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Effect of dietary supplementation of rumen-protected fat and bypass protein on feed intake, nutrient digestibility and plane of nutrition in transition buffaloes

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Abstract

The transition period in dairy buffaloes involves extensive physiological strain, characterized by depressed voluntary feed intake and heightened metabolic demand for nutrients. This study evaluated the effects of simultaneous dietary supplementation with rumen-protected protein and rumen-protected fat on feed intake, apparent nutrient digestibility, and the plane of nutrition in transition Murrah buffaloes (*Bubalus bubalis*). Ten healthy multiparous Murrah buffaloes were balanced according to parity and production potential, then allocated into two equal groups ($n = 5$): a control group (T_0) fed a standard basal diet, and a treatment group (T_1) supplemented with formaldehyde-treated groundnut cake (1.0 g formaldehyde/100 g CP) and prilled long-chain saturated palm fatty acids (15 g/L milk produced). The 120-day trial spanned from 30 days pre-partum through 90 days post-partum, featuring a 7-day total-collection digestion trial between days 45 and 60 post-calving. Supplemented buffaloes (T_1) demonstrated significantly higher voluntary total dry matter intake (13.84 kg/day vs. 12.65 kg/day in T_0 ; $P < 0.01$) without compromising roughage consumption. Apparent digestibility coefficients of crude protein (67.42% vs. 63.85%; $P < 0.05$) and ether extract (79.85% vs. 71.20%; $P < 0.01$) improved significantly in T_1 , whereas crude fiber digestibility remained unaffected ($P > 0.05$). Daily intake of digestible crude protein (1.173 kg vs. 0.945 kg; $P < 0.001$) and total digestible nutrients (8.77 kg vs. 7.65 kg; $P < 0.01$) were markedly enhanced in the treatment group. In conclusion, concurrent supplementation of bypass protein and fat significantly enhances nutrient utilization, feed intake, and plane of nutrition without disrupting ruminal cellulolysis in transition Murrah buffaloes.

Keywords: Murrah buffalo, transition period, rumen-protected fat, bypass protein, apparent digestibility, plane of nutrition

1. Introduction

Dairying is a foundational sector in tropical agriculture, with the water buffalo (*Bubalus bubalis*) providing over half of the national milk pool in the Indian subcontinent (Paul *et al.*, 2002; ICAR, 2013) [18, 28]. Among recognized riverine breeds, the Murrah buffalo is globally valued for high milk yield, substantial milk solids, and remarkable capacity for roughage bioconversion (Kumar, 2010) [23]. Despite this high genetic potential, maintaining optimal production and metabolic health is challenging during the transition period the interval spanning three to four weeks pre-partum to twelve weeks post-partum (Grummer, 1995; Drackley, 1999) [12, 16]. During this window, the metabolic demand for amino acids and glucogenic substrates accelerates rapidly to support late-stage fetal growth and the initiation of lactogenesis (Bell, 1995; Bauman and Currie, 1980) [4, 5]. Concurrently, voluntary dry matter intake (DMI) drops significantly due to hormonal shifts and physical compression of the rumen by the gravid uterus (Ingvarsen and Andersen, 2000; Hayirli *et al.*, 2002) [17, 19]. This disparity induces a state of negative energy and nitrogen balance, which prompts extensive mobilization of adipose lipids and skeletal proteins, elevating the risk of subclinical ketosis, hepatic lipidosis, and reduced lactation persistency (Goff and Horst, 1997; Sordillo and Aitken, 2009) [15, 36].

Traditional methods to increase dietary energy density by elevating cereal starch concentrations frequently lower ruminal pH, increasing the occurrence of subacute ruminal acidosis (SARA) and depressing cellulolytic microbial activity (Nocek, 1997; Russell and Rychlik, 2001) [25, 31]. Supplementing diets with conventional, unprotected plant oils is also problematic; free unsaturated fatty acids adhere to fibrous feed particles and exhibit toxic effects on cellulolytic bacteria such as *Fibrobacter succinogenes* and *Ruminococcus albus*, causing impaired fiber digestion and depressed voluntary intake (Jenkins, 1993; Palmquist and Jenkins, 1980) [20, 21, 27].

