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Effect of Palm Stearin Supplementation on the Haematological and Blood Biochemical Indices in Growing Buffaloes

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The present study aimed to assess the impact of Palm stearin (PS), a byproduct of palm oil refining industry on the haematological and blood biochemical profile of growing buffaloes. Eighteen healthy buffalo calves were randomly assigned to three dietary treatment groups (n=6 per group): T_0 (control), T_1, and T_2. The control group (T_0) was provided a basal diet without PS, while the T_1 and T_2 groups received concentrate mixtures supplemented with 3% and 6% PS, respectively. The feeding trial was conducted over 165 days and during which all animals were fed a balanced diet consisting of a 50:50 ratio of concentrate mixture and wheat straw, formulated

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according to the Indian Council of Agricultural Research (ICAR, 2013) standard. To fulfil the vitamin A requirements, each animal also received a small amount (1–2 kg) of green fodder twice a week. Blood samples were collected at three time points: day 0, day 80, and day 160 and the samples were analysed for various haematological and biochemical parameters. The results showed that supplementation with Palm stearin (PS) had no significant effect ($P > 0.05$) on most of the blood parameters including haemoglobin (Hb), packed cell volume (PCV), glucose, triglycerides, total protein, albumin, globulin, albumin-to-globulin ratio (A: G), urea, high density lipoprotein (HDL), low density lipoprotein (LDL), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). However, a significant increase ($P < 0.001$) in Total Cholesterol was observed in the groups supplemented with PS. These findings indicate that dietary addition of Palm stearin (PS) at levels of 3% and 6% in the concentrate mixture (approximately 1.5% and 3% of the total diet) alters cholesterol levels without negatively impacting liver function or other key blood indices. Thus, Palm stearin (PS) appears to be a safe and potentially beneficial fat source for growing buffaloes thereby offering a sustainable alternative energy source in the livestock feeding strategies.

Keywords: Byproduct; palm stearin (PS); growing buffaloes; haematological; blood biochemical parameters.

1. INTRODUCTION

Energy plays a vital role in sustaining the metabolic activities and productivity of ruminants. Due to the high energy demands associated with the production processes of animal products such as milk and meat, as well as the increased energy demand during pregnancy, the conventional energy sources in ruminant diets often fall short of meeting the elevated energy requirements. This shortfall can result in decreased animal performance and a higher risk of metabolic disorders leading to high economic losses. One effective approach to addressing this shortfall is by incorporating fat into the diet, as fats deliver approximately 2.25 times more energy per unit compared to carbohydrates (Behan et al., 2019; Tomkins & Drackley, 2010).

The selection of dietary fat sources is important, as the specific fatty acid profile can significantly affect digestion, nutrient absorption, metabolism and overall animal performance. While animal derived fats are high in energy content but their uses are often limited by high costs and competition with human consumption. This has led to a growing interest in alternative fat sources for ruminants, particularly from agro-industrial byproducts such as Palm stearin (PS). Derived from the palm oil refining process, Palm stearin is increasingly being considered a practical and economical fat supplement in ruminant diets. It offers a rich supply of essential fatty acids and significantly enhances the energy density of feeds (Tomkins & Drackley, 2010).

Palm stearin is mainly composed of long-chain saturated fatty acids, with palmitic acid being the predominant component. Due to their high melting point and chemical stability, these fats are unaffected by rumen microbial activity and thus allowing them to bypass ruminal degradation and making it a suitable energy source for ruminants (Norliza et al., 2016). Its efficient absorption and metabolism contribute to faster growth rates, improved milk production, enhanced milk quality, better reproductive outcomes, and reduced thermal stress in ruminants. Moreover, in monogastric animals such as pigs, fat-enriched diets have been associated with faster growth rates, better reproductive performance, and increased lactation efficiency. Owing to its nutritional benefits and cost-effectiveness, Palm stearin emerges as a promising alternative for enhancing animal health and productivity (Vranković, et al., 2018).

In addition to supplying energy, the dietary fat also influences key blood biochemical indicators, which reflect the metabolic health, liver function and lipid metabolism of livestock. Monitoring these parameters in response to Palm stearin supplementation can offer valuable insights into its physiological effects and nutritional potential. Consequently, this study aims to evaluate the impact of Palm stearin inclusion in ruminant diets on blood haematological and biochemical profiles, thereby providing a deeper understanding of its role in promoting animal health and production efficiency.

