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EFFECTS OF FEEDING CALCIUM SALTS OF FATTY ACIDS AND TWO LEVELS OF RUMEN UNDEGRADABLE PROTEIN ON PRODUCTION PERFORMANCE OF LACTATING *NILI-RAVI* BUFFALOES

R. Y. Arafat[1], Saima[2], Hifzulrahman[3], M. Akhtar[4], M. Naveed-ul-Haque[5]

[1, 2, 5]Department of Animal Nutrition, University of Veterinary and Animal Sciences, Outfall Road, Lahore 54000, Pakistan
[3]Department of Livestock Management, University of Veterinary and Animal Sciences, Outfall Road, Lahore 54000, Pakistan
[4]Livestock Production Research Institute, Bahadurnagar Okara, 56300, Pakistan
*Corresponding author: muhammad.naveed@uvas.edu.pk
Available ORCID;
https://orcid.org/0009-0000-4575-7646[1], https://orcid.org/0000-0003-2747-9691[2], https://orcid.org/0000-0002-4090-5053[3], https://orcid.org/0000-0003-3583-7647[4], https://orcid.org/0000-0003-1605-7629[5]

ABSTRACT

Production performance of lactating *Nili Ravi* buffalo can be improved with feeding of high rumen undegradable protein (RUP) and calcium salts of fatty acids (Ca-FA). Sixteen multiparous lactating *Nili Ravi* buffaloes were arranged in a 4×4 Latin square arrangement with (mean ± SD) 11.2±0.76 kg/d of milk yield, 6.41±0.23% milk fat, 583±26 kg of body weight (BW) and 161±24 days in milk (DIM). The dietary treatments were: (1) LPLF-low RUP low fat (2) LPHF-low RUP high fat (3) HPLF-high RUP low fat and (4) HPHF-high RUP high fat. The designed diets provided low and high levels of RUP (27.4% and 38.6%) with low (3.5%) and high fat (4.9%). The duration of each period was 21 days and milk sampling was done twice weekly. Statistical significance among treatment means was evaluated using a p-value of < 0.05. Increasing the RUP and fat supplies increased periodic BW and DMI ($P < 0.01$) with no interaction effect of RUP × fat ($P = 0.51$) on BW. Milk yield was increased by 3.8% and 14.0% by increasing RUP and fat supplement respectively. The RUP × fat interaction indicated that increase in milk yield was 51% higher when fat was supplemented with low RUP compared with the high RUP diet (RUP × fat, $P < 0.01$). Milk fat content was increased with the supplementation of fats by 22.0% and milk fat yield by 39.0% ($P < 0.01$). Milk protein content and yield increased with the increasing RUP level by 10.8% and 15.7%, respectively. Milk lactose yield increased by 5.8% and 17.0% with increasing RUP and fat levels, respectively ($P < 0.01$). Feed, nitrogen, and milk efficiencies including 4% fat corrected milk (FCM), energy corrected milk (ECM) and milk energy output were increased ($P < 0.01$) with HF and HP treatments. Plasma urea nitrogen decreased with HP diet compared with LP diet ($P < 0.01$), whereas the emission of enteric methane was decreased in HPHF group ($P < 0.01$). In conclusion, the RUP fraction of 38.6% in concentrate and 300 gram of Ca-FA per animal per day increased the production performance of lactating *Nili Ravi* buffalo, whereas milk fat increased with fat supplementation and milk protein increased with high RUP supplies.

