# EFFECT OF GRINDING SIZE OF CORN GRAINS AT TWO STARCH LEVELS ON LACTATION PERFORMANCE OF NILI RAVI BUFFALOES

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## ABSTRACT

The aim of the study was to investigate the effect of grinding size of corn grains at two starch levels on lactation performance, methane production, and blood metabolites of Nili Ravi buffaloes. Sixteen multiparous buffaloes in early lactation (32 ± 12 DIM; mean ± SE), were enrolled into four treatment groups using a 4 × 4 Latin square design and each period consisted of 21-d. The different experimental diets were LSCG = low starch coarse ground corn, LSFG = low starch fine ground corn, HSCG = high starch coarse ground corn, and HSFG = high starch fine ground corn. The treatments contained fine (FG; particle size (PS) = 1.5mm) and coarse (CG = 3.0 mm) ground corn in high- (HS; starch (ST) = 25%) and low- (LS= 15%) starch (dry matter basis), respectively. Milk yield was increased by 1.85% with increase in starch level and showed a tendency to increase with ST x PS. In response to ST, lactose and milk protein yield were increased by 4.50%, and 2.42% respectively. The HSFG diet exhibited the highest milk protein and lactose yield. The treatment diets showed no difference in milk fat contents and yield. Glucose was higher in FG compared to the CG diets. Gross, metabolic, milk nitrogen efficiencies and enteric methane production was decreased by feeding high - starch diet. Body weight increased with increase in starch level. Diets with 25 %starch level improved milk production, protein, lactose yields and decreased methane production. Starch level had a more pronounced effect than particle size, however, interaction was not observed between starch and particle size for any parameter.

## INTRODUCTION

Buffalo (*Bubalus bubalis*) is a major global source of milk production, with approximately 15.86 million milking individuals producing 36.45 billion liters of milk in Pakistan (FAOSTAT,2023). The Nili -Ravi buffalo is main dairy animal in Pakistan, contributing around 68% of total milk production (FAOSTAT,2023). Balancing optimal energy allowances in the ration of early lactating buffaloes have become important issue. The optimization is important not only for economic benefits but also for its role in reducing the environmental pollution.

Corn grains are the primary source of feed energy, with their main component being starch (Ferraretto et al. 2013). Starch contributes almost 75% of the energy value of corn grain

(NRC,2001). Thus, improving starch utilization may improve the lactation performance and minimize the feed expenses by reducing corn in diet or increasing income, specifically during periods of high grain prices (Ferraretto et al. 2013). The optimum starch content of diet for dairy buffaloes is not well defined but for cows, suggested range is 24 to 26% (DM basis) (Staples, 2007).

Grain processing has been used as a technique to increase the availability of starch in rumen (Theurer, 1986) Various methods of processing corn for feeding dairy cows have been investigated earlier (Ferraretto et al. 2013: Fredin et al. 2015). One of the methods is reducing the particle size (PS) of corn grain enhances the access of ruminal microbes to starch reserves, increases the surface area for bacterial attachment, or enzymatic degradation (Firkins et al. 2001) Consequently, this enhances the digestibility of starch and net energy for lactation $NE_L$ (Huntington et al. 1997). Starch utilization is improved by particle size reduction and well-studied in dairy cattle compared with buffalo.

Literature is available on cattle, but there are few studies evaluated one or more of these corn size or dietary factors, such as particle size effects, level of starch, on milk production in Nili Ravi buffaloes. Hence, the objective of the current study was to evaluate the effect of processing methods of corn grain and dietary starch levels on production performance, methane production and blood metabolites in early lactational dairy buffaloes. We hypothesized that optimum level of starch with fine ground corn, compared with coarse ground corn, would result in improved milk production and milk composition.

## MATERIALS AND METHODS

**Ethical approval, experimental animals, and husbandry practices:** An experiment was carried out from January to April 2021 in a tie-stall shed situated at the Livestock Experiment Station, Bhunikey, Pattoki, Pakistan (186 m altitude and 31.02°N, 73.85°E; Pattoki, Pakistan). All experimental procedures were performed in compliance with the authorized guidelines approved by the ethical committee for animal welfare at Livestock Experiment Station, Bhunikey Pattoki. Sixteen multiparous lactating Nili Ravi buffaloes, averaging (8. 21 ± 1. 56 kg/d of milk yield, 5. 00 ± 1. 61 % of milk fat, 496 ± 47 kg of BW, and 32. 5 ± 12. 2DIM, mean ± SD), were randomly used. Buffaloes were individually tied in well- ventilated tie-stalls. Cool and clean drinking water was made available around the clock.