2. MATERIALS AND METHODS

2.1 Experimental Location

The present study was undertaken to investigate the effects of dietary inclusion of Palm stearin

(PS), a byproduct of palm oil refining on the haematological and blood biochemical profile of growing buffaloes. The experimental trial was carried out at the Experimental Animal Shed of the Animal Nutrition Division, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh. The research facility is geographically situated at 28° 23′ 18.98″ N latitude, 79° 25′ 18.47″ E longitude, at an elevation of 240.00 meters above mean sea level.

2.2 Experimental Animal, Designs and Housing

A total of 18 growing buffalo calves were selected for the trial. The animals were randomly allocated into three dietary treatment groups—T_0 (control), T_1, and T_2—with six calves in each group. The allocation was carried out using a Randomized block design (RBD) to eliminate selection bias and ensure uniform distribution across the groups. The experimental study was conducted at the Experimental Animal Shed of the Animal Nutrition Division, ICAR-Indian Veterinary Research Institute (ICAR-IVRI), Izatnagar.

The feeding trial extended over a period of 165 days. Prior to the commencement of the study, all calves were subjected to a standard deworming protocol to eliminate internal parasites. Additionally, the animals were maintained under well-ventilated, clean, and hygienic housing conditions to promote animal welfare and minimize environmental stressors that could affect the experimental outcomes. These preparatory measures were undertaken to ensure that the health status of the calves remained optimal throughout the duration of the study, thereby allowing for accurate assessment of the dietary treatments under investigation.

2.3 Feeding Management

The growing buffaloes in the control group (T_0) were fed a standard diet consisting of a 50:50 ratio of concentrate and wheat straw, formulated to meet their nutritional requirement in accordance with ICAR (2013) feeding standards. In the experimental groups (T_1 and T_2), Palm stearin was incorporated into the concentrate mixture at 3.0% and 6.0%, respectively, by partially replacing maize and wheat bran, while ensuring the diets remained iso –nitrogenous. The formulation of the concentrate mixture followed the composition outlined in Table 1, containing maize, de-oiled soybean meal (DSBM), wheat bran, a mineral mixture, salt and varying levels of Palm stearin Additionally, green fodder was provided biweekly to fulfil the vitamin A requirements. Fresh and clean drinking water was supplied ad libitum twice daily at 10:00 AM and 3:00 PM throughout the experimental period.

2.4 Blood Sample Collection and Analysis

Blood samples were collected on day 0^{th}, day 80^{th}, and 160^{th} of the experimental period for the analysis of various haematological and blood biochemical parameters. A total of 10 ml of blood was drawn from each animal via the jugular vein in the morning, before feeding and watering. Of the 10 ml collected, 2 ml was placed in a clean, dry EDTA-coated vacutainer for haemoglobin and haematocrit measurement, while the remaining 8 ml was transferred into a clean, dry test tube and allowed to stand at an angle for 5-6 hours to separate the serum. The vacutainer tubes were then centrifuged at 2000 rpm for 10 minutes at 4°C. The resulting serum was transferred into Eppendorf tubes, labelled, and stored in a deep freezer for subsequent analysis of the blood biochemical parameters.

In this study, the whole blood was used for the estimation of hematological parameters, however, all the blood biochemical parameters were analysed from harvested serum. The biochemical parameters were analyzed spectrophotometrically (Multiskan™ FC Microplate Photometer with incubator, Thermo-scientific Ltd.) by using diagnostic kits of Coral Clinical Systems-A Division of Tulip diagnostics (P) Ltd. following the standard protocol.

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 (PS)	0	3	6

2.5 Statistical Analysis

The statistical analyses followed the standard methodology outlined by Snedecor and Cochran (2004) and were conducted using the SPSS software (version 26.0). A significance level of $P < 0.05$ was applied to all statistical tests.

3. RESULTS AND DISCUSSIONS

3.1 Haematological Profile

The haemoglobin concentrations and packed cell volume (PCV) percentages of the experimental animals are presented in Table 2. In the current study, with the inclusion of Palm stearin (PS) supplementation, haemoglobin (Hb) levels (g/dL) ranged from 11.17 to 12.39. The mean Hb values (g/dL) for the treatment groups T_0, T_1, and T_2 were recorded as 11.83, 12.03, and 12.23, respectively. Over the course of the trial, the haemoglobin levels (g/dL) measured were 11.63 at day 0^{th}, increasing to 12.07 by day 80^{th} and reaching 12.39 at day 160^{th}. The Hb concentrations remained comparable across the dietary treatments, with no statistically significant differences observed over time, nor was there any significant interaction between treatment and trial duration.