Key words: Buffalo, DMI, rumen undegradable protein, rumen by pass fat, milk yield

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INTRODUCTION

The digestion process of ruminants is different from monogastric animals for having four compartment stomach. Rumen, the first portion of stomach of ruminants is specialized fermentation vat where different colonies of microbes utilize dietary substrates by the process of fermentation degrading into nutrients of different categories mainly volatile fatty acids and ammonia. Ammonia is the end product of degradable fractions of protein sources in the diet of animal whereas dietary simple fats are hydrogenated in the rumen altering the profile and hence it's metabolic and physiological effects in ruminants (Fatima and Bayram, 2025). The ruminants usually require more nutritious and protected profiled nutrient in the diet that avoid rumen degradation converting it into less nutritious commodities in periods of high production usually early lactation (Caixeta and Omontese, 2021). Buffaloes like other ruminants in early lactation undergo drastic physiological adaptations to compensate higher nutritional demands to produce milk. Buffaloes are capable of altering dietary nutrients in rumen through hydrogenation of fats and degradation of proteins through fermentation that may have its own consequences on metabolic status of animal, hence during periods of high nutritional demands it is obvious to supplement rumen bypass nutrition like calcium salts of fatty acids (Ca-FA), the form of rumen bypass fat and rumen un-degradable proteins (RUP) providing nutrients of high quality without altering its nutritional value because of fermentation process. The RUP reach lower part of gastrointestinal tract where these are subject to the digestion and absorption with its end products as amino acids rather liberation of nitrogen N as in rumen serving a substrate for microbial protein synthesis (Reddy and Hyder, 2023). The amino acids so liberated from RUP are directly absorbed and metabolically utilized resulting in improved milk yield and quality (Katiyar *et al.,* 2019).

Rumen bypass fats in the form of Ca-FA when offered to ruminants remain unaffected from rumen fermentation process reaching small intestine where it is subject to enzymatic hydrolysis and degradation releasing fatty acids and glycerol that absorbs through intestinal wall (NRC, 2011). Nutrients from rumen bypass fat after absorption result into higher milk volumes and quality during early lactation in *Nili Ravi* buffaloes (Hifzulrahman *et al.,* 2020). Buffalo is the primary contributor of milk in Pakistan, however, to which extent the supplementation of Ca-FA alone and in combination with RUP could affect the performance of lactating buffaloes is not well established as the volume of work conducted to understand their combined effects is rare to establish any conclusive approach of feeding these nutrients in lactating *Nili Ravi* buffalo. We hypothesized that feeding lactating buffaloes diets with high RUP content and Ca-FA will improve milk production and composition with the synergistic response expected when both supplied together. The anticipated interaction of Ca-FA with RUP reflects that increased post ruminal amino acid supply can only be maximum utilized when accompanied by sufficient rumen protected energy source, enabling a positive combined effect on milk production. Therefore, the current study was designed to investigate the individual and combined effects of rumen bypass fat and RUP on production performance in lactating *Nili Ravi* buffalo.

MATERIALS AND METHODS

Animals: The experiment was conducted from May to August 2022 in a naturally ventilated tie stall barn located at the dairy sheds of Livestock Experiment Station, Bahadurnagar, Okara (30.801380^0 N and 73.448334^0 E with an altitude of 170 m (570 ft.). The entire experiment was conducted in accordance with regulations approved by ethical committee for animal welfare at University of Veterinary & Animal Sciences Lahore (DR/494). Sixteen lactating multiparous *Nili Ravi* buffaloes with (mean ± SD) 11.2±0.76 kg/d of milk yield, 6.41±0.23% milk fat, 583±26 kg of BW, and 161±24 DIM were used. The buffaloes had free access to fresh drinking water round the clock.

