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EFFECTS OF DIETARY STARCH REPLACEMENT WITH SUGAR ON LACTATION PERFORMANCE OF NILI RAVI BUFFALOES

H. Tahir[1], S. Naveed [1], N. Ahmad[2], M. A Tausif [3], B. E Azam[3], S. Qamar[4], S. Anwar[4], A. Jabbar[5], M. A Khan[6] M. Imran[7] and M. N. Haque[1]

[1]Department of Animal Nutrition, [2]Department of Livestock Production, University of Veterinary and Animal Sciences, 54000, Lahore, Punjab, Pakistan
[3]Livestock Experiment Station Bhunikey, Pattoki, District Kasur, 55300 Pakistan; [4]Buffalo Research Institute, 55300, Pattoki, Punjab, Pakistan; [5]Fodder Research Institute, 40100, Sargodha, Punjab, Pakistan
[6]Department of Livestock and Dairy Development, Govt of Punjab, Lahore.
[7] Department of Animal Nutrition, KBCMA College of Veterinary and Animal Sciences, Narowal 51600, Pakistan
*Corresponding author's E-mail: muhammad.naveed@uvas.edu.pk

ABSTRACT

Feeding high starch diet is a common practice to increase milk production and similarly feeding sugars helps in improving milk fats. However, the extent to which trade-off between dietary starch and sugar content is possible for optimal milk production and fats is not fully explored in lactating Nili Ravi buffaloes. Twelve multiparous early-lactating Nili Ravi buffaloes (52 ± 37 d in milk, mean ±SD) received 3 treatments in a 3 × 3 Latin square design and each period consisted of 28 d. The dietary treatments contained starch and sugars in the following manner: 1) 28.7% starch, 2.61% sugar; 2) 25.9 % starch, 4.29% sugar; 3) 22.9% starch, 5.73% sugar on DM basis. All dietary treatments were iso-nitrogenous. Replacing dietary starch with sugar increased milk fat content, milk fat yield and 4% fat-corrected milk (FCM) linearly by 7.29, 13.8 and 11.9% respectively, however, milk yield was not affected by treatments. Similarly, energy-corrected milk (ECM) was also increased by 10.3% by the replacement of dietary starch with sugar. Body weight increased by 2% linearly, whereas, the body condition score remained unaffected as sugar replaced starch. There was a linear increase in plasma cholesterol concentrations, whereas plasma urea nitrogen, glucose, and triglyceride concentrations remained unaffected by increasing sugar. The marginal efficiency of NEL consumed for milk yield, 4% FCM and ECM increased linearly with increasing sugar. Feeding diets containing 22.9% starch and 5.37% sugar increased milk fat content, fat yield, and body weight of Nili Ravi buffalo without impacting on milk production and blood metabolites. Using sugar as a substitute for starch could be a practical option for producers without negatively impacting production.

Keywords: Buffaloes, milk production, grain starch, sugar

INTRODUCTION

Buffaloes in Pakistan significantly contribute by adding 36.45 billion liters in milk value chain with a herd average of 6.30 liters per buffalo per day (FAO, 2023). This milk is primarily coming from feeding agriculture by products and crop residues. However, due to the rise in urban farming and growing feed mill business, a shift from traditional practices to more corn-based diets is gaining momentum. In dairy cows, corn feeding as NFC to high-producing dairy animals is a common diet-formulation approach to maximize milk production (Gao and Oba, 2016). Starch is the main source of NFC in dairy cow diets according to NRC (2001). The diets of high-yielding cows usually contain 20% to 30% starch (Akins *et al.*, 2014). Aside from benefits in milk production feeding high starch in dairy cows diets may increase the risk of subacute ruminal acidosis by decreasing rumen pH (Monnerat *et al.,* 2013), which is associated with decreased feed intake (Carmo *et al.,* 2014) and milk fat depression (Monnerat *et al.*, 2013). Corn prices have increased due to high demand over the past years and are expected to continue rising in the future (USDA-NASS, 2014; USDA-ERS, 2021). Industries such as bio fuel also use corn grain creating greater competition with livestock demand. One way to overcome such issues is to partially replace corn grains (starch) with molasses (sugar) while maintaining milk yield (Gao and Oba, 2016). Previous research in dairy cows indicated that partial replacement of starch with sugars produces many desirable production responses, including an increase in feed intake (Broderick *et al.,* 2008; Gao and Oba, 2016), higher milk production (Gao and Oba, 2016) and milk fat yield (Penner *et al.*, 2009; Gao and Oba, 2016). Research demonstrates that even though sugar ferments quickly in the rumen, the inclusion of sugar content up to 13% of DM did not lower the pH of the rumen in lactating cows when starch was substituted with either lactose (DeFrain *et al.,* 2004) or sucrose (Broderick *et al.,* 2008).