Similarly, the packed cell volume (PCV%) in the present study ranged from 33.50 to 38.30. The mean PCV values (%) for the treatment groups T_0, T_1, and T_2 were recorded as 35.50, 36.08, and 36.68, respectively. Over the course of the trial, the mean PCV values (%) were 34.90 at day 0, increasing to 36.20 by day 80 and reaching 37.17 at day 160. The PCV levels remained comparable across all dietary treatments, with no statistically significant differences observed over time. Additionally, no significant interaction effects were detected between treatment and trial duration. These findings align with previous research, including the study by Tyagi et al. (2010), which reported no significant ($P>0.05$) variation in haemoglobin levels in cows supplemented with bypass fat. Similarly, Savsani et al. (2015) observed no significant changes in haemoglobin and PCV levels in postpartum Jaffrabadi buffaloes following bypass fat supplementation. Katiyar et al. (2019) also reported that bypass fat supplementation in lactating Murrah buffaloes did not result in statistically significant ($P>0.05$) alterations in haemoglobin concentrations. Furthermore, Singh et al. (2016) found no significant ($P>0.05$) differences in haemoglobin levels between the control and treatment groups. In the present study, the supplementation of Palm stearin maintained stable haematological parameters, suggesting its potential role in supporting the overall health status of the animals.

3.2 Serum Biochemical Profile Related to Energy Metabolism

3.2.1 Blood glucose and triglyceride levels

Table 3 present the serum biochemical parameters related to energy metabolism, specifically glucose and triglycerides. Following Palm stearin (PS) supplementation, serum glucose levels (mg/dL) ranged from 62.95 to 67.49. The mean serum glucose concentrations (mg/dL) for the treatment groups T_0, T_1, and T_2 were 63.89, 65.06, and 66.40, respectively. Across the trial period, glucose levels (mg/dL) were recorded as 63.62 on day 0, 66.19 on day 80, and 65.56 on day 160. These findings are consistent with previous studies by Tyagi et al. (2009a) and Wadhwa et al. (2012), which reported no effect of bypass fat supplementation on blood glucose levels. Similarly, Katiyar et al. (2019) observed no significant differences ($P>0.05$) in glucose levels among groups at different time points, which was also in agreement with the study of Ranjan *et al*. (2012); Shelke et al. (2012a) in buffaloes. Ranjan et al. (2012) further reported that bypass fat supplementation in lactating buffaloes did not significantly alter glucose concentrations.

Similarly, serum triglyceride concentrations (mg/dL) ranged from 27.41 to 41.21. The mean triglyceride levels (mg/dL) for T_0, T_1, and T_2 were 33.07, 34.10, and 35.96, respectively. Over the trial period, triglyceride concentrations were 28.45 mg/dL on day 0, increasing to 36.71 mg/dL on day 80 and 37.96 mg/dL on day 160. While no significant differences ($P>0.05$) were observed between dietary treatments, triglyceride concentrations showed a significant increase ($P<0.001$) over time, particularly on days 80^{th} and 160^{th} compared to day 0^{th}. However, no significant interaction effects were detected between treatment and time for serum triglyceride values. These results are in agreement with Katiyar et al. (2019), who reported no significant variation ($P>0.05$) in triglyceride levels after supplementation with rumen-protected fat (RPF), with overall mean values remaining consistent across groups. Tyagi et al. (2009a) also reported no effect of

Table 2. Effect of different levels of dietary PS on HB and PCV profile in growing buffaloes

Attributes	Dietary groups			Period mean	SEM	P value		
Hb (g/dL)	**T_0**	**T_1**	**T_2**			**Treatment**	**Period**	**Treatment*Period**
0th day	11.17±0.61	12.08±0.47	11.65±0.66	11.63±0.33				
80th day	12.03±0.40	11.90±0.56	12.27±0.47	12.07±0.26	0.16	0.613	0.172	0.721
160th day	12.30±0.32	12.10±0.42	12.77±0.33	12.39±0.21				
Treatment man	11.83±0.28	12.03±0.27	12.23±0.30					
PCV (%)								
0th day	33.50±1.66	36.25±1.11	34.95±2.02	34.90±0.93				
80th day	36.10±1.21	35.70±1.01	36.80±1.33	36.20±0.65				
160th day	36.90±0.95	36.30±1.45	38.30±1.26	37.17±0.70	0.46	0.576	0.138	0.673
Treatment mean	35.50±0.79	36.08±0.66	36.68±0.92					

Control (T_0), Treatment (T_1) &Treatment (T_2): Dietary groups having concentrate containing (0, 3 & 6 % PS) along with wheat straw in the diet.