To resolve these digestive constraints, rumen-protected fats (RPF) particularly prilled saturated fatty acids or calcium salts of long-chain fatty acids have been adopted (Palmquist, 1984; Chilliard, 1993) [9, 26]. Because these lipid supplements remain chemically inert at normal ruminal pH (6.2 to 6.8), they pass into the abomasum without disturbing the microbial ecosystem (Jenkins and Palmquist, 1984; Naik, 2013) [21, 24, 26]. In the acidic abomasal environment (pH 2.0 to 3.0), the lipids dissociate, allowing long-chain fatty acids to pass into the duodenum where they are digested by bile salts and pancreatic lipases, providing dense energy without hindering structural carbohydrate breakdown (Shelke, 2012; Khadda, 2025) [22, 33]. Equally critical during early lactation is optimizing metabolizable protein delivery. Highly degradable protein sources, such as untreated groundnut cake (GNC), are rapidly broken down by ruminal microflora into peptides and free ammonia nitrogen (NH_3 -N) (Satter and Slyter, 1974; Broderick, 1995) [6, 32]. When the rate of ruminal proteolysis surpasses microbial assimilation capacity, excess ammonia enters portal circulation, where it is converted to urea by the liver at a net metabolic energy cost before being lost in the urine (Tamminga, 1992; Reynolds, 2006) [30, 37].

Treating oilseed cakes with formaldehyde (formalin, 40%) at a standardized rate of 1.0 to 1.2 g formaldehyde per 100 g crude protein protects dietary protein from ruminal loss (Ferguson *et al.*, 1967; Walli, 2005) [13, 39]. The formaldehyde forms methylene cross-linkages with free ϵ -amino groups (mainly

lysine residues), making the protein matrix insoluble and resistant to microbial enzymes at ruminal pH (Barry, 1976; Chatterjee and Walli, 2003) [3, 7, 39]. When these protected proteins reach the acidic environment of the abomasum, the cross-linkages hydrolyze, releasing intact amino acids and intact dietary protein for enzymatic digestion in the small intestine (Clark, 1975; Garg *et al.*, 2003; Sirohi *et al.*, 2013) [10, 14, 34].

Although the independent effects of bypass fat or bypass protein are documented in taurine cattle (*Bos taurus*), buffaloes differ markedly in their ruminal nitrogen recycling, fiber retention times, and rumen fermentation kinetics (Devendra, 1985; Puppo *et al.*, 2002) [11, 29]. Research examining the simultaneous dietary inclusion of formaldehyde-treated groundnut cake and bypass palm fatty acids during the peri-partum transition period in Murrah buffaloes remains limited (Shelke *et al.*, 2012; Chavda *et al.*, 2021) [8, 33]. Evaluating how this combined dietary regimen affects feed intake, total-tract apparent nutrient digestibility, and the plane of nutrition is essential for refining transition feeding systems.

Therefore, this experiment was conducted to evaluate the effect of dietary supplementation of rumen-protected protein and rumen-protected fat on feed intake, apparent nutrient digestibility, and overall nutrient utilization in transition Murrah buffaloes (*Bubalus bubalis*).

Materials and Methods

2.1 Animal Selection, Grouping, and Experimental Design

Ten healthy, multiparous Murrah buffaloes (*Bubalus bubalis*) in late gestation were selected from the institutional herd. Animals were matched and balanced based on their Most Probable Production Ability (MPPA ~1,800 L) and historical parity records, and then randomly allocated into two uniform dietary groups of five animals each ($n = 5$):

The experimental feeding trial lasted 120 days, beginning 30 days pre-partum and continuing through 90 days post-partum.

Table 1: Distribution of experimental Murrah buffaloes based on lactation number and Most Probable Production Ability (MPPA)

S. No.	Control Group (T0)	Lactation No.	MPPA (L)	Treatment Group (T1)	Lactation No.	MPPA (L)
1	M-1	4	1619.80	M-8	3	1704.70
2	M-7	3	1869.80	M-4	3	1959.50
3	M-5	4	1462.00	M-2	3	1868.70
4	M-14	1	1705.00	M-11	1	1669.60
5	M-12	1	1605.10	M-9	2	1581.60
Mean $\pm$ SE		2.60 $\pm$ 0.68	1652.34 $\pm$ 67.45		2.40 $\pm$ 0.40	1756.82 $\pm$ 68.49

2.3 Housing, Management and Feeding Regimens

Buffaloes were housed in well-ventilated, individual tie-stalls designed with separate mangers and water troughs to avoid cross-feeding and ensure accurate quantification of individual intake. Shed hygiene was maintained through twice-daily scrubbing and disinfection. Clean, potable drinking water was offered *ad libitum*.

