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EFFECTS OF DIETARY STARCH LEVELS WITH HIGH AND LOW FORAGE NEUTRAL DETERGENT FIBER IN DAIRY BUFFALOES

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ABSTRACT

The aim of the experiment was to determine the effects of starch, neutral detergent fiber from forage (FNDF) levels and their interaction on milk yield, milk composition, methane production, and blood metabolites in lactating buffaloes. Sixteen multiparous Nili Ravi buffaloes (averaging 9 ± 1.58 kg/d of milk yield, 5.00 ± 1.61% of milk fat, 534 ± 83 kg of BW, and 46 ± 20 DIM, mean ± SD at the start of the study) were enrolled and received 4 treatments with 2 different levels of FNDF according to a changeover design with 6-wk periods under restricted feed intake. Within each FNDF supply level, the buffaloes received 2 different starch levels of concentrate with 3-wk subperiods. Corresponding to the following treatments: (1) 39% FNDF and 33% starch, (2) 39% FNDF and 49 % starch, (3) 50% FNDF and 33% starch, and (4) 50% FNDF and 49% starch. Milk production was increased by 4.27%, when low FNDF was fed compared with high forage NDF. Milk fat and lactose yields were increased by 7.50, 14.6%, respectively, in low FNDF. Energy-corrected milk (ECM) was increased by 5.09% in low FNDF diets than high forage NDF. Predicted enteric methane intensity decreased with high starch and low FNDF level. It is concluded that milk production, milk fat, and lactose yield increased in early lactating buffaloes fed low FNDF compared to high FNDF. Production efficiencies also improved with the low FNDF diet. However, no interaction between FNDF and starch levels was observed for the parameters investigated.

Keywords: Buffalo, Forage neutral detergent fiber, Milk yield, Nili Ravi, Starch.

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INTRODUCTION

Buffalo ranks as the world's 2nd largest source of milk after cattle. Nili- Ravi is most popular buffalo breed in Pakistan, and its shares is 68% in total milk production (FAOSTAT, 2023). Buffalo milk has higher milk protein, fat, and lactose as compared to cows (Ahmad *et al*., 2013). Consequently, greater milk composition indicates higher energy demands for milk production in lactating buffaloes as compared to cows (Pegolo *et al*., 2017).

Carbohydrates are the main energy source for dairy animals, including components such as starch, fiber, and sugars (NRC, 2001). Their prime function is to furnish energy to rumen microorganisms as well as the host animal (Krause and Combs, 2003). These are converted into volatile fatty acids (VFAs) in the rumen and provide 80 % net energy for maintenance and milk synthesis to ruminants. Starch is the primary nutrient in ruminant diets used to support high levels of production (Weiss *et al.,* 2011). Amylolytic bacteria in the rumen ferment starch into propionate. It provides glucose, which is a precursor for lactose that withdraws water from the blood for milk synthesis. Propionate also serves as a competitive pathway for the use of H_2 in the rumen. Moreover, dietary starch treatments change the microbe's ecology by preferring propionate-producing bacteria over methanogens and reducing methanogenesis (Bannink *et al*., 2006; Benchaar *et al*., 2001), but high starch levels cause metabolic diseases like ruminal acidosis. To overcome this problem, fiber is required. which is usually called Neutral Detergent Fiber (NDF) fiber is essential to maintain proper rumen function, high intake, and normal milk fat percentage in lactating cows (Sarwar *et al*., 1992). Feeding a high-fiber diet to buffalo is a common practice, as it is a better converter of low-quality fiber. It is efficiently utilized by buffaloes due to a higher population of cellulolytic bacteria and fungal zoospores (Qingbiao *et al.,* 2022). According to the (NRC, 2001), at least 75% of dietary NDF in dairy diet should come from forages to maintain optimal rumen function. Neutral detergent fiber from forages causes variation in ration NDF and starch levels. Digestion of fiber-rich diets increases the H_2 and CO_2 production, which are substratum for methanogenesis. To overcome this issue dietary starch is used which utilize H_2 for propionate production. In addition, milk production and components synthesis are correlated with carbohydrates composition. When the diet rich in starch and low in NDF, that diet typically increases the dry matter intake and milk yield. Milk production increases with an increase in starch level (Silvestre *et al*., 2022; Qamar *et al*., 2024; Rebecca *et al*., 2025) in cows as well as in buffaloes. Improvement in milk yield was observed when there was a decrease in FNDF (Ben-Meir *et al.,* 2021; Kendall *et al*., 2009) in cattle. An increase in milk fat percentage is usually noticed when NDF concentration in diet is increased (Allen, 2009). On the other hand, content of lactose is generally improved when a starch-rich diet is fed (Khorasani and Kennelly, 2001; Sterk *et al*., 2011). Milk protein content usually increased when starch rich diets are fed, relatively due to improve in rumen-available energy intake, leading to higher microbial crude protein (MCP) synthesis and amino acid (AA) supply to the mammary gland (Hristov *et al*., 2004; Cantalapiedra-Hijar *et al*., 2014). Thus, dietary carbohydrate composition is strongly correlated by both the synthesis of milk production and composition of milk. It should reduce the occurrence of metabolic diseases like ruminal acidosis, maximize the yield of microbial protein synthesis, and maintain stable fermentation and rumen pH (Grant *et al.*, 1990).