Table 3. Effects of different levels of dietary PS on serum biochemicals related to energy metabolism in growing buffaloes

Attributes	Dietary groups			Period mean	SEM	P value		
Glucose (mg/dL)	**T_0**	**T_1**	**T_2**			**Treatment**	**Period**	**Treatment*Period**
0th day	62.95±1.34	63.29±3.63	64.62±3.39	63.62±1.62	0.82			
80th day	64.28±1.99	66.79±2.57	67.49±4.12	66.19±1.68		0.497	0.452	0.993
160th day	64.45±1.41	65.12±1.50	67.10±1.25	65.56±0.80				
Treatment mean	63.89±0.89	65.06±1.51	66.40±1.74					
Triglycerides (mg/dL)								
0th day	30.10±4.00	27.85±1.57	27.41±1.67	28.45±1.47^{P}	0.95	0.301	<0.001	0.331
80th day	34.54±1.72	36.33±2.40	39.27±1.54	36.71±1.14^{Q}				
160th day	34.55±1.48	38.11±2.80	41.21±2.19	37.96±1.38^{Q}				
Treatment mean	33.07±1.53	34.10±1.66	35.96±1.78					

Control (T_0), Treatment (T_1) &Treatment (T_2): Dietary groups having concentrate containing (0, 3 & 6 % PS) along with wheat straw in the diet.

^{PQ} *Means with different superscripts within a column differ significantly.*

supplementation on blood triglyceride levels, while Wadhwa et al. (2012) noted an increase due to bypass fat supplementation. Our observations also align with the findings of Delbecchi et al. (2001) in crossbred heifers and Shelke et al. (2012a) in buffaloes. Other studies, including those by Singh et al. (2014) and Kirovski et al. (2015) also found no significant differences in triglyceride levels with bypass fat feeding. Furthermore, research by Grewal et al. (2014) and Thakur & Shelke (2010) indicated no significant effects on triglyceride concentrations following supplementation with bypass fat and protein.

3.3 Serum Biochemical Profile Related to Protein Metabolism

The data on serum total protein, albumin, globulin, albumin-to-globulin (A:G) ratio, and urea concentrations are presented in Table 4. In the present study, following Palm stearin (PS) supplementation, the mean total protein concentrations (g/dL) in the treatment groups T_0, T_1 and T_2 were 8.23, 8.28, and 8.39. Over the course of the trial, total protein levels (g/dL) were recorded as 7.69 on day 0, 8.59 on day 80, and 8.61 on day 160. Statistical analysis revealed no significant differences ($P>0.05$) in total protein concentrations among dietary treatments. However, a significant increase ($P<0.001$) was observed on day 80^{th} and day 160^{th} compared to day 0^{th}. Additionally, there was no significant interaction between treatment and time for total protein levels. These findings are consistent with those of Rajan et al. (2012), who reported a similar significant increase ($P<0.001$) in total protein levels over time without any significant ($P>0.05$) differences among treatment groups when bypass fat was supplemented to lactating Murrah buffaloes. Similarly, Katiyar et al. (2019) found that bypass fat supplementation in lactating Murrah buffaloes had no significant effect on total protein concentrations ($P>0.05$). Wadhwa et al. (2012) also reported no improvement in total protein levels in Murrah buffaloes and crossbred cows supplemented with bypass fat. The observed increase in serum protein levels in the present study may be attributed to a positive energy balance resulting from improved nutritional status.