Experimental Design, Treatments and Feeding: Buffaloes were randomly assigned to a treatment sequence in a 4×4 Latin square design with 21-d periods for a total of 4 periods (comprising 84 days). The dietary treatments were as follows: low RUP low fat (LPLF), low RUP high fat (LPHF), high RUP low fat (HPLF) and high RUP high fat (HPHF). In HF treatments, 300 g of Ca-soap of FA (CaFA) was top-dressed. All the animals received all four treatments according to their respective periods, thereby substantially reducing between animal variability. This design increases statistical power and reliability of treatment comparison despite four animals per treatment at any given time. Therefore, total number of observations was 16 for each RUP and Ca-FA level. The experimental diets were formulated using Cornell Penn Miner CPM Dairy Beta volume 3 software 3.0.10 (Fox *et al.,* 2004) from Cornell University (Ithaca, NY), University of Pennsylvania (Philadelphia, PA) and Miner Institute (Chazy, NY). Composition and nutrient analysis of dietary treatments is presented in table 1. The diets were composed (on DM basis) of 41.4% corn silage, 16.3% wheat straw, 10.8% corn grain, 9.10% wheat bran, 9.37% and 8.52% canola meal, 9.36% and 8.7% soybean meal and 5.6% corn gluten meal (60.0%). To achieve the higher level of RUP from 27.4% to 38.6%, corn gluten meal (60.0%) was used in HP treatments. Buffaloes were relatively similar in body weight so, they were offered a similar quantity of DM (16.0 kg/buffalo/day) assuming similar lactation persistency throughout the experiment and were fed once daily at 0900 h.

Experimental Measures, Sample Collection and Analyses: Diets offered as well as individual orts were weighed daily. Diets samples collected weekly were pooled for each period for laboratory analysis. These samples were analyzed for DM, CP, NDF, ADF, ether extract, and ash following official methods of analysis (AOAC International, 2005). Buffaloes were milked twice daily at 0200 and 1400 h. Milk production was recorded at each milking. Individual milk samples were taken twice weekly basis and assayed by infrared analysis using Lactoscan-S Milk Analyzer (Lactoscan S-1720, Milkotronic, Nova Zagora, Bulgaria) for fat, protein, and lactose content. Blood samples were collected at end of each period from jugular vein following Haque *et al.,* (2012). Blood samples were collected in heparinized syringes and immediately centrifuged at 2000 × *g* for 15 min at 4°C. Plasma was separated, aliquot stored at −20°C to be assayed by enzymatic method and estimated for the analysis of plasma urea nitrogen (PUN; Fluitest Urea, Analyticon Biotechnologies AG, Lichtenfels, Germany), triglycerides (Fluitest TG, Analyticon Bio-technologies AG, Lichtenfels, Germany), and glucose (BioMed-Glucose L.S, Egy-Chem for lab technology, Badar city, Elrubaki, Egypt). Buffaloes were weighed on d 21 of each treatment before feed distribution.

Table 1. Ingredients and nutrient composition of experimental diets

Item	Treatments*			
Ingredient (% of DM)	LPLF	LPHF	HPLF	HPHF
Corn silage	41.4	42.0	41.3	40.6
Wheat straw	16.3	16.1	16.3	16
Corn grain	10.8	10.6	10.4	10.2
Canola meal	9.37	9.22	8.52	8.37
Soybean meal	9.36	9.2	8.7	8.6
Wheat bran	9.1	8.95	8.38	8.24
Soybean hulls	2.15	2.11	--	--
Urea	0.84	0.83	--	--
Mineral premix	0.33	0.32	0.32	0.32
Salt	0.38	0.37	0.38	0.37
Bypass fat	--	0.3	--	1.3
Calcium carbonate	--	--	0.33	0.3
Corn gluten meal 60%	--	--	5.69	5.59
Analyzed nutrient composition				
DM, %	54.6	55	54.7	55.1
OM, % of DM	95.7	95.5	94.2	94
CP, % of DM	15.8	15.9	16	15.7
NDF, % of DM	40.2	39.5	38.7	38.1
ADF, % of DM	26.0	25.6	25.0	24.6
Forage NDF, % of DM	29.8	29.3	29.8	29.3
NFC, % of DM	37.8	37.2	38.1	37.4
Ether extract, % of DM	3.55	4.92	3.53	4.90
Starch, % of DM	27.1	26.6	27.9	27.3
Ash, % of DM	4.33	4.52	5.79	5.96
Predicted nutrient value**				
MP, g/kg of DM	85.3	85.3	96.2	96.3
RUP, % of CP	27.4	27.5	38.6	38.69
RDP, % of CP	72.6	72.5	61.4	61.31
ME, Mcal/kg of DM	2.43	2.53	2.49	2.59
NE_L, Mcal/kg of DM	1.57	1.63	1.6	1.67