All animals received a basal roughage diet consisting of green maize (*Zea mays*), lucerne (*Medicago sativa*), sugarcane tops (*Saccharum officinarum*), and dry jowar stover (*Sorghum bicolor*). The concentrate mixture was formulated to meet standard nutrient requirements (ICAR, 2013) [18] and was fed in two equal portions during morning (05:00 h) and evening (16:30 h) milkings:

- **Control Diet (T_0):** Received an untreated basal concentrate mixture comprising crushed maize (35%), dal

chuni (22%), wheat bran (20%), untreated groundnut cake (20%), mineral mixture (2%), and common salt (1%).

- **Treatment Diet (T_1):** Received the basal concentrate mixture containing formaldehyde-treated bypass groundnut cake (20%) along with rumen-protected palm fatty acids top-dressed at 15 g per liter of actual daily milk production.

2.4 Preparation and Procurement of Rumen-Protected Nutrients

Rumen-Protected Protein (Bypass Protein):

Commercial groundnut cake (GNC) containing 38.01% crude protein (CP, DM basis) was procured from a single commercial batch. The cake was treated with commercial-grade formalin (40% formaldehyde solution) at a standardized rate of 1.0 g formaldehyde per 100 g CP of the cake (equivalent to

2.5 mL formalin per 100 g CP or 25 mL formalin per kg CP; Chatterjee and Walli, 2003) [7, 39].

The formalin was diluted in a measured volume of water, uniformly sprayed over the spread GNC, and mixed mechanically. The treated material was immediately sealed in airtight polyethylene bags and incubated at ambient temperature for a minimum of 7 days to promote stable methylene bridge cross-linkages before incorporation into the T_1 concentrate mash.

Rumen-Protected Fat (Bypass Fat):

Fractionated, prilled long-chain saturated palm fatty acids were procured from Berg + Schmidt Ingredients Pvt. Ltd. (India) and top-dressed on the concentrate allowance of individual animals in the T_1 group during daily milking.

2.5 Digestion Trial and Sample Collection

Midway through the post-partum experimental period (between days 45 and 60 post-calving), a 7-day total-collection digestion trial was conducted following a 3-day acclimation period. Individual animal body weights were recorded on two consecutive days immediately before and after the digestion trial using a calibrated digital platform balance.

- **Feed and Residue Sampling:** The daily allowances of green fodder, dry roughage, and concentrate mixture, as well as the unconsumed orts from each stall, were weighed every morning at 06:00 h. Representative sub-samples of each feed ingredient, roughage source, and refusal were collected daily, dried in a forced-air oven at 65°C to constant weight for dry matter (DM) determination, ground through a 1 mm screen in a Wiley mill, and stored in airtight containers for proximate analysis.
- **Fecal Collection and Aliquoting:** Total daily fecal output was quantitatively collected directly from the stall floor upon voiding over consecutive 24-hour cycles. The daily voiding was weighed and thoroughly mixed in an enamel trough. A 1/100th representative aliquot of the total wet feces from each animal was sampled daily:
- One portion was dried at 65°C to constant weight for moisture determination and pooled for proximate analysis.
- A second portion (1/500th of total output) was preserved in 1:4 diluted sulfuric acid (H_2SO_4) in glass containers for total fecal nitrogen estimation.

2.6 Chemical and Proximate Analysis

Representative pooled samples of feedstuffs, orts, and dried feces were analyzed according to standard AOAC (1995) [2] procedures:

- **Dry Matter (DM):** Method 934.01 (oven drying at 105°C for 3 h).
- **Crude Protein (CP):** Method 984.13 (micro-Kjeldahl method, total nitrogen $\times$ 6.25).
- **Ether Extract (EE):** Method 920.39 (Soxhlet extraction with petroleum ether, boiling range 40-60°C).