These dietary changes with the interaction of FNDF or starch were investigated by (Beckman and Weiss, 2005; Zhao *et al*., 2016) in cows but not evaluated in Nili Ravi buffaloes. We hypothesized that low FNDF coupled with high starch levels in diet could increase the milk production. The objectives of this experiment were to estimate the effect of different dietary FNDF levels, in diets with different levels of starch on production performance, composition, blood metabolites, and methane production of early lactating Nili Ravi buffaloes.

MATERIALS AND METHODS

Animals: The study was conducted from January to May 2022 at Livestock Experiment Station (LES), Bhunikey, Pattoki, (186 m altitude and 31.02°N, 73.85°E, Pattoki, Pakistan). All study practices were approved by the ethical review committee of LES, Bhunikey, Pattoki vide a letter no. 4016/R- 6 dated 02 .12 .21

Experimental Design, Treatments, and Feeding: Sixteen multiparous Nili Ravi buffaloes (averaging 9.0 ± 1.58 kg/d of milk yield, 5.00 ± 1.61% of milk fat, 534 ± 83 kg of BW, and 46 ± 20 DIM, mean ± SD at the start of the study) were enrolled. The 4 treatments diets were arranged in a 2 x 2 factorial design with 2 levels of forage NDF and 2 levels of starch. The buffaloes received either a low forage neutral detergent fiber FNDF (39%) or high FNDF (50%) diets according to a changeover design, with 6-wk period of each level. Each 6-wk period (macro period) was further divided into 2 subperiod (micro period) of 3-wk each so that animals received 1 of 2 starch levels of concentrate high-starch (49%) or low-starch (33%). Corresponding to the following treatments: (1) 39% FNDF and 33% starch, (2) 39% FNDF and 49 % starch, (3) 50% FNDF and 33% starch, and (4) 50% FNDF and 49% starch. Two types of concentrates were formulated. The FNDF levels were achieved by varying the level of wheat straw and corn silage. The diets were comprised (on a DM basis) of 55, 26% corn silage, 15.3, 47.2% wheat straw, and 29.5, 25.9 % concentrate in low and high fiber diets, respectively (Table 1). Animals were shifted from low to high starch within microperiods of 21 d. The overall duration of the trial was 94 d along with 10 d of the transition period, excluding the of adaptation period. The diets of the experiment were formulated with Cornell-Penn-Miner-Dairy 3.0.10 established on Cornell Net Carbohydrate and Protein System (CNCPS) 5.0.2 (Fox *et al*., 2004) on the basis of nutrient requirements established in previous studies in cattle (Cabrita *et.al*., 2007; Fredin *et al*., 2015) and (Qamar *et.al*., 2024) in starch and NDF levels (Ahmed *et al*., 2014) in buffalo. TMR was manually mixed and individually fed to buffaloes once a day at 0900 h. Lactating buffaloes were relatively same in BW; thus, offered the same amount of dry matter (12-14 kg per day per buffalo on a DM basis) considering same lactation persistence during the trial.