The mean serum albumin concentrations (g/dL) for treatment groups T_0, T_1, and T_2 were 3.69, 3.80, and 3.81, respectively, while the period-wise values were 3.45, 3.94, and 3.90 on days 0, 80, and 160. Similarly, mean serum globulin levels (g/dL) for T_0, T_1, and T_2 were 4.54, 4.47, and 4.57, respectively, while the corresponding values at different time points were 4.24,4.65, and 4.71. Statistical analysis indicated no significant differences ($P>0.05$) in serum albumin and globulin concentrations among treatment groups at any time point. Furthermore, no significant treatment-period interactions were observed. Serum albumin and globulin levels remained within the normal physiological range throughout the experimental period, with no significant differences observed among the treatment groups. These results align with those of the findings of Mane et al. (2016), who found no significant effect on albumin and globulin levels following bypass fat supplementation. Similar findings were reported by Shelke (2010), who concluded that bypass fat supplementation did not influence albumin concentrations. Additionally, Kirovski et al. (2015) observed no significant changes in albumin levels with bypass fat supplementation. Katiyar et al. (2019) also noted that serum globulin levels did not differ significantly ($P>0.05$) among treatment groups, a finding supported by Wadhwa et al. (2012), who reported no variations in globulin concentrations in crossbred cattle supplemented with bypass fat.

In the present study, mean blood urea concentrations (mg/dL) were 35.95, 35.52, and 34.74 for treatment groups T_0, T_1, and T_2 respectively, with no significant differences compared to the control group. Similarly, blood urea levels recorded on days 0, 80, and 160 were 34.29, 35.73, and 36.20 mg/dL, respectively, with no significant variations observed across the trial period ($P>0.05$). Additionally, no significant treatment-period interactions were noted for blood urea levels throughout the experimental duration. Furthermore, serum urea levels were not significantly affected by supplementation. Similar results were reported by Wadhwa et al. (2012) and Kirovski et al. (2015), both of whom found no significant changes ($P>0.05$) in blood urea nitrogen (BUN) levels following bypass fat supplementation. Mane et al. (2016) also observed no significant differences in BUN levels in crossbred cattle fed bypass fat. Likewise, Garg et al. (2012) reported a non-significant effect on BUN levels with bypass fat supplementation. However, in contrast to these findings, Yadav et al. (2015) reported a significant reduction in BUN levels following prilled fat supplementation in crossbred cows.

Table 4. Effects of different levels of dietary PS on serum biochemical profile related to protein metabolism in growing buffaloes

Attributes	Dietary groups			Period mean	SEM	P value		
Total Protein (g/dL)	**T_0**	**T_1**	**T_2**			**Treatment**	**Period**	**Treatment*Period**
0th day	7.80±0.06	7.58±0.09	7.69±0.05	7.69±0.04^P	0.09	0.660	<0.001	
80th day	8.46±0.21	8.59±0.16	8.73±0.09	8.59±0.09^Q				0.801
160th day	8.42±0.27	8.68±0.38	8.74±0.33	8.61±0.18^Q				
Treatment mean	8.23±0.13	8.28±0.18	8.39±0.16					
Albumin (g/dL)								
0th day	3.48±0.30	3.39±0.39	3.48±0.33	3.45±0.19	0.09	0.845	0.074	0.983
80th day	3.82±0.12	4.01±0.23	4.00±0.27	3.94±0.12				
160th day	3.76±0.24	3.99±0.42	3.96±0.13	3.90±0.16				
Treatment mean	3.69±0.13	3.80±0.20	3.81±0.15					
Globulin (g/dL)								
0th day	4.32±0.31	4.19±0.37	4.21±0.35	4.24±0.19				
80th day	4.64±0.18	4.59±0.17	4.73±0.31	4.65±0.13	0.10	0.948	0.169	0.997
160th day	4.66±0.40	4.69±0.34	4.78±0.40	4.71±0.21				
Treatment mean	4.54±0.17	4.47±0.18	4.57±0.20					
A/G								
0th day	0.85±0.13	0.88±0.16	0.89±0.15	0.87±0.08				
80th day	0.83±0.04	0.89±0.07	0.89±0.12	0.87±0.05	0.04	0.912	0.997	
160th day	0.86±0.13	0.89±0.12	0.88±0.12	0.88±0.07				1.000
Treatment mean	0.85±0.06	0.88±0.07	0.88±0.07					
Urea (mg/dL)								
0th day	35.27±1.09	34.01±1.56	33.58±1.52	34.29±0.78	0.38	0.431	0.115	
80th day	35.69±1.01	36.28±0.84	35.22±0.84	35.73±0.50				0.948
160th day	36.89±0.61	36.27±1.34	35.43±1.12	36.20±0.60				
Treatment mean	35.95±0.53	35.52±0.74	34.74±0.68					

Control (T_0), Treatment (T_1) &Treatment (T_2): Dietary groups having concentrate containing (0, 3 & 6 % PS) along with wheat straw in the diet.
^{PQ} Means with different superscripts within a column differ significantly.