- **Crude Fiber (CF):** Method 962.09 (sequential dilute acid and alkali digestion using a fiber analyzer).
- **Total Ash (TA):** Method 942.05 (muffle furnace ignition at 550°C for 4 h).
- **Nitrogen-Free Extract (NFE):** Calculated by subtraction:

$$NFE (\% DM) = 100 - (CP + CF + EE + TA)$$

- **Organic Matter (OM):** Calculated as:

$$OM (\% DM) = 100 - \text{Total Ash}$$

2.7 Calculations and Plane of Nutrition

The apparent digestibility coefficients of proximate nutrients were determined using the standard equation:

Apparent Digestibility Coefficient (%)

$$= \frac{\text{Nutrient Intake (g/day)} - \text{Fecal Nutrient Excreted (g/day)}}{\text{Nutrient Intake (g/day)}} \times 100$$

Digestible Crude Protein (DCP) and Total Digestible Nutrients (TDN) were computed as:

$$DCP (\%) = \text{Feed CP} (\%) \times \left(\frac{\text{CP Digestibility Coefficient}}{100} \right)$$

$$TDN (\%) = DCP (\%) + DCF (\%) + DNFE (\%) + [DEE (\%) \times 2.25]$$

Daily DCP intake (DCPI) and TDN intake (TDNI) were calculated and expressed in absolute amounts (kg/day) and relative to metabolic body size and live weight (% BW).

2.8 Statistical Analysis

Data gathered on nutrient intake, proximate composition, digestibility coefficients, and plane of nutrition were statistically evaluated using an independent two-sample Student's *t*-test in accordance with Snedecor and Cochran (1989) [35]. All results are reported as Mean $\pm$ Standard Error (SE). Treatment means were judged statistically significant at $P < 0.05$ and $P < 0.01$.

3. Results

3.1 Chemical Composition of Experimental Feeds

The proximate chemical composition of the basal concentrate mixtures, green forages, and dry roughage offered during the 120-day feeding trial is presented in Table 2. The crude protein (CP) contents of the control (T_0) and treatment (T_1) concentrate mixtures were formulated to be iso-nitrogenous, analyzing at 19.82% and 20.14% on a dry matter (DM) basis, respectively. The ether extract (EE) content of the T_1 concentrate mixture was elevated (5.85% vs. 3.40% in T_0) due to the incorporation of the fractionated prilled long-chain fatty acid supplement. The structural carbohydrate (CF) and mineral ash fractions were comparable across both concentrate mashes. The green fodder and dry stover fractions exhibited compositional values typical of tropical ruminant feedstuffs.

Table 2: Chemical and proximate composition of experimental feedstuffs (% DM basis)

Nutrients	Control Concentrate (T_0)	Treatment Concentrate (T_1)	Green Maize	Lucerne	Dry Jowar Stover	Sugarcane Tops
Dry Matter (DM)	89.45	89.80	22.30	20.15	91.20	26.50
Organic Matter (OM)	91.60	91.85	90.80	88.50	90.10	89.20
Crude Protein (CP)	19.82	20.14	8.25	18.60	3.45	5.10
Ether Extract (EE)	3.40	5.85	1.80	2.40	1.15	1.40
Crude Fiber (CF)	8.90	8.75	26.40	24.10	34.20	31.80
Nitrogen-Free Extract (NFE)	59.48	57.16	54.35	43.40	51.30	50.90
Total Ash (TA)	8.40	8.15	9.20	11.50	9.90	10.80

3.2 Voluntary Feed and Nutrient Intake

Data on voluntary feed consumption and daily nutrient intake obtained during the digestion trial are summarized in Table 3. Total dry matter intake (DMI) was significantly higher ($P < 0.01$) in Murrah buffaloes fed the combination of formaldehyde-treated GNC and rumen-protected fat (T_1 : 13.84 kg/day) compared to those on the untreated control diet (T_0 : 12.65 kg/day).

Expressed as a percentage of live body weight, DMI was 2.58% in T_0 versus 2.76% in T_1 ($P < 0.05$). Similarly, dry matter

intake per unit of metabolic body size ($W^{0.75}$) improved significantly from 122.46 g/kg $W^{0.75}$ in the control group to 132.88 g/kg $W^{0.75}$ in the treatment group ($P < 0.05$). Intake of crude protein was significantly higher in T_1 (1.74 kg/day) relative to T_0 (1.48 kg/day; $P < 0.01$). Daily ether extract intake increased by 67.7% in T_1 over T_0 (0.52 kg/day vs. 0.31 kg/day; $P < 0.001$), driven directly by bypass fat supplementation. Roughage DMI and crude fiber intake did not differ significantly between dietary groups ($P > 0.05$).