Various studies related to starch feeding (Akins *et al.,* 2014; Carmo *et al.,* 2014; Silvestre *et al.,* 2022) or sugar feeding (DeFrain *et al.,* 2004; DeFrain *et al*., 2006; Penner *et al.,* 2009) on the lactation performance of cattle have been conducted. As per author's knowledge, no study has reported the effect of replacing starch with sugar in lactating buffaloes. The objective of the current study was to determine how replacing starch with sugar affected the milk yield, milk composition and plasma metabolites of lactating Nili Ravi buffaloes.

MATERIALS AND METHODS

Experiment site, approval, research design and husbandry practices: The experiment was performed in a tie-stall barn of the Livestock Experiment Station (LES), Pattoki, from November 2020 to February 2021 in Pakistan. The experiment was conducted in compliance with the ethical rules and regulations for the animal welfare of LES Bhunikey, Pattoki. Twelve Nili Ravi buffaloes of early lactation averaging (± SD) 6.84 ± 1.47 kg/d of milk, 5.87 ± 0.51% of milk fat, 485 ± 47 kg of BW, and 52 ± 37 days in milk (DIM) were enrolled. Animals were tied individually in ventilated sheds and had free access to water throughout the day. Buffaloes were randomly assigned to a treatment sequence in a 3 × 3 Latin square design with a 28-d period. The experiment duration was 84 days. The treatments were as follows: 1) 28.7% starch, 2.61% sugar; 2) 25.9% starch, 4.29% sugar; 3) 22.9% starch, 5.73% sugar. The experiment diets were formulated using the Cornell-Penn-Miner-Dairy 3.0.10 system CNCPS (Fox *et al.,* 2003). The diet was composed (on a DM basis: Table 1) of 40%, corn silage, 13% wheat straw, and 47% concentrate. Each treatment diet was offered in a fixed amount (13.9 kg) and offered in the form of TMR after manual mixing. All buffaloes had almost the same body weight (BW), with only slight differences; therefore, similar lactation persistency was assumed during the experimental period, and no feed refusal was observed in any treatment group. Buffaloes were individually fed once in the morning at 0900 h.

Experimental measures, sample collection and analysis: The diet offered was weighed daily for each animal. Feedstuff samples (n=3) were taken twice in each experiment period to determine dry matter content by method 934.01. According to the standard procedures of AOAC International (2005), all feed samples were estimated for CP, EE and Ash using methods 984.13, N x 6.25, 920.39 and 942.05. Analysis of NDF (α-amylase + sodium sulfite) and ADF (sulphuric acid+ cetyltrimethylammonium bromide treated filtration, were conducted by Ankom-2000 fiber analyzer (Fairport, NY, USA) according to Akhtar *et al.* (2021). Corn and concentrate starch was estimated as method described by Hodge and Hofreiter, (1962). Molasses sugar was estimated by Kitinoja *et al.* (2005). For the experiment TMR formulation in the CNCPS system, all these values were used. Milk from each buffalo was recorded twice a day. In the first two weeks, milk samples were taken on an alternate day and daily from the 15th to 28th day of each period. Morning and evening milk samples were evaluated for fat, protein, and lactose content using an ultrasonic milk analyzer (Lactoscan S 1720, Milkotronic, Bulgaria).

Blood was sampled on the 25th day of each experiment period from the jugular vein into tubes containing sodium heparin (Haque *et al.,* 2012; Qamar *et al.* 2024). Immediately following collection, samples were centrifuged at 2,000 × g for 15 minutes at 4°C. Separated plasma from the blood and stored for further analysis at -20°C. These samples were assayed for concentration of glucose (GL), PUN (UR), triglyceride (TG), and cholesterol (CH) using a biochemical analyzer (RX Monza, Randox Laboratories Ltd). An esophageal polyethylene probe (10 mm internal, 14 mm exterior diameter and length, 3.6 m) was used to collect rumen fluid samples from buffaloes at the 28-day of each study period. Before feeding, samples were taken to evaluate the ruminal pH. Approximately 0.6 L of rumen fluid was filtered through two layers of cheesecloth, and the pH was immediately recorded (Pirondini *et al.*, 2015). Fresh fecal samples were scored using a visual fecal scoring scale (Irelanpperry *et al.*, 1993). Buffaloes were weighed before and at the end of each experiment period before feeding. Body condition score (BCS) was assessed at the beginning and the last day of each experiment period with the evaluation of three independent individuals and average scores were used (Anitha *et al.*, 2011).