Experimental Measures, Sample Collection, and Analysis: Diets offered were weighed individually on daily basis. Subsamples of TMR and refusals were collected for each buffalo in every period. There was no refusal of feed, as restricted feeding was offered on a DM basis. Three samples of every feedstuff were collected during the entire experiment. Two times per period and composited for chemical analysis in accordance with the methods of the AOAC International (2005) for dry matter (DM) (method 934.01), ash (method 942.05), ether extract (EE) (method 920.39), and crude protein (CP) (method 984.13, N X 625: Kjeldahl method). Non-Fibrous Carbohydrate (NFC) was calculated according to (NRC, 2001). The ADF (sulfuric acid + cetyltrimethylammonium bromide treated filtration) and NDF (sodium sulfite + α-amylase filtration) were evaluated using the Ankom A 200 fiber analyzer (Ankom Technology Corp., Fairport, NY, USA) based on the procedure of Van Soest, 1991. Starch of corn and concentrate was estimated using an Anthrone method by Hodge and Hofreiter, (1962) and molasses sugar was measured at the Nutrition Laboratory, PCSIR Islamabad by (Kitinoja *et al*., 2005). The NDF from corn silage and wheat straw was the forage NDF. These values were used in experimental TMR formulation by CNCPS system. Corn silage samples were collected on a weekly basis to determine the DM content. After determination of silage dry matter, the corn silage quantity offered was adjusted every week to ensure the same delivery of DM on each experimental day. Buffaloes were milked two times a day at 06.00 am and 06.00 pm. At every milking, record the production for each buffalo. Milk Samples were collected on every other day throughout the first fourteen days and consecutively from 15th to 21st day of each period. Samples from evening and morning milking were collected and evaluated by using a Lactoscan analyzer S 1720 (Milkotronic, Bulgaria) for protein, lactose, and fat. Blood samples of experimental animals were collected from the jugular vein on third last day of every period. Two blood samples per buffaloes were collected in heparinized syringes at 0800 and 0815 h i.e. (30 and 45 min before feed distribution, and 30 and 15 min after milking), and immediately centrifuged at 2,000 x g for 15 min at 4°C to separate the plasma, which were stored at −20°C for further analysis. The objective of sampling the blood at basal level (before feeding) was to avoid the hormonal changes related to the feeding times (Haque *et al*., 2012). The plasma samples were subsequently estimated by using enzymatic kits (Randox Laboratories Ltd.) for the absorption level of glucose (GL2623), triglycerides (TG; TR210), and cholesterol (CH201) using a biochemical analyzer (Anwar *et al*., 2024). Animals were weighed at the start and end of each period before feed distribution and after morning milking. Body condition score was examined at the start and end of every period followed by Anitha *et al*., 2001.

Table 1: Ingredient and nutrient composition of experimental diets.

Items	Dietary Treatments[1]			
	39% FNDF		50% FNDF	
	33% Starch	49% Starch	33% Starch	49% Starch
Ingredient (% DM)				
Corn silage	55.09	55.19	26.34	26.39
Wheat straw	15.36	15.39	47.13	47.2
Corn Grain	11.75	17.66	10.30	15.48
Molasses Cane	1.71	1.46	1.50	1.28
Wheat Bran	2.01	3.35	1.77	2.94
Soybean Meal	2.25	2.41	1.98	2.11
Soybean Hulls	8.51	1.07	7.46	0.93
Canola Meal	2.26	2.41	1.98	2.11
Salt	0.23	0.23	0.20	0.20
Sodium Bicarbonate	0.23	0.23	0.20	0.20
Dicalcium Phosphate	0.20	0.20	0.17	0.18
[2]Mineral Mixture	0.40	0.40	0.35	0.35
Urea	0.00	0.00	0.62	0.62
Nutrient composition, % of DM				
DM	42.79	42.71	59.19	59.1
Forage	70.45	70.57	73.47	73.59
CP	10.54	10.54	10.50	10.50
Ash	5.71	5.52	5.61	5.44
NDF	47.66	43.98	57.59	54.38
ADF	29.54	26.57	35.94	33.35
EE	3.00	3.13	2.47	2.58
[3]NFC	34.94	38.55	25.83	28.98
Predicted Nutritive Value				
Forage NDF%	39.11	39.18	50.09	50.17
RUP, % CP	29.44	29.55	30.06	30.20
RDP, % CP	70.56	70.45	69.94	69.8
ME, Mcal / kg	2.33	2.39	1.86	1.91
NEL, Mcal/ kg	1.50	1.54	1.20	1.23
Starch % of DM	25.53	30.21	17.26	21.35
Sugar % of DM	2.92	2.89	2.49	2.47