Table 3: Voluntary dry matter and nutrient intake in transition Murrah buffaloes

Parameter	Control (T0)	Treatment (T1)	t-value	P-value
Live Body Weight (kg)	490.20 ± 12.45	501.50 ± 14.10	0.60	0.562
Metabolic Body Size ($W^{0.75}$, kg)	104.28 ± 1.98	106.08 ± 2.22	0.61	0.559
Roughage DMI (kg/day)	7.92 ± 0.18	8.35 ± 0.21	1.55	0.159
Concentrate DMI (kg/day)	4.73 ± 0.11	5.49 ± 0.14	4.27	0.003
Total DMI (kg/day)	12.65 ± 0.22	13.84 ± 0.26	3.49	0.008
DMI (% Live Body Weight)	2.58 ± 0.04	2.76 ± 0.05	2.81	0.023
DMI (g/kg $W^{0.75}$)	122.46 ± 2.15	132.88 ± 2.80	2.95	0.018
Crude Protein Intake (kg/day)	1.48 ± 0.03	1.74 ± 0.04	5.20	0.001
Ether Extract Intake (kg/day)	0.31 ± 0.01	0.52 ± 0.02	9.39	<0.001
Crude Fiber Intake (kg/day)	2.82 ± 0.06	2.95 ± 0.07	1.41	0.196

3.3 Apparent Total-Tract Nutrient Digestibility

Apparent total-tract nutrient digestibility coefficients are detailed in Table 4. Digestibility of dry matter (61.42% vs. 63.15%) and organic matter (64.18% vs. 65.90%) showed minor improvements in T_1 that did not attain statistical significance ($P > 0.05$).

In contrast, the apparent digestibility of crude protein was significantly improved by 3.57 percentage units in the

treatment group (67.42 ± 0.90% in T_1 vs. 63.85 ± 0.82% in T_0 ; $P < 0.05$). Total-tract ether extract digestibility increased significantly in T_1 relative to controls (79.85 ± 1.25% vs. 71.20 ± 1.10%; $P < 0.01$). Digestibility of crude fiber (55.60% in T_0 vs. 56.45% in T_1) and nitrogen-free extract (67.80% in T_0 vs. 68.95% in T_1) remained unaffected by the dietary modifications ($P > 0.05$).

Table 4: Apparent total-tract nutrient digestibility coefficients (%) in experimental Murrah buffaloes

Nutrient Digestibility (%)	Control (T0)	Treatment (T1)	t-value	P-value
Dry Matter (DM)	61.42 ± 0.85	63.15 ± 0.78	1.50	0.172
Organic Matter (OM)	64.18 ± 0.74	65.90 ± 0.69	1.70	0.127
Crude Protein (CP)	63.85 ± 0.82	67.42 ± 0.90	2.93	0.019
Ether Extract (EE)	71.20 ± 1.10	79.85 ± 1.25	5.20	0.001
Crude Fiber (CF)	55.60 ± 0.95	56.45 ± 0.88	0.66	0.529
Nitrogen-Free Extract (NFE)	67.80 ± 0.71	68.95 ± 0.65	1.19	0.267

3.4 Plane of Nutrition and Nutrient Utilization

The overall plane of nutrition, calculated on the basis of digestible crude protein (DCP) and total digestible nutrients (TDN), is shown in Table 5. Both the DCP density (8.48% vs. 7.47%; $P < 0.01$) and TDN density (63.36% vs. 60.45%; $P < 0.01$) of the total consumed ration were significantly elevated in the treatment group.

Daily DCP intake increased by 24.1% in the T_1 group (1.173 kg/day vs. 0.945 kg/day; $P < 0.001$), corresponding to an intake of 11.06 g/kg $W^{0.75}$ compared to 9.06 g/kg $W^{0.75}$ in the control group ($P < 0.001$). Daily TDN intake also improved significantly ($P < 0.01$) from 7.65 kg/day (73.34 g/kg $W^{0.75}$) in T_0 to 8.77 kg/day (82.67 g/kg $W^{0.75}$) in T_1 .