*The treatments LPLF = low RUP low fat with RUP 27.4% and fat 3.55%, LPHF = low RUP high fat with RUP 27.5% and fat 4.92%, HPLF = high RUP low fat with RUP 38.6% and fat 3.53%, HPHF = high RUP high fat with RUP 38.69% and fat 4.9% of DM

**Values predicted using CNCPS evaluations, MP: metabolizable protein, RUP: rumen un-degradable protein, RDP: rumen degradable protein, ME: metabolizable energy and NE_L: net energy for lactation

Calculations and Statistical analysis: Milk efficiencies were calculated using equations: FCM (4%) fat corrected milk = (0.4×milk yield)+15×(milk fat content/1000)×milk yield, ECM energy corrected milk = (0.327×milk yield+12.95×milk fat yield/1000)+(7.65×milk protein yield/1000), MEO milk energy output = 0.00929×milk fat yield+0.00563×milk protein yield+.00395×milk lactose yield, 3.4% PCM protein corrected milk = milk yield × 0.294 × milk protein content. Methane emission was calculated using equation described by Patra (2016).

Data were analyzed using the MIXED procedure of SAS University Edition (SAS Institute, Inc., Carry. NC with the fixed effects of period and treatments whereas buffalo was designed as a random effect in this model. The following mathematical model was used for the analysis:

$$Y_{ijklm} = \mu + Buff_i + Per_j + RUP_k + FAT_l + RUP_k \times FAT_l + \varepsilon_{ijklm}$$

where Y is the response variable, μ is the overall mean, ε is the random error, $Buff_i$ represents the random effect of buffalo, Per_j represents the 21-d period, RUP_k represent the effects of RUP levels and FAT_l represents the fixed effects of supplementation of rumen-inert fat $Treat_k$ represents the treatment diets of experiment. Standard errors of the mean were reported and treatment differences were considered significant if $P \leq 0.05$ and as a trend for $0.05 > P \leq 0.10$.

RESULTS

DMI, milk yield and composition: The results on DMI, milk yield and composition are presented in Table 2. A small but significant increase was observed on DMI with HP and HF treatments ($P < 0.01$), while this increase was greater when fat supplementation was combined with low RUP diet compared with the high RUP diet (RUP × fat interaction, $P < 0.01$). Milk yield was increased in HF diets by 14% and HP diets by 3.8% ($P < 0.01$) with significant RUP × fat interaction ($P < 0.01$), which indicated that increase in milk was more pronounced when fat was supplemented with low RUP diet. The RUP feeding increased milk protein content and yield by 10.8% and 15.7% ($P < 0.01$), respectively, independent of the fat supplementation ($P > 0.10$). Milk lactose content and yield and 1.5% and 5.8% respectively, with increasing RUP levels ($P < 0.01$), respectively. Milk fat yield tended to be higher ($P = 0.07$) with increasing RUP levels (P = 0.07). The supplementation of fat increased milk fat content and yield by 22% and 39%, respectively ($P < 0.01$) without showing any interaction with RUP levels ($P > 0.10$). Milk lactose content and yield were increased by 3% and 17% with the increasing Fat levels ($P < 0.01$). Milk protein yield was increased by 14% with the supplementation of fat ($P < 0.01$). The BW was increased by 0.5% in RUP and 1% in fat treatments ($P < 0.01$) without showing any RUP x Fat interaction (*P* = 0.51).