**Research design, treatments, and feeding:** Buffaloes were randomly assigned to four treatment groups, consisting of four animals. Each group received four treatment diets with two levels of starch (% DM basis). Experimental diets included two types of concentrates, low starch (LS), and a high starch (HS) concentrate. Starch levels were altered by replacing soy hulls with ground corn. Low and high starch levels were 15 (**LS**) and 25% (**HS**), with coarse and fine ground corn having particle size 3.0 mm and 1.5 mm, respectively. The HS and LS treatment diets included either fine ground (**FG**) or coarse ground (**CG**) corn. The four treatments were as follows: (1) HS with FG corn (**HSFG**), (2) HS with CG corn (**HSCG**), (3) LS with FG corn (**LSFG**), and (4) LS with CG corn (**LSCG**), in accordance with 4×4 Latin square design. Total duration of the experiment was 84 days, with each period of 21- d. Ingredient and nutrient composition of the experimental diets are presented in Table 1. The treatment diets were formulated using Cornell-Penn-Miner-Dairy (CPM) 3.0.10 based on CNCPS 5.0.2 (Fox et al. 2003). The HS and LS treatments, (on a DM basis) comprised of 7.04 and 7.09% corn silage, 40.9 and 41.2% wheat straw, and 51.9 and 51.6% concentrate, respectively. The forage-to concentrate ratio was 49:51on DM basis. Buffaloes were offered a total mixed ration (TMR) one week before the start of the experiment as an adaptation period (pre-experiment). Buffaloes were individually offered a manually mixed TMR once a day at 0900 h. Buffaloes were relatively similar in BW; therefore, they fed the same amount of dry matter (13 kg per buffalo per day on a DM basis), assuming consistent lactation persistency throughout the study. The DM contents of corn silage was analyzed on weekly basis, and the as-fed amount of feed was adjusted as required to maintain the DM percentage of the ingredients in the TMR.

**Corn processing:** The coarse and fine ground corn grains were prepared from the same batch of shelled corn. Coarse ground (CGC) or fine ground corn grains (FGC) were prepared by passing dry corn through a hammer mill (Shakeel engineering Company, Lahore, Pakistan) using screen sizes 3.0 mm and 1.5mm, respectively.

**Experimental measures, sample collection, and analysis:** Diets offered were weighed individually on daily basis. We collected three sample of each feedstuff twice per period for DM evaluation and for further analysis. Feed samples were analyzed for DM (method 934.01), CP (Kjeldahl method 984.13, N X 625), Ether Extract (method 920.39), and ash (method 942.05) following the standard procedures of (AOAC, 2005). Non fibrous carbohydrate (NFC) was calculated according to method described by (NRC, 2001). The NDF (Sodium sulfite + α-amylase filtration) and ADF (Sulphuric acid + Cetyltrimethylammonium bromide treated filtration) were estimated by using Ankhom A 200 fiber analyzer (Ankom Technology Corp., Fairport, NY, USA). Starch analysis of corn and concentrate was estimated at Agripak laboratory, Rawalpindi, Pakistan by Anthrone method described by (Hodge and Hofreiter, 1962). These starch values were then used in experimental TMR formulation by CNCPS system.

**Production performance:** Buffaloes were milked two times a day at 6.00 am and 6.00 pm. Milk yield was recorded every day during the experimental study. Samples of milk were collected on every other day during the initial 2 weeks and consecutively from d 15 to 21 of each period for milk composition. Samples of milk were obtained from morning and evening milking. These samples were analyzed with ultrasonic milk analyzer Lactoscan S 1720 (Milkotronic, Bulgaria) for protein, fat, and lactose.