[1]Dietary treatments consisted of 4 diets with 2 levels of forage NDF (39 and 50%) and 2 levels of starch concentrate (33 and 49%) on dry matter DM basis in diet; [2]Mineral Mixture contained 0.7% DCP, 0.23% salt, 0.05% $MgSO_4$, 0.007% , $FeSO_4$, 0.005% $ZnSO_4$, 0.005% $MnSO_4$, 0.0013% $CuSO_4$, 0.001% CoCl, 0.005% KI.; [3]NFC = 100 – (CP + NDF + ash + EE)

Statistical analysis: Experimental data were evaluated by using the PROC MIXED of SAS (On-Demand for Academics, SAS, Institute Inc., Cary, NC), represents major effects of treatments and period, while buffaloes in the model were considered as a random effect. Following statistical model was applied for the study:

$$Y_{ijklm} = \mu + Buff_i + Period_j + wk_k\,(Period_j) + FNDF_l + ST_m + FNDF_l \times wk_k\,(Period_j) + FNDF_l \times ST_m + \varepsilon_{ijklm}$$

Where Y_{ijklm} is the dependent variable. μ is the grand mean, $Buff_i$ represents the random effect of buffalo (i = 1 to 16), $Period_j$ represents the macro period of 6-wk period (j = 1 to 2), wk_k ($Period_j$) represents the 3-wk subperiod (k = 1 to 2) within the 6-wk macro periods, $FNDF_l$ represents the (l = 1 to 2), ST_m represents the fixed effect of starch (m = 1 to 2), $FNDF_l \times ST_m$ represents the interaction between FNDF and ST effects. The ε_{ijklm} was the residual random error. This model conferred the considerable accuracy in the statistical test for the ST supply and limited the power of the FNDF effect because of less degree of freedom and the largest residual error used (Steel and Torie, 1980). The Standard error of the means was observed, and the treatment differences were declared significant if $p \leq 0.05$ and considered trend for $0.05 < p \leq 0.10$.

RESULTS

Milk production and composition: Lactation responses of experimental animals fed different levels of starch and FNDF are illustrated in Table 2. The buffaloes fed a 39% FNDF diet produced 4.27% more milk than those fed a 50% FNDF diet (p<0.01). Milk yield was not affected by different starch levels (p>0.10). Milk contents and yields remained unaffected (p>0.10) in response to ST, whereas milk fat, and lactose yields were higher by 6.01%, 4.88 %, respectively, in response to 39% FNDF diets (p≤0.01). Milk production and milk contents were unaffected by starch and forage NDF the interaction (p>0.10). Energy-corrected milk (ECM) was increased 5.09% in low forage NDF diets than high forage NDF (p ≤0.01).

Table 2: Milk production and contents of lactating buffalo's feds with different diet levels of starch and forage neutral detergent fiber.

Items	Dietary Treatments[1]					*P-value*[2]		
	39% FNDF		50%FNDF					
	33% Starch	49% starch	33% starch	49% starch	SEM	ST	FNDF	ST×FNDF
Yield,								
Milk, kg/d	8.19	8.18	7.79	7.88	0.33	0.50	0.01	0.45
Fat, g/d	500	498	462	476	12.71	0.53	0.01	0.38
Protein, g/d	318	322	305	312	11.16	0.46	0.15	0.81
Lactose, g/d	420	420	394	405	8.82	0.36	0.01	0.34
Composition, %								
Fat	6.16	6.12	6.06	6.08	0.12	0.93	0.54	0.74
Protein	3.86	3.90	3.96	3.94	0.08	0.84	0.24	0.57
Lactose	5.11	5.12	5.13	5.12	0.04	0.96	0.83	0.73
ECM[3]	11.59	11.59	10.87	11.13	0.24	0.44	0.01	0.44

[1]Dietary treatments consisted of 4 diets with 2 levels of forage NDF (39 and 50%) and 2 levels of starch concentrate (33 and 49%) on dry matter DM basis in diet
[2]ST= Effect of starch [2]FNDF= Effect of forage neutral detergent fiber
[2]ST × FNDF = Interaction effect of starch and Forage Neutral Detergent Fiber
[3]ECM = Energy Corrected Milk, ECM = [0.327 × milk (kg)] + [12.95 × fat (kg)] + [7.65 × true protein (kg)] Tyrrel and Reid (1965)

Body weight and body condition score: Treatment means for BW and BCS are presented in Table 3. No effect of BCS and BW were observed in response to the dietary treatments (p >0.10).