Production efficiencies: The data on milk efficiencies is presented in Table 3. Fat corrected milk, ECM, and MEO were increased by 5.4%, 7.6%, and 8.1%, respectively, with increasing RUP levels ($P < 0.01$). Similarly, 4% FCM, ECM, and MEO were increased by 33%, 29%, and 29% respectively ($P < 0.01$) without showing any RUP × fat interaction ($P > 0.10$). The ratio of 4%FCM-to-DMI, 3.4% PCM-to-DMI, feed efficiency were increased by 4.6%, 13.7% and 2.0%, respectively with increasing RUP ($P < 0.01$), whereas, it was 32%, 14%, and 13% with increasing fat levels, respectively ($P < 0.01$). The efficiency of N utilization was increased by 16% in RUP and 17% in FAT treatment ($P < 0.01$) without showing any RUP × fat interaction ($P > 0.10$).

Blood metabolites: The results on plasma metabolites including plasma glucose, triglycerides and plasma urea nitrogen are presented in Table 4. Plasma glucose and triglycerides remained unaffected by HF or HP ($P > 0.05$) whereas plasma urea nitrogen was significantly low ($P < 0.01$) in high RUP diets. In high RUP diets the PUN was recorded to be 34.89% low as compared to low RUP diets. There was no interaction of high RUP and Ca-FA diets on any of the blood metabolites analyzed in the current study ($P > 0.05$).

Methane emission: Results on methane emission are presented in table 5. Methane emission as MJ, Mcal and CH_4 gram per day remained unaffected ($P < 0.05$) of HF and HP feeding whereas the interaction showed a significant decrease in CH_4 gram per day when fed LF diet as compared to HF diet under low RUP treatment ($P < 0.01$). Methane emission as gram per kg of DMI was significantly decreased ($P < 0.01$) by HF and HP and a weak decreasing interaction was also found ($P = 0.09$).

Table 2. Effects of Ca-FA and RUP on milk yield and composition in lactating *Nili Ravi* buffalo

Item	**Treatments***				**SEM**	**P-value**		
	LPLF	**LPHF**	**HPLF**	**HPHF**		**RUP**	**Fat**	**RUP × fat**
BW (kg)	586	592	589	595	19.183	<0.01	<0.01	0.51
DMI (kg)	15.78	16.05	16.08	16.09	0.043	<0.01	<0.01	<0.01
Milk yield (kg/d)	7.2	8.49	7.72	8.57	0.119	<0.01	<0.01	<0.01
Milk fat (%)	6.83	8.26	6.95	8.54	0.187	0.29	<0.01	0.67
Milk protein (%)	3.49	3.53	3.88	3.90	0.042	<0.01	0.52	0.89
Milk lactose (%)	4.76	4.93	4.84	5.00	0.025	<0.01	<0.01	<0.01
Milk fat yield (g)	491	702	542	729	21.9	0.07	<0.01	0.58
Milk protein yield (g)	251	297	302	332	6.3	<0.01	<0.01	0.2
Milk lactose yield (g)	343	415	377	425	6.8	<0.01	<0.01	0.07

*The treatments LPLF = low RUP low fat with RUP 27.4% and fat 3.55%, LPHF = low RUP high fat with RUP 27.5% and fat 4.92%, HPLF = high RUP low fat with RUP 38.6% and fat 3.53%, HPHF = high RUP high fat with RUP 38.69% and fat 4.9% of DM.

Table 3. Effects of Ca-FA and RUP on production efficiencies in lactating *Nili Ravi* buffalo

Item	**Treatment***				**SEM**	***P*-value**		
	LPLF	LPHF	HPLF	HPHF		RUP	Fat	RUP × fat
4% FCM	10.27	13.97	11.14	14.42	0.348	0.05	<0.01	0.53
ECM	10.63	14.14	11.86	14.79	0.348	<0.01	<0.01	0.39
MEO	7.33	9.83	8.23	10.32	0.251	<0.01	<0.01	0.41
PCM	5.86	6.46	6.7	7.21	0.626	0.19	0.36	0.94
4% FCM:DMI	0.65	0.87	0.69	0.90	0.021	0.09	<0.01	0.67
3.4% PCM:DMI	0.47	0.55	0.55	0.61	0.011	<0.01	<0.01	0.40
Feed Efficiency	0.46	0.53	0.48	0.53	0.007	0.0136	<0.01	0.06
N Efficiency	9.49	11.48	11.41	12.94	0.229	<0.01	<0.01	0.30