**Plasma analysis, Body condition score measurements, pH, and body weight calculation:** Third last day of each period, blood samples were collected from the jugular vein of all the buffaloes following (Haque et al. 2012). The samples were immediately centrifuged at 2,000 × *g* in heparinized syringes for 15 min at 4°C and subsequently preserved at −20°C for future 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). Plasma metabolites were analyzed by using commercially available enzymatic kits (Randox Laboratories Ltd.) for the concentration of glucose (GL2623), PUN (UR107), triglyceride (TG; TR210), and cholesterol (CH201) with a biochemical analyzer (RX Monza, Randox Laboratories Ltd.). The rumen fluid samples were obtained from the buffaloes in the last of each period using an esophageal polyethylene probe with internal and external diameters of 10 and 14 mm, respectively, and a length of 3.6 m. To evaluate the ruminal pH, samples were collected immediately before the morning feeding. Almost 0.6 L of rumen fluid was strained through two layers of muslin cloth, and pH was measured immediately after filtration following (Pirondini et al. 2015). Buffaloes were weighed before and in the end of each period after morning milking and before feed distribution. Assessment of body condition score BCS was carried out before and at the end of each period followed by (Anitha et al. 2001). Three individuals independently assess the buffaloes during the experiment, and a median score was used for each buffalo.

**Calculations:** Energy-corrected milk ECM = [0.327 × milk (kg)] + [12.95 × fat (kg)] + [7.65 × true protein (kg) Tyrrell and Reid (1965), 3.4% protein corrected milk = 3.4% PCM = milk (kg/d) × 0.294% CP (Charbonneau et al. 2006), 4% Fat Corrected Milk = 4% FCM = (0.4 × milk yield) + [15 × (fat/100) × milk yield (Chung et al. 2007), Milk nitrogen= milk protein/3.98, Milk nitrogen efficiency% = (N in milk / N intake) x 100 (Barros et al. 2017), MkN: MkE (g/Mcal) = Milk nitrogen (g/ d /Milk energy (Mcal/ d), 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), milk nitrogen/milk energy, gross efficiency MP= Milk protein yield/ MP intake, metabolic efficiency MP = Milk protein yield/ MP intake – (MP for growth + MP for maintenance+ MP for pregnancy), Feed efficiency = Milk yield/ DMI were calculated by the following equations presented previously by (Akhtar et al. 2021). Enteric methane production was calculated using the following Patra 's equations (Patra, 2016). Methane production Mega joule $CH_4$ MJ = 0.436+0.678 x DMI+0.697 x NDF intake, Methane production Mega Calories $CH_4$ Mcal= $CH_4$ MJ/4.18, Methane production gram/day $CH_4$ g/d= (0.671/40) x $CH_4$ MJ x1000, Methane production in gram /kg of dry matter intake = $CH_4$ /DMI (kg)= $CH_4$ g/d/DMI, Methane production in gram /kg of milk yield= $CH_4$ g/d/ kg of MY.

**Statistical analysis:** The experimental data thus obtained were analyzed using MIXED procedure of SAS (SAS Institute Inc., Cary, NC) (SAS, 2004) considering the main and fixed effects of period square and treatments, while buffaloes were nested within square and included as a random effect in the model. Mathematical model used for the analysis was described below:

$$Y_{ijklm} = \mu + S_i + Buff_{j(i)} + Per_k + ST_l + PS_m + (ST \times PS)_{lm} + \varepsilon_{ijklm}$$

where $Y_{ijklm}$ = response variable (variable of interest), $\mu$ = overall mean, $S_i$ is the random effect of square (i= 1to 4), Buff $_{j(i)}$ = random effect of buffalo within square (j = 1 to 4), $Per_k$ shows the fixed effect of period ($k$ = 1 to 4), and $ST_l$ = fixed starch effect ($l$ = 1 to 2), $PS_m$= fixed particle size effect ($m$ = 1 to 2), $(ST \times PS)_{lm}$ = interaction between ST and PS fixed effect and $\varepsilon_{ijkl}$ = residual random error term. The mean of standard errors was calculated, and treatment differences considered significant if $P \leq 0.05$ and as a trend for $0.05 < P \leq 0.10$.

## RESULTS

### Milk production and composition:

There was no refusal of feed in any experimental group due to fixed DM offered. The DMI was similar among treatments, with an average of 13.0 kg/d. Lactation responses of buffaloes fed different levels of starch and ground corn particle size are presented in Table 2. The buffalo fed the HS diet produced 1.85% more milk than those fed the LS diet (P = 0.03). Production was not affected by varying corn particle size (P > 0.10). A decrease trend for fat content was observed towards high starch diet (P = 0.01), whereas milk lactose and protein contents were unaffected (P > 0.10). Lactose and protein yield were increased by 4.50 and 2.42 %, respectively, with ST (P ≤ 0.01). Milk yield or components were unaffected by particle size in the treatment diets (P >

0.10). Milk production tend to be increased (P = 0.07), whereas milk contents were not affected by the interaction of ST and PS (P > 0.10). ECM, 3.4%PCM, and 4% FCM were not affected by the treatment diets (P > 0.10).