Feed and production efficiencies: Feed and production efficiencies are presented in Table 4. Milk nitrogen (MkN) remained unaffected by the treatment diets (p>0.10). Feed efficiency (milk yield per unit of DMI), ratio of 4 % FCM to DMI and ratio of 3.4 % PCM to DMI, decreased (p<0.01) by 17 %, 23 % and 20 %, respectively, with 50% FNDF diet compared to 39% FNDF diet; however, they were did not affected by ST (p>0.10). Milk nitrogen efficiency (MNE) has tendency to increase (p=0.08) by 4.40 % in response to low forage NDF. Milk nitrogen to milk energy ratio (MKN: MKE) g/Mcal remained unaffected (p>0.10). Gross MP, metabolic MP efficiency remained unaffected by the dietary treatments. The 4% FCM yield decreased (p<0.01) by 5.8 % with 50% FNDF diet compared with 39% FNDF diet, however; did not influence by ST (p>0.10). The 3.4 % PCM yield increased (p<0.03) with

39% FNDF diet by 20 % compared with the 50% FNDF diet but remained unaffected by ST and starch to forage NDF interaction. Milk energy (MKE) decreased by 5.6 % with increasing FNDF level from 39 % to 50 % of DM ($p<0.05$). No interaction was found between starch and forage NDF on any efficiency.

Methane production: The results of methane production per milk yield are presented in Table 4. Interaction was not observed between starch and forage NDF for methane production to milk yield, whereas methane production was decreased by 28 % with decreasing forage NDF level ($p<0.01$). However, methane production to milk yield decreased by 3.4 % with increasing the starch level ($p<0.02$).

Table 3: Body weight, and Body condition score of lactating buffalos' feds with different diet levels of starch and forage neutral detergent fiber.

Items	Dietary Treatments					P-value		
	39% FNDF		50%FNDF					
	33% starch	49% starch	33% starch	49% starch	SEM	ST	FNDF	ST× FNDF
BCS[1]	3.03	3.09	3.03	3.03	0.05	0.44	0.37	0.44
BW[2], kg	487	494	490	491	6.0	0.44	0.94	0.50

[1]BCS= Body Condition Score, [2]BW = Body Weight

Table 4: Feed and Production efficiencies of lactating buffaloes' feds with different diet levels of starch and forage neutral detergent fiber.

Items	Dietary Treatments					P-value		
	39% FNDF		50%FNDF					
	33% starch	49% starch	33% starch	49% starch	SEM	ST	FNDF	ST× FNDF
[1]Feed efficiency	0.68	0.68	0.56	0.57	0.02	0.51	<0.01	0.52
4% FCM: DMI	0.91	0.90	0.72	0.74	0.03	0.49	<0.01	0.37
3.4%PCM: DMI	0.67	0.68	0.53	0.54	0.05	0.71	<0.01	0.88
Gross efficiency MP	0.02	0.02	0.02	0.02	0.00	0.87	<0.01	0.87
Metabolic efficiency MP	0.02	0.02	0.02	0.02	0.00	0.88	<0.01	0.87
[2]Milk nitrogen efficiency (%)	27.42	27.77	26.10	26.76	1.25	0.45	0.08	0.81
[3]MkN: MkE (g/Mcal)	6.10	6.18	6.26	6.23	0.07	0.78	0.18	0.46
[4]MkN(g/d)	49.77	50.39	47.74	48.92	2.29	0.46	0.15	0.81
[5]4% FCM, kg/d	10.78	10.74	10.05	10.29	0.41	0.48	<0.01	0.35
[6]3.4%PCM, kg/d	8.02	8.06	7.40	7.54	0.67	0.72	0.03	0.85
[7]MkE (Mcal/d)	8.10	8.10	7.57	7.78	0.32	0.40	0.01	0.41
[8]CH_4/MY (kg)	26.46	25.60	34.06	32.89	1.30	0.02	<0.01	0.56