Calculations and statistical analysis: Non-fibrous carbohydrates (NFC) were computed using the NRC (2001) methods. Values of energy-corrected milk (ECM), 3.4 % protein-corrected milk (PCM), milk energy (MkE), milk nitrogen (MkN) and milk nitrogen efficiency (MNE) were calculated by the equations presented (Akhtar *et al.,* 2021; Barros *et al.,* 2017). The metabolic efficiency of MP and gross efficiency MP were calculated according to (INRA, 2007; Anwar *et al.,* 2024). The marginal efficiency for net energy of lactation (NEL) consumed for milk, 4% FCM and ECM was calculated according to Moallem. (2016). The predicted value of enteric methane intensity was estimated by using the equation of Patra. (2016).

Table 1. Ingredient and analyzed chemical composition of the experiment diets.

Items	Treatments		
	28.7% starch 2.61% sugar	25.9% starch 4.29% sugar	22.9% starch 5.73% sugar
Ingredients (DM %)			
Corn silage	39.5	39.8	40.0
Wheat straw	12.8	12.9	12.9
Corn grains ground	21.6	15.8	11.0
Wheat bran	12.9	14.9	14.5
Canola meal	2.67	3.47	3.88
Corn gluten meal 60%	1.70	1.81	2.02
Soybean hulls	5.95	4.98	6.01
Molasses	0.94	4.35	7.55
Urea 46%	0.59	0.54	0.49
Mineral mixture[a]	0.54	0.54	0.54
Dicalcium phosphate	0.54	0.54	0.54
Salt (NaCl)	0.27	0.27	0.27
Oil[b]	0.07	0.16	0.27
Nutrient composition (DM %)			
CP	10.7	10.8	10.7
Ash	4.88	5.43	5.90
ADF	27.8	27.8	28.2
NDF	42.6	42.7	43.1
NFC[c]	40.4	39.8	39.0
EE	2.87	2.90	2.94
Predicted values			
Forage	52.3	52.7	52.9
RUP (CP %)	30.0	29.9	30.1
RDP(CP %)	70.0	70.1	69.9
MP(g/kg)	87.7	87.3	86.4
ME (Mcal/kg)	2.42	2.40	2.38
NEL(Mcal/kg)	1.56	1.55	1.53
Sugar (% of DM)	2.61	4.29	5.73
Starch (% of DM)	28.7	25.9	22.9

[a]Mineral mixture contained 0.23% Salt, 0.05 % $MgSO_4$, 0.7% DCP, 0.005 % $ZnSO_4$, 0.007% $FeSO_4$, 0.005 % $MnSO_4$, 0.0013 % $CuSO_4$, 0.0005 % KI,0.001 % $CoCl_2$, .; [b]Musturd seed oil.; [c]NFC = non-fibrous carbohydrates [100-(CP + NDF + ash + crude fat]

Data were analyzed by using the GLIMMIX procedure of SAS University Edition (Institute Inc., Cary, NC), with the buffalo treated as random variable whereas, period and treatments were representing the main effect in the following mathematical model.

$$Y_{ijk} = \mu + Buff_i + Per_j + Treat_k + \varepsilon_{ijk}$$

Where Y is the response variable, μ is the overall mean, $Buff_i$ represents the random effect of buffalo, Per_j represents period of 28 days, and Treat k represents the treatment diets and ε is the random error. The polynomial contrast effects were used to compare treatments with linear and quadratic effects. To determine appropriate coefficients for the contrast statement, SAS's ORPOL function Interactive Matrix Language procedure was used to uneven intervals among the treatments. Treatment differences were considered significant when $P \leq 0.05$, and as a trend for $0.05 < P \leq 0.10$.

RESULTS

Milk yield and composition: Treatment effects on production parameters are presented in Table 2. Milk production averaging 8.73 kg/d showed no difference among the treatments ($p > 0.10$). Milk fat content and yield increased linearly by 7.29 and 13.8% respectively, with increasing dietary sugar levels from 2.61 to 5.73% of DM ($p < 0.05$). Milk lactose content tended to decrease by 3.17%, whereas lactose yield, protein contents and yield remained unaffected ($p > 0.10$). Likewise, increasing sugar linearly increased ECM by 10.3% ($p < 0.04$), 4% FCM by 11.9% ($p < 0.03$) and milk energy by 10.1% ($p < 0.05$), however, 3.4% PCM remained unaffected ($p > 0.10$).