*The treatments LPLF = low RUP low fat with RUP 27.4% and fat 3.55%, LPHF = low RUP high fat with RUP 27.5% and fat 4.92%, HPLF = high RUP low fat with RUP 38.6% and fat 3.53%, HPHF = high RUP high fat with RUP 38.69% and fat 4.9% of DM

FCM (4%) fat corrected milk = (0.4×milk yield)+15×(milk fat content/1000)×milk yield, ECM energy corrected milk = (0.327×milk yield+12.95×milk fat yield/1000)+(7.65×milk protein yield/1000), MEO milk energy output = 0.00929×milk fat yield+0.00563×milk protein yield+.00395×milk lactose yield, 3.4% PCM protein corrected milk = milk yield × 0.294 × milk protein content.

Table 4. Effects of Ca-FA and RUP on blood metabolites in lactating *Nili Ravi* buffalo

Item	Treatment*				SEM	*P*-value		
	LPLF	LPHF	HPLF	HPHF		RUP	Fat	RUP × fat
Plasma glucose (mg/dL)	77.79	79.92	77.6	84.96	2.441	0.33	0.06	0.29
Triglycerides (mg/dL)	91.12	85.31	87.16	85.98	4.512	0.71	0.43	0.61
PUN (mg/dL)	20.42	20.07	13.15	13.21	0.841	<0.01	0.86	0.81

*The treatments LPLF = low RUP low fat with RUP 27.4% and fat 3.55%, LPHF = low RUP high fat with RUP 27.5% and fat 4.92%, HPLF = high RUP low fat with RUP 38.6% and fat 3.53%, HPHF = high RUP high fat with RUP 38.69% and fat 4.9% of DM.

Table 5. Effects of Ca-FA and RUP on methane emission in lactating *Nili Ravi* buffalo

Item	Treatment*				SEM	*P*-value		
	LPLF	LPHF	HPLF	HPHF		RUP	Fat	RUP × fat
CH4, MJ	14.7	14.9	14.8	14.7	0.04	0.93	0.12	<0.01
CH4, Mcal	3.5	3.6	3.5	3.5	0.01	0.93	0.12	<0.01
CH4, g/d	246	249	248	247	0.7	0.93	0.12	<0.01
CH4, g/kg DMI	15.6	15.5	15.4	15.4	0.001	<0.01	<0.01	0.09

* The treatments LPLF = low RUP low fat with RUP 27.4% and fat 3.55%, LPHF = low RUP high fat with RUP 27.5% and fat 4.92%, HPLF = high RUP low fat with RUP 38.6% and fat 3.53%, HPHF = high RUP high fat with RUP 38.69% and fat 4.9% of DM.

DISCUSSION

Our objective was to investigate the effects of feeding low and high levels of Ca-FA and RUP on milk yield and composition in lactating *Nili Ravi* buffalo. Four dietary treatments; 1) low RUP low fat (LPLF); 2) low RUP high fat (LPHF); 3) high RUP low fat (HPLF) and 4) high RUP high fat (HPHF) were offered. Rumen bypass fat, source of both saturated (C16:0) and unsaturated (C18:1) FAs was fed in the form of Ca-FA (calcium-fatty acid) complex to bypass the rumen as the bond of FAs with calcium slows down its degradation in rumen. Increasing levels of RUP were fed through corn gluten meal. The idea of feeding bypass protein in combination with bypass fats in lactating animals was based on the fact that buffalo milk contain more solids than cows and response of these nutrients has been improved in cows as per literature. Moreover, the study was conducted in summer, while heat stress is known to reduce feed intake and milk production of the dairy animals. Under such conditions, supplementing rumen protected fat can help maintain energy supply while RUP supports in post rumen amino acid availability to maintain productive performance during thermal stress (Piscopo *et al.,* 2024).