3.4 Serum Biochemical Profile Related to Lipid Metabolism

Table 5 presents the serum biochemical parameters related to lipid metabolism, including total cholesterol, HDL and LDL. The findings indicate that the average cholesterol concentration (mg/dL) was significantly higher ($P<0.05$) in the supplemented groups. Moreover, a significant increase ($P<0.01$) in cholesterol levels was observed across all dietary groups on the 80th and 160th days of the trial compared to the baseline (0th day). The mean cholesterol concentrations (mg/dL) for treatments T_0, T_1, and T_2 were recorded as 106.82, 115.90 and 126.03, respectively. Similarly, the mean cholesterol concentrations measured on the 0th, 80th, and 160th days were 94.44, 122.15, and 132.16 mg/dL, respectively.

Serum HDL levels (mg/dL) during the experimental period ranged from 31.80 to 50.57. The average HDL concentrations for treatments T_0, T_1 and T_2 were 39.37, 42.54, and 43.65 mg/dL, respectively. Across the trial period, the mean HDL levels were recorded as 33.77 mg/dL on day 0, 43.37 mg/dL on day 80, and 48.42 mg/dL on day 160. Although no significant differences ($P>0.05$) in serum HDL levels were found between dietary treatments, a significant increase ($P<0.001$) was noted on days 80 and 160 compared to the baseline. Furthermore, there was no observed interaction between treatment and time for HDL levels. Similarly, serum LDL levels (mg/dL) ranged from 51.94 to 88.67 throughout the experimental period. The mean LDL concentrations for treatments T_0, T_1 and T_2 were recorded as 60.84, 66.54, and 75.20 mg/dL, respectively. The mean LDL values measured on days 0, 80, and 160 were 54.99, 71.44, and 76.15 mg/dL, respectively. No significant differences ($P>0.05$) in LDL levels were observed among dietary treatments; however, a significant increase ($P<0.001$) in serum LDL concentration was recorded on days 80 and 160 compared to the 0th day. Additionally, no interaction effects between treatment and time were found for LDL levels during the study.

Fat supplementation has generally been associated with elevated cholesterol levels (Son et al.,1996). In the present study, the mean cholesterol concentration (mg/dL) remained significantly higher ($P<0.001$) in the supplemented groups. Additionally, cholesterol levels showed a marked increase across all dietary groups on the 80th and 160th days of the experiment compared to the baseline values recorded on day 0th. These findings are in agreement with those of Mane et al. (2016), who observed significantly elevated cholesterol levels in crossbred cattle supplemented with protected fat. Similarly, Ranjan et al. (2012) reported a progressive increase in serum cholesterol levels in Murrah buffaloes following bypass fat supplementation. Fahmy et al. (2016) also demonstrated a significant ($P<0.05$) rise in blood cholesterol concentrations in buffaloes fed varying levels of palm oil (0%, 5%, and 10%). These results are further supported by studies conducted by Singh et al. (2014) and Kirovski et al. (2015), both of which reported significant increases in cholesterol levelswith bypass fat supplementation. The observed elevation in cholesterol levels is likely attributable to enhanced dietary fatty acid absorption, as suggested by research conducted by Peter and Corah (1993) and Kumar and Thakur (2007), all of whom reported that dietary fat supplementation can facilitate fatty acid absorption, leading to increased cholesterol levels. However, Sharma et al. (2016) examined the effects of bypass fat supplementation in transitional Murrah buffaloes and found that while cholesterol levels rose significantly from the prepartum to postpartum period, no significant differences were observed among the treatment groups at specific time points. Thakur and Shelke (2010) also observed elevated blood cholesterol concentrations in experimental groups compared to controls, a finding consistent with the results of Kumar and Thakur (2007).

Furthermore, Singh et al. (2014) supplemented bypass fat to crossbred cows and noted no significant ($P>0.05$) increase in total cholesterol levels, despite the supplemented group exhibiting higher mean cholesterol levels than the control group.