Feed and production efficiencies: The ratio of FCM to DMI increased linearly by 13% with increasing dietary sugar ($p < 0.02$); Table 3). A linear decrease of 3.8 % was observed in milk nitrogen to milk energy ratio ($p < 0.04$), whereas, a tendency was observed for gross and metabolic efficiency with increasing dietary sugar ($p = 0.06$). However, no effect was found ($p \geq 0.10$), when efficiencies were represented as milk N/N-intake or milk yield/DM intake and PCM/DMI intake. However, methane intensity CH_4/MY (g/d kg of milk) did not show any affect by replacing dietary starch with sugar ($p \geq 0.10$). The marginal efficiency of NEL consumed for milk yield, 4% FCM and ECM increased linearly with increasing sugar ($p < 0.03$).

Body weight, body condition score, rumen pH and manure scoring: Body weight, body condition score, rumen pH and manure scoring are presented in Table 4. Body weight increased linearly by 2% with increasing sugar ($p < 0.01$). However, body condition scoring, rumen pH and manure scoring remained unaffected by dietary treatment ($p > 0.10$).

Blood metabolites: The effects of treatments on plasma metabolites are shown in Table 5. Blood cholesterol concentration increased linearly by 11.2% with increasing dietary sugar ($p \leq 0.05$). Dietary treatment did not affect on plasma glucose, plasma urea nitrogen, or triglyceride concentrations ($p > 0.10$).

Table 2. Effect of replacing starch with sugar for lactating buffaloes on milk production.

Items	Treatments			SE	*P*	
	28.7%starch	25.9%starch	22.9%starch			
	2.61% sugar	4.29% sugar	5.73% sugar		Linear	Quadratic
Milk (kg/d)	8.43	8.79	8.99	0.41	0.20	0.81
Yield (g/d)						
Fat	557	590	634	31	0.02	0.89
Lactose	437	448	451	20.2	0.47	0.83
Protein	337	348	358	16.5	0.20	0.93
Milk composition (%)						
Fat	6.58	6.72	7.06	0.16	0.01	0.46
Lactose	5.20	5.10	5.04	0.07	0.08	0.77
Protein	4.00	3.97	3.97	0.04	0.77	0.91
ECM[a] (kg/d)	12.6	13.2	13.9	0.64	0.04	0.97
4.0% FCM[b] (kg/d)	11.7	12.4	13.1	0.62	0.03	0.96
3.4% PCM[c](kg/d)	8.68	9.27	9.69	0.82	0.19	0.87
MkN[d] (g/d)	52.7	54.5	56.0	2.59	0.20	0.93
MkE[e] (Mcal/d)	8.79	9.21	9.68	0.46	0.05	0.98

[a] ECM= energy corrected milk = (12.95 × fat yield) + (7.65 × true protein yield) + (0.327 × milk yield)

[b] FCM= fat corrected milk, 4% = (0.4 × milk yield) + [15 × (fat/100) × milk yield]

[c] PCM= protein corrected milk, 3.4 % = milk (kg) × 0.294% CP

[d] MkN = milk nitrogen = milk protein /3.98

[e] MkE = milk energy = 0.00929 × g of fat/d + 0.00563 × g of true protein/d + 0.00395 × g of lactose/d

Table 3. Effect of replacing starch with sugar for lactating buffaloes on feed and nitrogen efficiencies

Items	Treatments			SE	*P*	
	28.7%starch	25.9%starch	22.9%starch			
	2.61% sugar	4.29% sugar	5.73% sugar		Linear	Quadratic
Feed efficiency[a]	0.60	0.63	0.65	0.03	0.15	0.86
4% FCM : DMI	0.84	0.89	0.95	0.04	0.02	0.91
4% PCM : DMI	0.62	0.67	0.70	0.06	0.16	0.89
Gross efficiency MP[b]	0.28	0.29	0.30	0.01	0.06	0.99
Metabolic efficiency	0.39	0.41	0.43	0.01	0.06	0.98
MNE[c] (%)	22.3	22.7	23.6	1.09	0.21	0.82
MkN:MkE[d] (g/Mcal)	6.00	5.93	5.78	0.10	0.04	0.68
CH_4/MY[e] (g/kg)	28.9	27.6	26.6	0.41	0.20	0.81
Marginal efficiency for NEL consumed[f]						
Milk Yield (kg/Mcal)	0.62	0.67	0.71	0.03	0.03	0.98
4% FCM (kg/Mcal)	0.87	0.94	1.92	0.05	<0.01	0.78
ECM (kg/Mcal)	0.93	0.99	1.09	0.05	0.01	0.80