**Feed and production efficiencies:**

Feed and production efficiencies are summarized in Table 3. Feed efficiency (milk yield per unit of DMI), ratio of FCM to DMI, ratio of PCM to DMI, Milk nitrogen (MkN) to milk energy ratio and MkN remained unaffected by treatment diets (P > 0.10). Gross, metabolic, and milk nitrogen efficiency decreased (P < 0.01) by 4.83%, 6.52% and 7.27%, respectively, with the HS diet compared with the LS diet; however, they were not affected by PS (P > 0.10). Interaction was not observed between ST and PS on any efficiency.

**Body weight, body condition score and pH:**

Treatment means for BW, BCS, and pH are presented in Table 4. Body weight was increased by 1.86% for buffaloes fed high ST diets as compared with low starch diets (P < 0.01). Moreover, BW was increased with the interaction of ST and PS (P < 0.01). However, no effect of BCS and pH was observed in response to the dietary treatments (P > 0.10).

**Blood metabolites:**

No interaction was observed between ST and PS for blood metabolites except triglycerides which showed a tendency to increase (P =0.04). These findings are summarized in Table 5. Glucose increased by 10.43% with decreasing PS from coarse to fine ground corn (P = 0.01), but it was not altered by ST and ST x PS (P > 0.10). However, triglycerides, cholesterol, and PUN were similar across the treatments (P > 0.10).

**Methane production:**

Enteric methane production results are presented in Table 6. Methane production was decreased by 5.44% with increasing starch level. However, Methane production remained unaffected by different PS ($P > 0.10$). Interaction was not observed between ST and PS for methane production.

## DISCUSSION

There was no refusal of feed in any experimental group due to restricted DM offered. The DMI was similar among treatments, with an average of 13.0 kg/d. The fixed feeding was offered because in commercial farm practices, the farmers in Pakistan feed buffaloes restricted feeding especially in intensive farming. In our study, buffalo fed the HS diet produced 1.85% more milk than those fed the LS diet. These results are in agreement with the findings of (Carmo et al. 2014: Fredin et al. 2015) who observed that 11%, 4.51% higher milk yield in cows offered high-starch diet respectively. These findings are mainly associated with increased energy partitioning to the mammary glands and higher production of glucogenic nutrients(propionate)as a proportion of VFA. Which increases the glucose and milk lactose synthesis, thus increase in milk yield. Another possible reason is improved microbial synthesis or a combination of both may have bolstered milk yield with increasing starch level (Carmo et al. 2014). However, no effect of starch levels was observed by (Gencoglu et al. 2010).

Milk yield did not differ for PS of corn. Our results are in accordance with Fredin et al. (2015), which was confirmed by the meta review performed by (Ferraretto et al. 2013). It associated with no effect of grain processing on DMI and in our study DMI was fixed. However, in a study MY was increased for treatment diets with FGC compared to CGC (Rémond et al. 2004). The yield of FCM and ECM was not affected by starch levels are in agreement with the findings of Ipharraguerre et al. (2003). Milk production showed tendency to increase by the interaction of ST and PS.

In our study, milk protein yield was increased by 2.42% in response to ST. Similar findings have been reported by (Carmo et al. 2014: Fredin et al. 2015), where they observed that protein yield was increased quadratically, 2.54% and 14%, respectively, higher for cows supplied with high- starch diets. In the present study, increase in protein yield might be due to the higher energy available to rumen microbes for protein synthesis Silvestre et al. (2022) or it may be associated with increase in mammary protein synthesis McGuire et al. (2007). Milk protein contents remained unaffected. Our results are in accordance with Fredin et al. (2015) concluded no effect by dietary starch concentration due to short-term response or nutrient supply for milk protein synthesis was unaffected for all treatments. However, in other studies milk protein content had a trend to decrease observed by Gencoglu et al. (2010) or decreased for the cows fed LS diets reported by Agle et al. (2010). Which was attributed to lower starch intake, decreasing ruminal microbial protein production. However, contrary to these results (Carmo et al. 2014: Ferraretto et al. 2011), who observed that milk protein contents increased as increase in starch levels. It might be associated with increase in microbial protein synthesis in rumen due to greater intake of starch (Leiva,2000). Milk protein content and yield did not differ for PS of corn. Our findings are in accordance with (Ferraretto et al. 2013).

