementation. Alexander *et al.* (2002) [1] also reported no significant alterations in TVFA concentrations in sheep fed with protected fat. Baumann *et al.* (2016) [4] examined the effects of a 450 g/day saturated fatty acid (SFA) supplement (containing 36% palmitic acid (C16:0) and 40% stearic acid (C18:0)) directly administered into the rumen and found that neither total nor individual VFA concentrations were affected.

Szumacher-Strabel *et al.* (2002) [19] investigated the inclusion of bypass fat in dairy sheep diets and reported no significant effects on individual VFA levels at a 4% dietary inclusion rate (containing 47-53% palmitic acid and 39-44% stearic acid). However, at a 6% inclusion rate, a significant increase in the molar proportion of acetate was observed compared to the control group, suggesting that higher levels of supplementation might influence specific VFA ratios. Furthermore, *in vitro* studies conducted by Hristov *et al.* (2004) [12] demonstrated that palmitic and stearic acids did not exert significant effects on total or individual VFA concentrations.

Collectively, these studies support the findings of the present research, indicating that ruminal fermentation parameters, including $\text{NH}_3\text{-N}$, TVFA and the molar proportions of propionate and butyrate, remain largely unaffected by dietary fat supplementation. The lack of significant changes suggests that rumen protected fat (RPF) remains inert within the rumen environment and does not disrupt microbial activity at the tested inclusion levels. These results further reinforce the hypothesis that fat based dietary interventions, including PS supplementation, do not adversely impact ruminal fermentation dynamics, as supported by Behan *et al.* (2019) [5].

Conclusion

The findings of the present study indicate that supplementation with palm stearin does not significantly influence rumen fermentation characteristics in ruminants. Specifically, measurements of rumen pH, ammonia nitrogen ($\text{NH}_3\text{-N}$) concentration, total volatile fatty acid (VFA) concentrations, and the molar proportions of individual VFAs namely acetate, propionate, and butyrate remained statistically unaltered among the different dietary treatment groups ($p>0.05$). Additionally, the ratio of acetate to propionate, a commonly used indicator of fermentative balance and energy metabolism in the rumen, was not significantly affected by palm stearin supplementation. These results suggest that the supplementation of ruminant diets with palm stearin does not interfere with the normal microbial activity or fermentation dynamics within the

rumen, suggesting its potential as a viable dietary fat source in ruminant nutrition.

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Declaration of Competing Interest

We certify that there are no competing interests of financial or personal nature with regard to the present experiment.

References

- Alexander BM, Hess BW, Hixon DL, Garrett BL, Rule DC, McFarland M, *et al.* Influence of prepartum fat supplementation on subsequent beef cow reproduction and calf performance. *The Professional Animal Scientist*. 2002 Dec;18(4):351-357.
- Allen MS. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *Journal of Dairy Science*. 2000 Jul;83(7):1598-1624.
- Bae H, Ghoorchi T, Toghdory A, Mokhtarpour A. Growth performance, ruminal fermentation characteristics and microbial protein synthesis of lambs fed palmitic and stearic acid. *Animal Feed Science and Technology*. 2023 Aug;302:115674.
- Baumann E, Chouinard PY, Lebeuf Y, Rico DE, Gervais R. Effect of lipid supplementation on milk odd- and branched-chain fatty acids in dairy cows. *Journal of Dairy Science*. 2016 Aug;99(8):6311-6323.
- Behan AA, Loh TC, Fakurazi S, Kaka U, Kaka A, Samsudin AA. Effects of supplementation of rumen protected fats on rumen ecology and digestibility of nutrients in sheep. *Animals*. 2019 Jun;9(7):400.
- Bhatt RS, Sahoo A, Shinde AK, Karim SA. Change in body condition and carcass characteristics of cull ewes fed diets supplemented with rumen bypass fat. *Livestock Science*. 2013 Oct;157(1):132-140.
- Bhatt RS, Soren NM, Tripathi MK, Karim SA. Effects of different levels of coconut oil supplementation on performance, digestibility, rumen fermentation and carcass traits of Malpura lambs. *Animal Feed Science and Technology*. 2011 Feb;164(1-2):29-37.
- Cottyn BG, Boucque CV. Rapid method for the gas-chromatographic determination of volatile fatty acids in rumen fluid. *Journal of Agricultural and Food Chemistry*. 1968 Jan;16(1):105-107.
- Demirel G, Wachira AM, Sinclair LA, Wilkinson RG, Wood JD, Enser M. Effects of dietary n-3 polyunsaturated fatty acids, breed and dietary vitamin E on the fatty acids of lamb muscle, liver and adipose tissue. *British Journal of Nutrition*. 2004 Apr;91(4):551-565.
- Grummer RR. Influence of prilled fat and calcium salt of palm oil fatty acids on ruminal fermentation and nutrient digestibility. *Journal of Dairy Science*. 1988 Jan;71(1):117-123.
- Hightshoe RB, Cochran RC, Corah LR, Kiracofe GH, Harmon DL, Perry RC. Effects of calcium soaps of fatty acids on postpartum reproductive function in beef cows. *Journal of Animal Science*. 1991 Oct;69(10):4097-4103.