Ca-FA and RUP did not induce satiety in lactating buffalo: In current study DMI was slightly increased with HF and HP treatments. The finding agrees with Gomes *et al.* (2016), Tacoma *et al.* (2017), Savari *et al.* (2018), Tiwari *et al.* (2019) who reported either higher or similar intakes when high RUP diets were offered in contrast with varying levels of rumen degradable protein. Increase in DMI, in current study can be attributed to fair balance of RUP and RDP with starch sources produce a conducive rumen environment for the proliferation of rumen microbes producing more digestive enzymes and rapid utilization of nutrients (Rosmalia *et al.,* 2022). The HF treatment increased the DMI in current study, similar to the findings of Ranjan *et al.* (2012). On contrary to our finding Hifzulrahman *et al.* (2019) reported a quadratic decrease in DMI when Ca-FA was fed 600 gram per animal per day. This could be attributed to inclusion of Ca-FA 300 gram per animal per day in current study is efficiently cleared by the rumen to bypass without altering the gut kinetics.

Ca-FA and RUP improved milk yield, composition and production efficiencies: In the current study, we observed increased milk yield, milk constituents and production efficiencies with HF and HP treatments. The finding is in agreement with Nisa *et al.* (2008), Gomes *et al.* (2016) and Tiwari *et al.* (2019) who reported high milk yield at varying RUP levels when fed with different forages and RDP levels to lactating buffalo. The RUP can increase milk yield either directly or indirectly as reported previously (Rehman *et al.,* 2020). Direct increase in milk yield can be attributed to increased supply of limiting amino acids to mammary tissues synthesizing more milk while indirect increase could be due to altered hormonal concentrations in plasma favoring release of growth hormone resulting in higher weight gains and milk production instead of fat deposition into adipose tissue (Danes *et al.,* 2023; Zhao *et al.,* 2024). The increased milk yield with HP and HF in the current study might be due high DMI and energy provided by additional Ca-FA added in rations with improved availability of amino acids due to high RUP fraction as similar finding were observed by Gulati *et al.*

(2005). High milk fat content in HF treatment might be due to availability of more bypass nutrients such as fatty acid and amino acids as suggested by Adnan and Bilal (2025), who reported milk fat is not only the function of effective fiber digestion but the availability of other nutrients to mammary tissue. High milk protein content with HP treatment in current study is because of high RUP intake resulting in availability of more limiting and total amino acids to mammary tissue for milk protein synthesis. High lactose content in current study is possibly due to improved digestion of feedstuff in rumen (Chumpawadee *et al.,* 2005) leading to availability of more nutrients including amino acids and glucose to mammary tissue for uptake and synthesis of milk lactose. Our findings are in line with previous studies conducted on feeding fat (Shelke *et al.,* 2012; Hifzulrahman *et al*., 2020; Öz and Küçükersan, 2024) and protein (Bartocci and Terramoccia, 2010; Haque *et al.,* 2018; Akhtar *et al.,* 2021) to lactating buffaloes indicating that rumen protected fat and increased dietary energy density mainly improves milk fat and milk production, while post rumen protein supply improves milk protein output.

Ca-FA and RUP improved body weight and feed efficiency: In current study HF and HP treatments increased body weight and feed efficiency. The finding agrees with Katiyar *et al.* (2019) and Tiwari *et al.,* (2019) who reported increase in body weight with dietary treatments high in bypass fat and RUP when fed to lactating buffaloes. The reason for high body weight in current study might be high DMI, more energy and positive nitrogen balance as reported by Pattanaik *et al.* (2003) and Paengkoum *et al.* (2004) leading to gains in body weight. On contrary to our finding Savari *et al.* (2018) reported no change in body weight when fed RUP against control diet. HF treatment improved feed efficiency, might be due to high concentration of energy in Ca-FA per unit of DMI. Average N efficiency was 11.3% which was lower compared to earlier reports (Haque *et al.,* 2018) and could be probably due to higher CP contents in the diet leading to poor N-conversion-to milk. However, as compared to lactating Holstein cow the N efficiency in lactating buffalo is low (Jonker *et al.,* 2002).