[1]Feed efficiency = (Milk production/ DMI) (Akhtar *et al.,* 2021) , [2] Milk Nitrogen Efficiency % = {(N in milk / N intake) x 100} Barros *et al*. (2017), [3]Milk nitrogen /milk energy = MkN: MkE (g /Mcal) = {Milk nitrogen (g/ d) /Milk energy (Mcal/ d)}, [4]Milk nitrogen MkN = (milk protein /3.98), [5]4% Fat Corrected Milk = [(0.4 × milk yield) + 15 × (fat/100) × milk yield)] (Chung *et al.,* 2007),[6]Protein Corrected Milk(PCM)=(milk (kg/d) × 0.294% CP)Charbonneau *et al.* (2006), [7]Milk energy MkE = {(0.00929 x g of Fat / d) + (0.00563 x g of true protein/d) + (0.00395 x g of Lactose/d)}Barros *et al.* (2017),[8]Enteric methane intensity in gram /kg of milk production = CH_4 g/d/ kg of MY(kg) (Patra, 2016).

Table 5: Blood metabolites of lactating buffalo's feds with different diet levels of starch and forage neutral detergent fiber.

Items	Dietary Treatments					P-value		
	39% FNDF		50%FNDF					
	33% Starch	49% Starch	33% starch	49% starch	SEM	ST	FNDF	ST × FNDF
Glucose, mg/dl	77.33	78.13	79.11	78.63	1.14	0.91	0.42	0.65
Cholesterol, mg/dl	140	134	160	151	6.12	0.23	0.03	0.74
Triglycerides, mg/dl	105	102	102	103	1.73	0.49	0.47	0.42

Plasma metabolites: Response of blood metabolites is presented in Table 5. Plasma glucose and triglyceride concentrations were unaffected with treatment diets ($p>0.10$), whereas the high- forage NDF diets increased the concentration of cholesterol by 13.5 % ($p<0.03$) compared with low- forage NDF diets. We found no effect of starch levels and its interaction with forage NDF on the blood glucose, triglyceride, and cholesterol concentrations ($p>0.10$).

DISCUSSION

Lactation responses of buffaloes fed different levels of starch and forage NDF are presented in (Table 2). The buffalo fed 39% FNDF diet produced 4.27% more milk than those fed 50% FNDF diet. These results are in accordance with Kendall *et al*. (2009) where observed that diet containing 28% NDF consumed by cows produced more milk and fat, than those fed diets containing 32% NDF. The present study is also in accordance with the findings of Zhao *et al*. (2016) conducted on dairy cows. It was attributed to increases in NE_L in diet, which increases milk production (Weiss and Pinos-Rodriguez, 2009). Our results are in agreement with Ahmed *et al*. (2014), who observed comparatively high milk production in Nili Ravi buffaloes with low NDF level. Which is an indication that low- fiber diets increase milk yield. An appropriate NDF ratio in diet of dairy animals is important to obtain optimum milk production. An increase in dietary NDF in dairy animals' diet can partly lower the milk yield and increases the milk fat associated with increase in net energy intake (Ahmed *et al*., 2014). However, contrary to our findings, Alzahal *et al*. (2009) in cows as well as Anwar *et al*. (2025) in Nili Ravi buffaloes, observed unaffected milk production with different level of NDF due to similar DMI, resulting in similar NE_L, or maybe due to rumen fill effect and subacute ruminal acidosis. In the present study, milk yield was unaffected by different dietary starch levels. Same findings were reported by Dyck *et al*. (2011), who observed no change in milk production after feeding different concentrations of starch. It might be due to similarity in DM and energy intake among the treatment groups of cows. In the present study, milk protein contents and yield remained unaffected with different starch, FNDF levels, and their interaction. These results agree with the study of Dann *et al*. (2010), where observed no effect by dietary starch concentration in dairy cows due to short-term response or nutrient supply for milk synthesis. These results agree with Kendall *et al*. (2009), who observed no change in milk protein fed different NDF levels in cows. Milk fat yield was increased by 6.01% in the group fed 39% FNDF diet in the current findings are in agreement with (Kendall *et al*., 2009). They concluded that greater milk fat yield with low NDF 28% than 32% diets was associated with greater milk production. Contrary to our findings, Anwar *et al*. (2025) in buffaloes and Oba and Allen, (2000a) in cows reported no effect of dietary FNDF on milk fat content and yield Milk fat percentage and yield remained unaffected in response to ST among the dietary treatments. Current results are in agreement with previous study on cows (Akins *et al*., 2014).