12. Hristov AN, Ivan M, McAllister TA. *in vitro* effects of individual fatty acids on protozoal numbers and on fermentation products in ruminal fluid from cattle fed a high-concentrate, barley-based diet. *Journal of Animal Science*. 2004 Sep;82(9):2693-2704.
13. Jenkins TC. Lipid metabolism in the rumen. *Journal of Dairy Science*. 1993 Dec;76(12):3851-3863.
14. Ngidi ME, Loerch SC, Fluharty FL, Palmquist DL. Effects of calcium soaps of long-chain fatty acids on feedlot performance, carcass characteristics and ruminal metabolism of steers. *Journal of Animal Science*. 1990 Aug;68(8):2555-2565.
15. Norliza S, Zahariah I, Ahmadilfitri MN, Mohd Suria AY, Mardhati M, Wong HK. Effect of feeding fractionated RBD palm stearin on milk yield and quality of dairy cattle. *Malaysian Journal of Animal Science*. 2016 Jun;19(1):9-13.
16. Patra AK, Yu Z. Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. *Applied and Environmental Microbiology*. 2012 Jun;78(12):4271-4280.
17. Patra AK. The effect of dietary fats on methane emissions, and its other effects on digestibility, rumen fermentation and lactation performance in cattle: A meta-analysis. *Livestock Science*. 2013 Aug;155(2-3):244-254.
18. Schauff DJ, Clark JH, Drackley JK. Effects of feeding lactating dairy cows diets containing extruded soybeans and calcium salts of long-chain fatty acids. *Journal of Dairy Science*. 1992 Nov;75(11):3003-3019.
19. Szumacher-Strabel M, Cieslak A, Potkanski A, Kowalczyk J, Czauderna M. The effects of different amounts and types of fat on rumen microbial protein synthesis in sheep. *Journal of Animal and Feed Sciences*. 2001 Jun;10:97-102.
20. Teh TH, Trung LT, Jia ZH, Gipson TA, Ogden KB, Sweeney TF. Varying amounts of rumen-inert fat for high producing goats in early lactation. *Journal of Dairy Science*. 1994 Jan;77(1):253-258.
21. Tomkins T, Drackley JK. Applications of palm oil in animal nutrition. *Journal of Oil Palm Research*. 2010 Dec;22(3):835-845.
22. Vandoni SL, Dell'Orto V, Sgoifo Rossi CA. Effects of administration of three different by-pass lipids on growth performance, rumen activity and feeding behaviour of beef cattle. *Italian Journal of Animal Science*. 2010 Feb;9(2):e44.
23. Weatherburn M. Phenol-hypochlorite reaction for determination of ammonia. *Analytical Chemistry*. 1967 Aug;39(8):971-974.
24. Wu D, Xu L, Tang S, Guan L, He Z, Guan Y, *et al.* Influence of oleic acid on rumen fermentation and fatty acid formation *in vitro*. *PLoS One*. 2016 Jun;11(6):e0156835.