In the current study milk lactose yield was increased by 4.50 %, respectively in response to ST whereas, lactose contents remained unaffected. Our results are in accordance with (Fredin et al. 2015: Carmo et al. 2014) where lactose yield was increased quadratically in cows feeding different starch levels and 5% increase in lactose yield at 25 % starch level, respectively. Milk lactose yield increase with higher starch level is associated with higher transport of propionate to the liver for glucose and milk lactose synthesis (Piccioli et al. 2014). Other possible reason is increase in starch contents enhanced glucose availability might enhance the capacity of absorption of nutrients and stimulate the synthesis of lactose Arndt et al. (2014) However, lactose concentration was decreased with supplemental starch (Dyck et al. 2011).

Similar to our findings (Fredin et al. 2015: Hanlon et al. 2020) observed a trend for higher milk fat content in cows fed low starch diet, which was attributed to higher NDF concentration. Milk fat yield and content remained unaffected in response to interaction of ST and PS among the treatment diets. Our results are in accordance with previous findings of (Silvestre et al. 2022). One potential factor is the animal ̒s stage of lactation, which naturally exhibit a tendency to produce more milk in the early lactation. Energy is more partitioned to milk yields rather than milk fat. In present study, we have early lactating buffaloes with higher tendency of milk yield.

Our results are in agreement with Ferraretto et al. (2013) who observed that milk fat percentage was not affected by PS. However, Firkins et al. (2001) reported that milk fat content was greater for those cows fed CG corn. Lactose content was not affected by the dietary particle size among the treatments.

In the current study, no effect of BCS and pH were observed in response to the treatments. Our results are in agreement with (Gencoglu et al. 2010). They were feeding different levels of dietary starch, BCS was unaffected. In the present study BCS was unaffected because energy partitioned a greater proportion toward milk production as we found increased tendency for milk yield. Similarly, we observed no interaction between starch and particle size for BCS. Our outcomes were similar to those of Fredin et al. (2015). In agreement with our results, BW increased with high starch diet due to the energy partitioned a greater proportion toward body tissue gain (Piccioli et al. 2014).

In the current study predicted enteric methane production $CH_4$ was decreased in response to an increase in starch level. These results agree with the (Pirondini et al. 2015: Benchaar et al. 2014) reported that $CH_4$ production lower with high starch diet. It may be due to rapid fermentation and increase in the production of propionate as a portion of VFAs absorbed from the rumen. High starch diets change the microbe's ecology by preferring propionic-acid producing bacteria over methanogens (Bannink et al.2006). In contrast to our findings Aguerre et al. (2011) observed that $CH_4$ yield unaffected with the starch level. In the present study methane production was unaffected by PS. However, Wang et al. (2022) estimated decrease in $CH_4$ production with PS. No interaction was observed between ST x PS.

## CONCLUSION

In our study, production performance was increased for early lactating buffaloes fed HS diet compared to LS diet. Lactose and milk protein yield were increased due to increase in starch level. Milk production have a tendency to increase by the interaction of starch and particle size. Methane production was decreased with increasing starch levels. At metabolic level glucose increased with decreasing particle size. Gross, metabolic, milk nitrogen efficiencies and enteric methane production was decreased by feeding high - starch diet. Body weight increased with increase in starch level. Interaction was not observed between starch and particle size. Starch level at 25 % will be used in total mixed ration. These findings will provide additional support for dairy farmers in identifying economically viable dietary strategies during period of elevated corn grains prices. It will reduce enteric methane production which further improves the environment, buffalo productivity and national economy.

## IMPLICATIONS

The implications of study are twofold, firstly it underscores the importance of considering synergistic effect between starch levels and corn particle size in dairy animals' nutrition. Secondly, the study suggests a more efficient and cost-effective approach to lactating buffaloes' nutrition management. Overall, the findings of the current study emphasize on the importance of precision in dietary formulation. Further research in this area could lead to refined dietary strategies maximizing production of lactating buffaloes.