Regarding serum lipoproteins, HDL levels (mg/dL) did not significantly differ between the treatment groups in this study. However, a significant increase in HDL concentrations was observed on the 80th and 160th days of the trial compared to the control group. Similarly, LDL levels did not show significant variation among treatment groups but were significantly elevated on the 80th and 160th days relative to baseline levels. These findings align with Sharma et al. (2016), who reported no significant differences in HDL and VLDL cholesterol levels among treatment groups but observed changes at different time points during the trial. Katiyar et al.

Table 5. Effects of different levels of dietary PS on serum biochemical parameters related to lipid metabolism in growing buffaloes

Attributes	Dietary groups			Period mean	SEM	P value		
Cholesterol(mg/dL)	**T_0**	**T_1**	**T_2**			**Treatment**	**Period**	**Treatment*Period**
0th day	96.04±13.40	93.12±11.02	94.17±16.30	94.44±7.46^{P}				
80th day	109.79±3.66	120.23±6.04	136.44±5.76	122.15±3.57^{Q}	3.62	0.028	<0.001	0.306
160th day	114.64±3.13	134.36±4.33	147.49±1.85	132.16±3.57^{Q}				
Treatment mean	106.82±4.85^{a}	115.90±5.76^{ab}	126.03±7.58^{b}					
HDL (mg/dL)								
0th day	31.80±2.98	35.62±0.53	33.89±0.76	33.77±1.05^{P}	1.14	0.078	<0.001	0.850
80th day	40.25±2.30	43.38±1.74	46.48±3.43	43.37±1.53^{Q}				
160th day	46.07±2.28	48.63±2.40	50.57±2.88	48.42±1.44^{R}				
Treatment mean	39.37±1.98	42.54±1.60	43.65±2.23					
LDL (mg/dL)								
0th day	58.22±12.99	51.94±10.75	54.80±16.69	54.99±7.46^{P}	3.06	0.660	<0.001	0.801
80th day	62.63±2.04	69.58±4.50	82.11±6.64	71.44±2.97^{Q}				
160th day	61.66±3.89	78.11±4.54	88.67±2.31	76.15±3.22^{Q}				
Treatment mean	60.84±4.32	66.54±4.63	75.20±6.54					

Control (T_0), Treatment (T_1) &Treatment (T_2): Dietary groups having concentrate containing (0, 3 & 6 % PS) along with wheat straw in the diet.
^{abc}Means with different superscripts within a row differ significantly.
^{PQR}Means with different superscripts within a column differ significantly.

Table 6. Effects of different levels of dietary PS on serum enzymatic profile in growing buffaloes

Attributes	Dietary groups					P value		
AST (IU/L)	**T_0**	**T_1**	**T_2**	**Period mean**	**SEM**	**Treatment**	**Period**	**Treatment*Period**
0th day	90.94±2.18	95.51±3.02	93.91±3.53	93.45±1.67				
80th day	93.91±2.13	96.94±1.62	97.09±4.59	95.98±1.70	0.95	0.074	0.134	0.889
160th day	93.46±1.44	99.49±2.55	101.36±2.55	98.10±1.47				
Treatment mean	92.77±1.10	97.31±1.39	97.45±2.11					
ALT (IU/L)								
0th day	26.27±2.98	27.88±1.86	27.73±1.11	27.29±1.17	0.56	0.712	0.069	
80th day	24.51±1.66	27.59±1.01	29.94±0.50	27.35±0.83				
160th day	26.91±1.96	28.30±1.67	29.70±1.06	28.30±0.91				0.818
Treatment mean	25.90±1.26	27.92±0.85	29.12±0.56					

Control (T_0), Treatment (T_1) &Treatment (T_2): Dietary groups having concentrate containing (0, 3 & 6 % PS) along with wheat straw in the diet

(2019) also found no significant differences (P>0.05) in mean HDL values at monthly intervals during the supplementation of bypass protein (BPP) and bypass protein and fat (BPPF); however, one month after discontinuing supplementation, all treatment groups exhibited significantly higher (P<0.001) HDL levels compared to controls. Additionally, a significant reduction (P<0.05) in LDL levels was noted on day 60th in the BPF group. Singh et al. (2014) also reported no significant changes in HDL cholesterol levels, although LDL levels increased with bypass fat supplementation.