[a] Feed efficiency = milk yield (MY) / dry matter intake (DMI)

[b] Gross efficiency MP = milk protein yield / MP intake

[c] Milk nitrogen Efficiency, MNE = (N in milk / N intake) x 100

[d] MkN:MkE = milk nitrogen / milk energy

[e] Methane intensity (g/kg milk)= CH_4(g/d)//MY

[f] Marginal efficiency for NEL consumed= milk yield/ (NE_C-NE_M), 4%FCM/ (NE_C-NE_M), ECM/ (NE_C-NE)

Table 4. Effect of replacing starch with sugar for lactating buffaloes on blood metabolites

Items	Treatments			SE	*P*	
	28.7%starch	25.9%starch	22.9%starch			
	2.61% sugar	4.29% sugar	5.73% sugar		Linear	Quadratic
BW (kg)	483	489	493	10.5	< 0.01	0.52
BCS	3.04	3.00	3.04	0.05	0.94	0.27
Rumen pH	6.90	6.87	6.95	0.04	0.43	0.25
Manure scoring	3.00	2.92	3.00	0.05	1.00	0.28

Table 5. Effect of replacing starch with sugar for lactating buffaloes on blood metabolites.

Items	Treatments				*P*	
	28.7%starch	**25.9%starch**	**22.9%starch**			
	2.61% sugar	**4.29% sugar**	**5.73% sugar**	**SE**	**Linear**	**Quadratic**
Glu[a] (mg/dl)	78.6	76.6	77.3	2.41	0.56	0.48
Chol[b] (mg/dl)	124	130	138	11.0	0.05	0.86
PUN[c] (mg/dl)	22.5	21.3	21.3	2.23	0.61	0.76
TG[d] (mg/dl)	136	127	142	11.0	0.62	0.26

[a]Glu=Glucose
[b]Chol= Cholesterol
[c]PUN = Plasma urea nitrogen
[d]TG= triglyceride

DISCUSSION

The present study aimed to evaluate the impacts of replacing dietary starch with sugar on milk yield, milk composition, MNE and blood metabolites in early lactating Nili Ravi buffaloes.

Lactation responses documented in different studies on replacing starch with sugar are inconsistent. In the present study, ECM and 4% FCM were increased linearly with increasing sugar from 2.61 to 5.37% possibly due to an increase in milk fat yield. Similar results are reported in dairy cattle by Gao and Oba, (2016) with sugar feeding. However, ECM and 4% FCM were not affected when starch was replaced with sugar (Broderick *et al*., 2008). Milk fat content and yield increased linearly with increasing dietary sugar in the present study. These findings are in accordance with other studies conducted on dairy cows, where feeding molasses (sugar) or disaccharides increases milk fat. Such results were even reported in a study where starch was replaced with other sugars as well (Gao and Oba, 2016). The contrary to starch favors the higher production of acetate and butyrate. Both acetate and butyrate are the main precursors associated with milk fat synthesis (Jenkins and McGuire, 2006). This increase in fat content could be attributed to increased hydroxybutyrate production (Gao and Oba, 2016). Conversely, some other studies reported that feeding starch and sugar to lactating cows had no impact on fat content (Chibisa *et al.,* 2015; Sun *et al.,* 2019).

In the present study, milk yield was not changed by dietary treatments. Similar results are reported in the study of cows, where starch was used in the replacement of sugars (Broderick *et al.,* 2008). However, when sucrose was fed up to 10% of the total amount of sugar in the diet, the milk and protein changed quadratically and this shift was linked to mainly change in feed intake (Broderick and Radloff, 2004). The DMI in our study was not modified primarily due to a restricted feeding strategy which may partially explain the similar milk and protein yields. Starch and sugar digestion differ in the yield of glucogenic (propionate) or lipogenic (acetate) nutrients (Akins *et al.,* 2014). Digestion of starch produces more glucogenic nutrients, the amount of lactose produced regulates milk production and more accessibility of glucogenic precursors in diet, which is a major factor that possibly affects milk yield (Leiva *et al.,* 2000). No change in milk production could be attributed to similarities in feed intake, nutrient digestibility, and rumen fermentation as reported in the literature (Wanapat *et al.,* 2012). Similarly, many other factors can also impact milk composition including animal breed, parity, lactation stage, diet formulation and environmental conditions (Sun *et al.,* 2019).