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**Table 1: Diet ingredient and chemical composition of Total mixed ration-based diets**

| | **Dietary Treatments[1]** | | | |
|---|---|---|---|---|
| **Item** | **LSCG** | **LSFG** | **HSCG** | **HSFG** |
| Ingredient (% DM) | | | | |
| Corn silage | 6.87 | 6.87 | 6.78 | 6.78 |
| Wheat straw | 42.1 | 42.1 | 41.6 | 41.6 |
| Coarse ground corn | 10.0 | ---- | 24.0 | ---- |
| Fine ground corn | ---- | 10.0 | ---- | 24.0 |
| Sugarcane molasses | 3.55 | 3.55 | 3.60 | 3.60 |

| | | | | |
|---|---|---|---|---|
| Wheat bran | 10.1 | 10.1 | 7.72 | 7.72 |
| Canola meal | 5.67 | 5.67 | 5.75 | 5.75 |
| Corn gluten 60% | 3.71 | 3.71 | 4.30 | 4.30 |
| Soybean hulls | 15.6 | 15.6 | 3.85 | 3.85 |
| Mineral mixture[2] | 0.68 | 0.68 | 0.69 | 0.69 |
| Dicalcium phosphate | 0.57 | 0.57 | 0.58 | 0.58 |
| Salt (NaCl) | 0.37 | 0.37 | 0.38 | 0.38 |
| Sodium bicarbonate | 0.37 | 0.37 | 0.38 | 0.38 |
| Urea 46% | 0.28 | 0.28 | 0.40 | 0.40 |
| Nutrient composition, % of DM | | | | |
| DM | 79.5 | 79.5 | 79.5 | 79.5 |
| Forage | 49.0 | 49.0 | 48.4 | 48.4 |
| CP | 11.0 | 11.0 | 11.2 | 11.2 |
| Ash | 7.51 | 7.51 | 6.80 | 6.80 |
| NDF | 54.3 | 54.3 | 46.3 | 46.3 |
| ADF | 33.5 | 33.5 | 27.9 | 27.9 |
| NFC | 26.7 | 26.7 | 34.7 | 34.7 |
| EE | 2.59 | 2.59 | 2.77 | 2.77 |
| Predicted Nutritive Value | | | | |
| RUP, % CP | 36.6 | 36.6 | 37.6 | 37.6 |
| RDP, % CP | 63.4 | 63.4 | 62.5 | 62.5 |
| ME, Mcal / kg | 2.05 | 2.05 | 2.20 | 2.20 |
| $NE_L$, Mcal/ kg | 1.32 | 1.32 | 1.42 | 1.42 |
| Sugar % of DM | 4.54 | 4.54 | 4.60 | 4.60 |
| Starch % of DM | 15.2 | 15.2 | 25.0 | 25.0 |

[1] 1) LSCG= Low starch coarse grains, 2) LSFG = Low starch fine grains, 3) HSCG = High starch coarse grains, 4) HSFG = High starch fine grains. low and high starch levels are 15 & 25% while, coarse and fine grains are 3.0 & 1.5 mm, respectively in diets.

[2] contained 70kg DCP,23 kg salt, 5kg $MgSO_4$, 0.7 kg $FeSO_4$, 0.5 kg $ZeSO_4$, 0.5kg $MnSO_4$, 0.13kg $CuSO_4$, 0.1 kg CoCl,0.05kg KI.

**Table 2: Dry matter intake, Milk production and contents of lactating buffalo's feds with different diet levels of starch and particle size**