Milk lactose percentage and yield remained unaffected in response to ST and starch to FNDF interaction among the dietary treatments. However, lactose yield was increased by 4.88 % in response to 39% FNDF diet. Current study results are in line with the previous findings (Kendall *et al*., 2009; Zhao *et al*., 2016). It was directly related to milk production. Energy-corrected milk (ECM) was 5.09% higher in low forage NDF diets than high forage NDF diets. These results agree with the findings of Zhao *et al*. (2016), who observed decreased in ECM with the increase in NDF level. In contrast to our results. Harmison *et al*. (1997) observed no effect on ECM. The yield of ECM was not affected by starch levels in cows (Dann *et al*., 2008). These results are in agreement with our current findings. Decreasing NDF mainly forage NDF with high starch from corn grains in the diet of dairy cows reduced enteric CH_4 production (g/d) and intensity (g/kg of milk). Our findings are in accordance with the results of Aguerre *et al*. (2011), who reported reduced emission of enteric methane in diets of cows fed lowest NDF level. It was attributed to low NDF intake (Aguerre *et al*., 2011). Our results are also in line with Anwar *et al*. (2025) in buffaloes. It was possibly due to H_2 formation, which is produced by the fermentation of structural carbohydrates to acetate and butyrate. Thus, methane production can be reduced by depriving methanogens of hydrogen. Higher dietary FNDF increased the acetate production in the rumen. In our study CH_4 production / milk yield showed tendency to decrease with increase in starch levels which is in line with (Benchaar *et al*., 2001). Mostly higher starch level led to lower NDF level (Albornoz *et al*., 2019). Starch diets change the microorganism's ecology by preferring propionic-acid producing microbes over methanogens, or it was due to high propionic acid, which reduced methanogenesis (Benchaar *et al*., 2001; Bannink *et al*., 2006). No effect of BCS and BW were observed in response to the dietary treatments. Our results are in line with Adin *et al*. (2009) where observed no effect of FNDF on BW in lactating cows. It was possibly due to the energy utilized for milk yield as a result no energy is available for body reservoirs (Dann *et al*., 2014). In contrast to our study (Harmison *et al*., 1997) observed that body weight decreased linearly as forage NDF decreased because DMI decreased as FNDF decreased, BW loss might have occurred to maintain milk production.

BCS was unaffected with dietary starch level, which is in accordance with the study of (Dyck *et al*., 2011). However, Oba and Allen, 2003 observed that change in BCS was positive for cows on high starch diets. Glucose and triglycerides were not affected by the dietary treatments. However, cholesterol shows increasing trend when FNDF level increased from 39% to 50% FNDF. The present study findings, are in agreement with the study of Mahmoud *et al.* (2014) in cows, possibly due to increase in fatty acids intake when fed alfa alfa silage than corn silage. The plasma metabolites were unaffected with different starch levels and its interaction with FNDF levels. The current findings are in accordance to the study of (Akhlaghi *et al*., 2022) in cows.

In the current study, production, fat and lactose increased in lactating buffaloes fed low FNDF (39%) as compared to high FNDF (50%). In addition, production efficiencies also increased for low FNDF diet. However, predicted enteric methane intensity was decrease with decreasing FNDF and increasing starch levels. Despite of all these changes in production no interaction affect is observed between FNDF and starch levels on any parameter. Based on the current findings, it can be concluded that diet containing low FNDF improves production performance of buffalo irrespective to starch levels in early lactating Nili Ravi buffaloes.

Conclusion: In the present study, milk production was increased in early lactating buffaloes fed low FNDF compared to high FNDF. Fat and lactose yield were increased with decrease in forage NDF level. Predicted enteric methane intensity was reduced with decreasing forage NDF and increasing starch levels. Based on the current findings, it was concluded that low FNDF (39%) with high starch in TMR diets, improves milk production, composition and reduced predicted methane production in early lactating Nili- Ravi buffaloes.

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