Table 5: Plane of nutrition and digestible nutrient intake in transition Murrah buffaloes

Parameter	Control (T0)	Treatment (T1)	t-value	P-value
Dietary DCP Content (%)	7.47 ± 0.12	8.48 ± 0.15	5.26	0.001
Dietary TDN Content (%)	60.45 ± 0.62	63.36 ± 0.58	3.43	0.009
Daily DCP Intake (kg/day)	0.945 ± 0.02	1.173 ± 0.03	6.32	<0.001
DCP Intake (g/kg $W^{0.75}$)	9.06 ± 0.18	11.06 ± 0.22	7.03	<0.001
Daily TDN Intake (kg/day)	7.65 ± 0.14	8.77 ± 0.18	4.91	0.001
TDN Intake (g/kg $W^{0.75}$)	73.34 ± 1.12	82.67 ± 1.35	5.32	0.001

4. Discussion

4.1 Dry Matter and Nutrient Intake

Transition ruminants experience a marked drop in voluntary dry matter intake during the final weeks of gestation that extends into early lactation, driven by ruminal compression

from the gravid uterus, elevated estrogens, and elevated circulating non-esterified fatty acids (Allen, 2000; Ingvarsen and Andersen, 2000) [1, 19]. In the current study, feeding formaldehyde-treated groundnut cake along with prilled palm fatty acids did not cause hypophagia; instead, it supported a

significant increase in total voluntary DMI (13.84 kg/day in T_1 vs. 12.65 kg/day in T_0 ; $P < 0.01$).

Conventional supplemental fats particularly unprotected vegetable oils rich in free unsaturated fatty acids often impair intake by depressing ruminal motility and exerting detergent-like antibacterial effects on the cellulolytic microbiota (Jenkins, 1993; Allen, 2000) [1, 20]. In contrast, the prilled long-chain saturated palm fatty acids supplied to the T_1 group remain chemically inert and insoluble at physiological ruminal pH (6.2 to 6.8). They pass into the abomasum without adhering to forage particles or perturbing microbial fermentation kinetics, thus circumventing the chemostatic and physical mechanisms that downregulate appetite (Palmquist and Jenkins, 1980; Naik, 2013) [24, 27].

Concurrently, the protection of groundnut cake with 1.0 g formaldehyde per 100 g CP prevented premature ruminal proteolysis, attenuating peak ruminal ammonia-nitrogen concentrations and shifting the delivery of intact amino acids to the duodenum (Walli, 2005; Sirohi *et al.*, 2013) [34, 39]. An enhanced supply of post-ruminal metabolizable protein, particularly essential amino acids like lysine and methionine, helps coordinate metabolic flux in the liver (Clark, 1975; Broderick, 1995) [6, 10]. As demonstrated by Garg *et al.* (2003) [14] and Shelke *et al.* (2012) [33], balancing the intestinal availability of glucogenic and lipogenic precursors relieves the metabolic burden of early lactation, promoting voluntary feed intake in Murrah buffaloes. Similar observations of sustained or elevated DMI following bypass fat and bypass protein supplementation in lactating buffaloes have been reported by Chavda *et al.* (2021) and Tyagi *et al.* (2009) [8, 38].

4.2 Apparent Total-Tract Nutrient Digestibility

Apparent crude protein digestibility improved significantly from 63.85% in control animals to 67.42% in supplemented animals ($P < 0.05$). In untreated groundnut cake diets (T_0), highly soluble oilseed proteins are rapidly fermented by rumen microflora into peptides and free ammonia at rates that frequently exceed microbial assimilation capacity (Satter and Slyter, 1974; Tamminga, 1992) [32, 37]. This excess ammonia traverses the rumen epithelium into portal circulation, requiring hepatic conversion to urea and resulting in substantial urinary nitrogen excretion (Reynolds, 2006) [30].

Treatment with formaldehyde establishes cross-linkages specifically methylene bridges with free ϵ -amino groups of lysine residues (Barry, 1976; Chatterjee and Walli, 2003) [3, 7, 39]. These covalent bonds are stable at the near-neutral pH of the rumen, preventing enzymatic degradation by microbial proteases (Ferguson *et al.*, 1967; Walli, 2005) [13, 39]. Upon exposure to the gastric secretions of the abomasum (pH 2.0 to 3.0), these methylene bridges hydrolyze, permitting normal enzymatic proteolysis by pepsin and pancreatic trypsin in the duodenum (Clark, 1975; Sirohi *et al.*, 2013) [10, 34]. The shift in the primary site of protein degradation from the reticulorumen to the small intestine explains the improved apparent crude protein digestibility observed in the T_1 cohort.

Ether extract digestibility showed an 8.65 percentage unit gain in the treatment group (79.85% vs. 71.20%; $P < 0.01$). This response reflects the high intestinal bioavailability and digestibility of prilled saturated palm fatty acids (Palmquist, 1984) [26]. Saturated free fatty acids dissociate cleanly in the acidic abomasum and form mixed micelles in the presence of taurocholate and pancreatic juice, allowing efficient absorption through the intestinal mucosa (Chilliard, 1993) [9].