| | Dietary Treatments[1] | | | | SEM | P value[2] | | |
|---|---|---|---|---|---|---|---|---|
| **Items** | **LSCG** | **LSFG** | **HSCG** | **HSFG** | | **ST** | **PS** | **ST ×PS** |
| Yield, | | | | | | | | |
| Milk, kg/d | 9.20$^{ab}$ | 9.12$^{b}$ | 9.23$^{ab}$ | 9.44$^{a}$ | 0.392 | 0.03 | 0.43 | 0.07 |
| Fat, g/d | 602 | 599 | 594 | 603 | 20.0 | 0.84 | 0.73 | 0.51 |
| Protein, g/d | 351$^{ab}$ | 349$^{b}$ | 354$^{ab}$ | 363$^{a}$ | 15.9 | 0.02 | 0.39 | 0.11 |
| Lactose, g/d | 414 | 408 | 423 | 436 | 31.2 | 0.01 | 0.68 | 0.23 |
| Composition % | | | | | | | | |
| Fat | 6.62 | 6.63 | 6.55 | 6.45 | 0.209 | 0.09 | 0.57 | 0.41 |
| Protein | 3.81 | 3.82 | 3.83 | 3.84 | 0.029 | 0.23 | 0.57 | 0.80 |
| Lactose | 4.44 | 4.41 | 4.48 | 4.57 | 0.195 | 0.16 | 0.67 | 0.45 |
| ECM[3], kg/d | 13.4 | 13.4 | 13.4 | 13.6 | 0.47 | 0.46 | 0.55 | 0.24 |
| 4% FCM[4], kg/d | 12.7 | 12.6 | 12.6 | 12.8 | 0.41 | 0.74 | 0.63 | 0.33 |
| 3.4% PCM[5], kg/d | 9.64 | 9.88 | 10.1 | 10.0 | 0.82 | 0.24 | 0.77 | 0.50 |
| MkE[6] (Mcal/d) | 9.15 | 9.21 | 9.20 | 9.06 | 0.331 | 0.70 | 0.73 | 0.42 |

[1]1) LSCG= Low starch coarse grains, 2) LSFG = Low starch fine grains, 3) HSCG=High starch coarse grains,4) HSFG= High starch fine grains. Low and high starch levels are 15 & 25% while, Coarse and fine grains are 3.0mm & 1.5% respectively in diets.

[2]Treatment effects Probability: ST= effect of starch, PS= effect of Particle size, ST×PS= Interaction effect of starch and particle size

[3]ECM = Energy corrected milk

[4]4% FCM= 4% Fat corrected milk

[5]PCM = 3.4% Protein corrected milk

[6]MkE= milk energy

**Table 3: Feed and Production efficiencies of lactating buffaloes fed diets with different diet levels of starch and particle size.**

| | Dietary Treatments[1] | | | | | P value[2] | | |
|---|---|---|---|---|---|---|---|---|
| **Items** | **LSCG** | **LSFG** | **HSCG** | **HSFG** | **SEM** | **ST** | **PS** | **ST × PS** |
| Feed efficiency | 0.72 | 0.72 | 0.72 | 0.71 | 0.028 | 0.69 | 0.19 | 0.41 |
| 4% FCM: DMI | 0.98 | 0.97 | 0.97 | 0.99 | 0.032 | 0.74 | 0.63 | 0.33 |
| 3.4% PCM: DMI | 0.74 | 0.76 | 0.78 | 0.77 | 0.065 | 0.24 | 0.77 | 0.50 |
| Gross efficiency MP | 0.31 | 0.31 | 0.30 | 0.29 | 0.012 | 0.01 | 0.79 | 0.44 |
| Metabolic efficiency MP | 0.46 | 0.47 | 0.43 | 0.43 | 0.019 | 0.01 | 0.69 | 0.67 |
| Milk nitrogen efficiency (%) | 25.8 | 26.4 | 24.5 | 23.9 | 1.10 | 0.01 | 0.92 | 0.22 |
| MkN: MkE[3] (g/Mcal) | 5.89 | 6.01 | 6.08 | 6.09 | 0.132 | 0.21 | 0.54 | 0.62 |
| MkN[4] (g/d) | 54.4 | 55.5 | 55.7 | 55.1 | 2.38 | 0.66 | 0.80 | 0.44 |

[1]1) LSCG= Low starch coarse grains, 2) LSFG = Low starch fine grains, 3) HSCG = High starch coarse grains, 4) HSFG = High starch fine grains. low and high starch levels are 15 & 25% while, coarse and fine grains are 3.0mm & 1.5% respectively in diets

[2]Treatment effects Probability: ST= effect of starch, PS= effect of Particle size, ST×PS= Interaction effect of starch and particle size

[3]MkN: MkE (g/Mcal) = Milk nitrogen (g/ d) /Milk energy (Mcal/ d)

[4]MkN (g/d) = Milk nitrogen (g/ d)