Sundram et al. (1990) conducted an experiment in which semi-purified diets containing 20% fat were fed to 18 male rats over 15 weeks. Their results showed that plasma HDL cholesterol levels were higher in rats fed palm oil, palm olein, and Palm stearin compared to those fed soybean oil. Additionally, Shelke et al. (2012) reported a significant increase in HDL cholesterol in treatment groups compared to controls, attributing this to the presence of long-chain and unsaturated fatty acids such as palmitic, stearic, oleic, and linoleic acids incorporated into calcium salts of fatty acids. Since elevated HDL cholesterol is generally associated with beneficial health effects, these findings suggest potential advantages of dietary fat supplementation. Moreover, Shelke et al. (2012) reported higher lipid metabolite concentrations in animals fed bypass fat, which was linked to increased secretion of lipoproteins such as HDL and LDL in the intestines. The inclusion of long-chain and unsaturated fatty acids in the diet appeared to improve lipid metabolism, thereby enhancing the absorption and utilization of these fatty acids. Likewise, Ranjan et al. (2012) did not observe significant differences in LDL levels among treatment groups supplemented with bypass fat, though HDL cholesterol showed a significant increase.

Overall, while the present study did not find significant differences in HDL and LDL levels amongtreatment groups, it did reveal significant temporal increases in these lipid parameters over the course of the trial. These findings are consistent with previous studies, including those by Sharma et al. (2016) and Singh et al. (2014), which also reported changes in lipid profiles over time rather than treatment-induced differences.

3.5 Liver Enzymes

Liver markers serve as essential indicators of liver function and its capacity to process various substances, particularly nitrogenous compounds such as proteins and urea. The data on serum ALT and AST levels are presented in Table 6. The average serum AST (IU/L) remained consistent across the different treatment groups, with values recorded at 92.77 for T_0, 97.31 for T_1, and 97.45 for T_2. Over the course of the study, serum AST levels were measured at 93.45, 95.98, and 98.10 IU/L on day 0th, 80th, and 160th, respectively. These findings indicate that AST levels did not differ significantly (P>0.05) between the treatment groups or across time, with no notable interaction between treatment and study duration. Similarly, the mean serum ALT (IU/L) values were comparable among the dietary treatment groups, measuring 25.90 for T_0, 27.92 for T_1, and 29.12 for T_2. Throughout the trial, the mean ALT levels were recorded as 27.29 IU/L on day 0, 27.35 IU/L on day 80, and 28.30 IU/L on day 160, demonstrating stability across both treatments and the study period. No statistically significant differences (P>0.05) were observed between groups or over time, nor was there any significant interaction between treatment and duration.

In this study, the levels of AST and ALT enzymes did not exhibit any significant differences compared to the control group. These findings align with those of Ranjan et al. (2012), who similarly reported no notable changes in liver enzyme activity in animals supplemented with bypass fat. The absence of significant enzymatic alterations indicates that the supplementation with bypass fat did not have any detrimental effects on liver or kidney function (Kaneko et al., 2008).

4. CONCLUSION

The findings of this study indicate that incorporating Palm stearin (PS) into livestock diets does not significantly impact the critical haematological indices, such as haemoglobin (Hb) concentration, packed cell volume (PCV), blood glucose levels or total protein content across the different dietary treatments (P>0.05). However, dietary supplementation with Palm stearin (PS) led to a significant increase in total cholesterol concentrations (P<0.001), with a progressive rise observed in both high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels as the inclusion rate increased from 0% to 3% and 6%. Despite this upward trend, the differences in HDL and LDL levels among the treatment groups were not

statistically significant (P>0.05). Similarly, triglyceride levels exhibited a mild increasing tendency, though the changes were not significant across treatments (P>0.05). In addition, the activities of the hepatic enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) remained consistent across all experimental groups (P>0.05), suggesting no hepatotoxic effects associated with Palm stearin inclusion. Overall, while Palm stearin (PS) appears to modulate lipid metabolism by elevating total cholesterol, it does not markedly influence other blood biochemical markers or liver function, supporting its potential as a safe and viable fat source in livestock diets.

5. FUTURE SCOPE

Future investigations may focus on evaluating the long-term impact of Palm stearin on various production parameters and overall health in buffaloes. Research across different physiological stages and among other ruminant species could enhance its applicability. Moreover, economic and environmental analyses are essential to substantiate its viability as a sustainable feed ingredient in livestock production systems.

ETHICAL APPROVAL

The experiment was conducted in strict compliance with the guidelines of 'Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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