High RUP decreased plasma urea nitrogen: A decreased level of plasma urea nitrogen was observed in high RUP diets despite having similar CP contents across treatment. This could be associated with urea supplementation in Low RUP diets leading more PUN in those treatments. Interestingly, the finding was in agreement with Nisa *et al.* (2008), Sultan *et al.* (2009), Gomes *et al.* (2016), Akhtar *et al*. (2017), Tacoma *et al.* (2017) and Savari *et al.* (2018) they reported low plasma urea N with increasing levels of RUP in their studies. A possible reason of low PUN against high RUP diets is that high RUP diets are usually accompanied with low rumen degradable protein (RDP) which leads to low ammonia release in rumen due to microbial degradation of RDP substrates (Santos *et al.,* 2016). Subsequent low ammonia synthesis in rumen leads to low plasma urea nitrogen content in animals consuming high RUP diets. However, blood glucose is not affected by treatment diets. The findings of Savari *et al.* (2018) agreed with the finding of current study as ground corn based didn’t alter glucose level in the plasma. Possible reason for constant blood glucose level may be higher utilization of corn starch in rumen than in small intestine because increased availability of glucose in small intestine elevates blood glucose levels as compared to higher starch utilization in rumen (Abbas *et al.,* 2020).

Reduced methane emission with feeding of Ca-FA and RUP to lactating buffalo: Methane emission (CH4, grams/kg of DMI) is reduced in current study as a result of feeding HF and HP diets. Our findings were in line with Hossain *et al.* (2017) and Lamba *et al.* (2019) who reported linear decrease in methane emission with increasing level of RUP. Higher level of RUP offered low amount of substrates available for fermentation in rumen (da Silva soares *et al.,* 2025). Phuoc Thanh *et al.* (2023) reported decrease in methane emission with RUP and fat feeding. On contrary to our finding, Sun *et al.* (2019) reported no change in methane emission with increasing RUP level when replaced with RDP. Increase in RUP fraction eventually decreases the DM proportion of rapidly fermentable sources in the rumen. Methane emission is related with hydrogen availability in rumen. The possible reason for reduction in methane emission in current study is low availability of rumen hydrogen on account of feeding high RUP diets with combination of Ca-FA, both of which are rumen bypass, ultimately reduction in the rapidly fermentable sources in the rumen and reduce methane production, as these finding were consistent with the study of Lamba *et al.* (2014).

Conclusion: The findings of the study showed an increase in milk yield and milk composition in terms of milk fat percentage and yield, milk protein and its yield along with milk lactose and its yield in response to RUP and fat supplementation. The results are interesting because low producing animals are typically thought not to be responsive against dietary manipulation. Therefore, it is suggested that RUP fraction of 38.6% in concentrate part of ration and an addition of 300 gram of Ca-FA per animal per day could be a beneficial strategy to enhance the production performance of dairy type *Nili Ravi* Buffalo. Further studies are warranted to explore the beneficial effects of RUP and Ca-FA and their interactions to establish nutrient requirements.

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Authors Contribution: Conceptualization: RYA and MNH conceptualized the study including design of experiment and formulation of dietary treatments. Data collection: RYA and MA collected milk, blood and feed samples from animals under trial and data thus collected was arranged and tabulated by RYA and H. Statistical analysis: RYA, S and MNH arranged, analyzed and explained the inferences from data results. Editing of manuscript: RYA, S and H completed the write up and editing of manuscript.

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