**Table 4: Body condition score, pH and body weight of lactating buffaloes feds with different diet levels of starch and particle size**

| | Dietary Treatments[1] | | | | | P value[2] | | |
|---|---|---|---|---|---|---|---|---|
| **Items** | **LSCG** | **LSFG** | **HSCG** | **HSFG** | **SEM** | **ST** | **PS** | **ST × PS** |
| BCS[3] | 2.93 | 2.92 | 2.99 | 2.95 | 0.062 | 0.26 | 0.51 | 0.71 |
| BW[4], kg | 483 | 477 | 486 | 501 | 10.4 | 0.01 | 0.17 | 0.01 |
| pH[5] | 6.75 | 6.78 | 6.72 | 6.74 | 0.027 | 0.36 | 0.17 | 0.70 |

[1]1) LSCG= Low starch coarse grains, 2) LSFG = Low starch fine grains, 3) HSCG = High starch coarse grains, 4) HSCG = High starch fine grains. Low and high starch levels are 15 & 25% while, Coarse and fine grains are 3.0mm & 1.5% respectively in diets.

[2]Treatment effects Probability: ST= effect of starch, PS= effect of Particle size, ST×PS= Interaction effect of starch and particle size

[3]BCS = body condition score

[4]BW = body Weight

[5]pH = negative logarithm of hydrogen ion

**Table 5: Blood metabolites of lactating buffalo's feds with different diet levels of starch and particle size**

| | Dietary Treatments[1] | | | | | P value[2] | | |
|---|---|---|---|---|---|---|---|---|
| **Items** | **LSCG** | **LSFG** | **HSCG** | **HSFG** | **SEM** | **ST** | **PS** | **ST × PS** |
| [3]Glu, mg/dl | 61.0 | 66.0 | 59.7 | 67.3 | 2.46 | 1.00 | 0.01 | 0.60 |
| [4]Chol, mg/dl | 95.8 | 99.3 | 95.1 | 99.7 | 5.81 | 0.97 | 0.34 | 0.91 |
| [5]PUN, mg/dl | 16.8 | 23.1 | 16.2 | 18.4 | 3.20 | 0.43 | 0.21 | 0.56 |
| [6]TG, mg/dl | 134 | 121 | 112 | 137 | 7.59 | 0.75 | 0.48 | 0.04 |

[1]1) LSCG= Low starch coarse grains, 2) LSFG = Low starch fine grains, 3) HSCG = High starch coarse grains, 4) HSFG = High starch fine grains. low and high starch levels are 15 & 25% while, coarse and fine grains are 3.0mm & 1.5%, respectively in diets.

[2]Treatment effects Probability: ST= effect of starch, PS= effect of Particle size, ST×PS= Interaction effect of starch and particle size

[3]Glu = glucose, [4]Chol = Cholesterol, [5]PUN= Plasma Urea Nitrogen, [6]TG= Triglycerides

**Table 6: Methane production of lactating buffalo's feds with different diet levels of Starch and particle size**

| Items | Dietary Treatments[1] | | | | SEM | P value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | LSCG | LSFG | HSCG | HSFG | | ST | PS | ST× PS |
| [3]$CH_4$ MJ | 13.2 | 13.2 | 12.5 | 12.5 | 0.01 | <0.01 | 0.55 | 0.81 |
| [4]$CH_4$ Mcal | 3.16 | 3.16 | 3.00 | 3.00 | 0.001 | <0.01 | 0.55 | 0.81 |
| [5]$CH_4$ g/d | 222 | 222 | 210 | 210 | 0.02 | <0.01 | 0.55 | 0.81 |
| [6]$CH_4$ /DMI (kg) | 17.1 | 17.1 | 16.2 | 16.2 | 0.01 | <0.01 | 055 | 0.81 |
| [7]$CH_4$ / MY (kg) | 24.3 | 24.5 | 23.2 | 23.6 | 0.94 | 0.01 | 0.28 | 0.79 |

[1]1) LSCG= Low starch coarse grains, 2) LSFG = Low starch fine grains, 3) HSCG = High starch coarse grains, 4) HSFG = High starch fine grains. low and high starch levels are 15 & 25% while, coarse and fine grains are 3.0mm & 1.5% respectively in diets.

[2]Treatment effects Probability: ST= effect of starch, PS= effect of Particle size, ST×PS= Interaction effect of starch and particle size

[3]$CH_4$ MJ = Methane production Mega joule

[4]$CH_4$ Mcal= Methane production Mega Calories

[5]$CH_4$ g/d= Methane production gram/day

[6]$CH_4$ /DMI (kg)= Methane production in gram /kg of Dry matter intake

[7]$CH_4$ / MY (kg)= Methane production in gram /kg of Milk yield