Crucially, total-tract crude fiber digestibility remained unaffected (55.60% in T_0 vs. 56.45% in T_1 ; $P = 0.529$). In

diets supplemented with unprotected oils, the coating of fibrous matrices and toxicity to fibrolytic bacteria such as *Fibrobacter succinogenes* and *Ruminococcus albus* lead to marked fiber digestion depressions (Jenkins and Palmquist, 1984; Russell and Rychlik, 2001) [21, 27, 31].

The maintenance of crude fiber digestibility in this study demonstrates that neither the prilled palm fatty acids nor the formaldehyde-treated GNC disrupted fibrolytic activity or altered the normal microbial fermentation balance in the rumen. Similar retention of cellulolytic degradation without impairment of fiber digestion has been recorded in dairy buffaloes supplemented with bypass fat by Khadda *et al.* (2025) [22].

4.3 Plane of Nutrition and Transition Metabolic Bioenergetics

The transition period between late gestation and peak lactation is marked by a wide disparity between nutrient intake and metabolic demands for lactopoiesis (Bauman and Currie, 1980; Drackley, 1999) [4, 12]. Dairy ruminants mobilize significant amounts of non-esterified fatty acids (NEFA) from subcutaneous adipose depots and amino acids from skeletal muscle reserves to offset this deficit, increasing the incidence of ketosis, hepatic steatosis, and metabolic disorders (Goff and Horst, 1997; Sordillo and Aitken, 2009) [15, 36].

In this trial, the combined supplementation of rumen-protected protein and fat increased dietary energy and protein density, raising the plane of nutrition. Daily DCP intake rose from 0.945 kg/day in the control group to 1.173 kg/day in the treatment group, and daily TDN intake rose from 7.65 kg/day to 8.77 kg/day. Scaled to metabolic body size, animals in the T_1 group received an additional 2.00 g DCP and 9.33 g TDN per kg $W^{0.75}$ each day.

The bioenergetic efficiency of this dietary regimen stems from complementary metabolic pathways:

- Bypassing ruminal proteolysis spares the metabolic energy usually expended by the liver to synthesize urea from ammonia via the ornithine cycle (an expenditure of 4 ATP equivalents per molecule of urea synthesized; Tamminga, 1992; Reynolds, 2006) [30, 37].
- Supplying dietary long-chain fatty acids for direct incorporation into milk triglycerides reduces the requirement for *de novo* fatty acid synthesis from acetate and β -hydroxybutyrate in the mammary gland, lowering the net energetic demand of milk production (Chilliard, 1993; Bell, 1995) [5, 9].
- Sparing systemic amino acids from gluconeogenesis allows their utilization for structural tissue maintenance and milk protein synthesis, limiting postpartum negative nitrogen balance (Bell, 1995; Drackley, 1999) [5, 12].

These findings demonstrate that simultaneous dietary inclusion of formaldehyde-treated groundnut cake and prilled bypass fat provides an effective nutritional intervention to elevate nutrient intake, improve protein and fat digestibility, and reinforce the plane of nutrition in transition Murrah buffaloes without disturbing rumen cellulolytic function.

Conclusion

Dietary supplementation of transition Murrah buffaloes with formaldehyde-treated groundnut cake (1.0 g formaldehyde per 100 g CP) and prilled long-chain saturated palm fatty acids (15 g per liter of milk produced) significantly enhanced voluntary dry matter intake without impairing roughage consumption. The bypass characteristics of the prilled fat and the protected protein preserved normal ruminal cellulolysis, maintaining

crude fiber digestibility while significantly improving the apparent total-tract digestibility of crude protein and ether extract.

Consequently, the combined dietary regimen elevated daily digestible crude protein (DCP) and total digestible nutrient (TDN) intake, substantially boosting the overall plane of nutrition during the metabolically challenging peri-partum transition period. These findings demonstrate that concurrent feeding of rumen-protected protein and bypass fat is a practical and effective nutritional intervention to alleviate negative energy and protein balances, improve nutrient utilization, and sustain performance in lactating Murrah buffaloes.

Conflict of Interest

The authors declare that they have no competing financial or non-financial interests that could have influenced this work.

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