In the current study, 4% FCM/DMI increased linearly with increasing sugar attributed to milk fat yield. Similarly, the marginal efficiency of NEL consumed for milk yield, 4% FCM and ECM was also increased linearly with increasing sugar**.** Previous research reported that the marginal efficiency of NEL consumed for milk yield, 4% FCM and ECM is associated with NEL intake, with an increase in NEL intake leading to decreased marginal efficiency (Moallem, 2016). Similarly, in the current study, a decrease in NEL intake resulted increase in marginal efficiency. In our study gross and metabolic efficiency for MP tended to increase with sugar might be due to a numerical increase in milk protein yield. The efficiency of milk production (milk yield /dry matter intake) was similar among diets. These findings are in accordance with Carmo *et al.* (2014) and Akhlaghi *et al.* (2022) where feeding starch did not affect the feed efficiency. On the other hand, feed efficiency tended to decrease (Gencoglu *et al*., 2010) or decrease (Ferraretto *et al.,* 2011; Akins *et al.,* 2014). In the current study, feed efficiency was not changed, accompanied by similar milk yield and restricted feeding. Predicted methane production was 0.234g/d among treatment groups. Methane intensity was not affected in our study. These findings are consistent with previous studies on cattle fed diets containing starch and sugar (Hatew *et al.,* 2015; Sun *et al.,* 2019).

Despite the relatively short duration of the experiment, body weight increased linearly by 2% when dietary starch was replaced with sugar. These results align with those of Silveira et *al*. (2007), who found that cows fed 22% starch had higher body weights than those fed 25% starch. Dann *et al.* (2015) reported similar results. One possible explanation for this increase in body weight is the shift of energy utilization toward weight gain compared to milk production (Potts *et al.,* 2015). Contrary to this, body weight was not affected by replacing dietary starch with sugar (Broderick and Radloff, 2004; Broderick *et al.*, 2008; Penner and Oba, 2009).

Rumen pH was not affected by replacing starch with sugar. These findings are consistent with several studies in dairy cows that reported no effect on rumen when starch was partly replaced with either 13% lactose (DeFrain *et al.,* 2004) or 7.5% sucrose (Broderick *et al.,* 2008). Likewise, another previous study reported no significant effect on rumen pH when sugar was added up to 5% (McCormick *et al.,* 2001). Many in vitro studies have reported sugar feeding increases the molar concentration of butyrate in the rumen (Vallimont *et al.*, 2004; Ribeiro *et al.,* 2005) which was one of the possible reasons of no change in the rumen pH (Oba, 2011). In contrast to other studies rumen pH tended to increase Penner *et al.* (2009) or decrease Gao and Oba, (2016) with increasing sugar. Alves et *al.* (2009) reported that buffaloes have a greater buffering capacity in the rumen compared to cows. In our study, the fixed feeding, inclusion level of grains (46%) and more buffering capacity of rumen in buffalo could be the factors resulting in no change in rumen pH.

Decreasing starch and increasing sugar did not affect the blood metabolites except blood cholesterol concentration. Blood cholesterol concentration was increased linearly by 11% with increasing dietary sugar which may be due to more need of cholesterol required for the transport of fatty acids for higher milk fat contents and yield (Piccioli-Cappelli *et al.,* 2014; Gao and Oba, 2016).

Conclusion: Based on the results, feeding diets containing 22.9% starch and 5.73% sugar to lactating buffaloes increased milk fat content, yield and body weight compared to high starch diet. In addition, rumen pH remained unaffected for buffaloes fed high-sugar diets. The marginal efficiency of NEL consumed for milk yield, 4% FCM and ECM increased linearly with increasing sugar. Despite these changes, no effect was observed in feed efficiency, milk nitrogen efficiency (MNE) and methane intensity when starch was replaced with sugar. These results indicate that sugar as a substitute for starch could be a practical option for producers without negatively impacting production.

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Availability of data and material: Data generated of the current study are obtainable from the corresponding author on reasonable request.

Conflict of interest: Authors declare no conflict or competing interest of a financial or personal nature.

Author's contribution: Conceptualization: MNH, SN, NA; Methodology: HT, AT, BA, MAT, SA, SQ; Formal data analysis: MNH, HT; Writing and original draft preparation: HT, MNH, SN, MI. Final drafting and revisions: HT, MNH, MS, MAK. Supervision: HT, MNH, NA, AJ.

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