HSJ

Health Sciences Journal
EISSN: 2959-2259; PISSN:2959-2240

Vol.3, Issue. 2

Received: 30 NOVEMBER 2024 Accepted: 30 JANUARY 2025 Published: 12 FEBRUARY 2025

MDRI

Research Article

Effects of Urea-Molasses Treated Wheat Straw Fermented with Rumen Digesta on the Production Performance of Lactating Nili-Ravi Buffaloes

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Citation
Siddiq K, Najeeb S, Nisa MU, Maris H, Rustam SA, Ullah S, Ahmad Z, Ain NU, Saleem Z, Javaid A. Effects of urea-molasses treated wheat straw fermented with rumen digesta on the production performance of lactating nili-ravi buffaloes. Health Sciences Journal, 2025;3(2): 79-88

ABSTRACT:

Background and Objectives: A research investigation was conducted involving a cohort of nine multiparous early lactating Nili-Ravi buffaloes, employing a randomized complete block design to ensure a systematic approach. The primary objective of the study was to evaluate the influence of different levels of urea-molasses treated wheat straw, which was ensiled with bovine rumen digesta, on various parameters. These parameters included the intake of nutrients, the digestibility of those nutrients, nitrogen balance, overall milk production, and the compositional elements of the milk produced by the buffaloes. The findings aimed to provide insights into how dietary interventions might improve the health and productivity of these animals during the early stages of lactation. **Methodology:** Wheat straw was mixed with rumen digesta at an 80:20 dry matter DM ratio. A solution of 4% urea and 4% molasses was applied to this mixture, which was then sealed and allowed to ferment for 21 days. Three iso-caloric and iso-nitrogenous diets were created with varying fermented wheat straw FWS levels: 0%, 50%, and 70% designated as FWS-0, FWS-50, and FWS-70, respectively. The FWS-0 and FWS-50 diets maintained a forage-to-concentrate ratio of 50:50, while FWS-70 had this ratio changed to 70:30 by substituting 20% of the concentrate with FWS. Each treatment was assigned to three buffaloes, and the experiment lasted for 120 days. **Results:** Buffaloes on the FWS-50 diet had significantly greater $P<0.05$ DM and crude protein CP intake compared to those on the other diets. In the course of this study, it was observed that individuals adhering to the FWS-70 diet demonstrated a higher intake of both neutral detergent fiber (NDF) and acid detergent fiber (ADF) when compared to participants on other dietary regimens. The differences in fiber consumption among the groups were statistically significant, with a p-value of less than 0.05. This suggests that the FWS-70 diet is associated with increased fiber intake, highlighting its unique impact on dietary fiber consumption compared to the other diets evaluated. The highest DM digestibility was noted in the FWS-50 group, with similar findings observed for CP, NDF, and ADF digestibility. Nitrogen balance was also greater $P<0.05$ for the FWS-50 and FWS-70 groups compared to FWS-0. Milk yield, 4% fat-corrected milk, lactose percentage, solids-not-fat, total solids, and blood urea nitrogen levels showed no significant differences across the diets. The FWS-50 and FWS-70 groups showed significantly higher percentages of milk fat and milk protein ($P<0.05$) when compared to the FWS-0 group. Milk yield and 4% fat-corrected milk were comparable for buffaloes given FWS-0 and FWS-70 diets. **Conclusion:** The results indicate that fermenting wheat straw treated with urea and molasses alongside rumen digesta improves nutrient absorption, digestibility, and nitrogen retention, while also boosting the percentages of milk fat and protein. Furthermore, it indicates that buffaloes can achieve similar milk production by substituting up to 20% of concentrate with FWS.

KEYWORDS:

Buffaloes, Rumen Digesta, Fermented Wheat Straw, Feed Intake, Digestibility

1 | INTRODUCTION

Ruminants get their energy needs by breaking lingo-cellulosic bond and have the unique ability to convert low quality dietary fiber into high quality and valuable animal products in the form of milk and meat.[1] Nutritionists are trying to be fulfilled nutritional needs of ruminants from seasonal fodders and crop residues which are abundantly available in the country.[2] Wheat straw WS is one of the major crop residues. The WS has less fermentable carbohydrates as well as protein contents which results to decrease its digestibility and ultimately decreases the productivity of ruminants.[3] Non-conventional feed resources need to be explored and nutritional value of crop residues should be improved for economical production of livestock and to fulfill the existing gaps of nutrients.[2,4] Nitrogen N from animal waste by-products can also play a key role in fulfilling the gap between demand and supply.[5] Nutritive value of crop residues has been improved by different methods like physical, chemical and biological treatments. [5-7] Urea treatment [8] and ensilation of urea treated straws with fermentable sugars like cane molasses [6] are the most common among the chemical treatments. Urea treatment of WS causes the hydrolysis of urea into ammonia and carbon dioxide.[9] About 75 % of ammonia has been reported to escape causing environmental pollution in addition to loss of N.[10] Ammonia losses in urea treated WS reduce the efficiency of urea treatment.[11] To solve the problem of loss of N content of ammonia and increase N fixation can be overcome through ensiling WS with fermentable carbohydrates like cane molasses.[12-13] non-conventional source of nutrients like bovine rumen digesta RD, poultry litter and cattle manure have been used in ruminant feeds. The RD contains quality nutrients and rumen microbes.[14] These contents are disposed off as a waste thus causing the environment pollution[15], water streams pollution and soil acidification resulting health hazards for human population due to proliferation of harm full bacteria.[16] These hazards can be overcome by reusing it in ruminant feeds, because it contains high amount of nutrients and microbial population. This can be used as biological substances for inoculating WS treated with urea-molasses during ensiling. [14, 17] Use of RD with urea molasses treated WS, may decrease the feed price resulting to decrease the cost of milk production. The use of RD in ruminant diet will not only meet the nutritional requirements but its reuse also decreases the pollution threats.[18] Ruminant diets can incorporate roughage diets RD in various forms, such as dry, ensiled with whole crop silage WS, or mixed with other feed ingredients. One effective method to enhance the nutritional quality of WS is through fermentation with urea and molasses, combined with bovine RD. Utilizing bacterial inoculants during the ensiling process of urea-molasses treated WS can lead to increased lactic acid production and reduced dry matter DM losses.[19] RD serves as a viable alternative to conventional microbial inoculants, consisting of partially digested feed that is rich in nutrients and includes rumen microorganisms. These microorganisms, particularly various Lactobacillus species, are known for producing lactic acid, which lowers the pH of silage.[20] Some studies indicate that ensiling RD with urea-molasses treated WS can enhance its nutritional value and improve the digestibility of WS.[17] However, there is limited information regarding the use of RD as an inoculum in urea-molasses treated WS for boosting its nutritional profile. The objective of this research was to evaluate how varying levels of urea-molasses treated wheat straw (WS) that has been fermented with rice distillers (RD) can influence several key aspects of nutrition in early lactating Nili-Ravi buffaloes. This study specifically focused on examining factors such as nutrient intake, the digestibility of feed components, nitrogen balance in the animals, milk production, and the composition of the milk produced. Additionally, this investigation included an analysis of how a partial replacement of concentrate feed affects these parameters. Through this comprehensive assessment, the study aims to provide insights into the benefits of utilizing treated wheat straw in the diets of lactating buffaloes.

2 | MATERIAL AND METHODS

2.1 | Preparation of Fermented Wheat Straw Utilizing Bovine Rumen Digesta

Wheat straw (WS) was obtained from agricultural fields at the University of Agriculture, Faisalabad. Meanwhile, bovine rumen digesta (RD) was sourced from a nearby slaughterhouse. The WS and RD were then mixed together in a designated facility, adhering to a specific dry matter ratio of 80:20. This blending process took place in designated pits designed for this purpose. To enhance fermentation, the mixture was treated with 4% urea and 4% molasses, which were first dissolved in 40 liters of water to achieve a 50% moisture content in the WS and RD mixture. This solution was evenly distributed over the blend, which was then compacted, sealed with plastic sheeting to create anaerobic conditions, and allowed to ferment for 21 days to produce fermented wheat straw FWS.

2.2 | Animal and Ration Management

Nine multiparous buffaloes in early lactation approximately 20±5 days postpartum were assigned to three blocks, each consisting of three animals, using a randomized complete block design. The diets categorized as FWS-0, FWS-50, and FWS-70 contained 0%, 50%, and 70% FWS on a dry matter basis, respectively. The buffaloes were provided with feed ad libitum. The experiment was conducted over 120 days, with the initial 30 days serving as an adaptation phase, followed by a 90-day collection phase.

2.3 | Sample and Data Collection

Daily measurements of feed offered and orts were taken to assess dry matter intake. Feces and urine were collected according to [21] for determining nutrient digestibility and nitrogen balance. Each buffalo's feces were collected daily in plastic drums, weighed, and composited. A 20% sample of the mixed feces was then taken for drying at 55°C. At the conclusion of the total collection period, the dried feces were mixed, and 10% of each animal's fecal sample was reserved for further analysis. Urine was collected using small metal funnels fitted with plastic tubing designed to surround the vulva and channel urine into a large container. Before the collection period, fresh urine was titrated against 50% H2SO4 to establish the amount of acid required to maintain the urine pH at approximately 3.0, thereby minimizing ammonia nitrogen loss. Urine produced over 24 hours was collected, and 50% H2SO4 was added according to titration results, with 20% of the urine collected set aside for analysis at −20°C. At the end of the collection period, urine samples were thawed, mixed, and 10% of this composite was used for analysis. Blood samples were taken from each animal's jugular vein two hours post-feeding to collect serum, which was then stored in aliquots at -20°C for subsequent analysis. Daily records of dry matter intake and milk production were maintained, with buffaloes being milked twice per day. Biweekly milk samples were gathered during two consecutive milking sessions at 5:00 AM and 5:00 PM, with a 12-hour interval between them, and were preserved at 6°C using 2-bromo-2-nitro-propane-1-3-diol until they were analyzed. A comprehensive examination of milk samples was conducted in order to evaluate their composition. This extensive analysis aimed to determine the concentration of key components present in these samples, including fat content, protein content, lactose levels, solids-not-fat percentages, and overall total solids content. To gain further insights into the nutritional aspects of the milk samples, the composition of the feed provided to the animals was also investigated, as well as the composition of the leftovers collected from the daily meals. By analyzing the combined samples from these sources, researchers were able to evaluate various critical parameters, such as dry matter content, crude protein percentages, and the relative concentrations of both neutral detergent fiber (NDF) and acid detergent fiber (ADF).[22]

2.4 | Chemical Analysis

The WS, FWS, and experimental diets were subjected to a comprehensive analysis to assess their composition, specifically focusing on dry matter, crude protein, ash, neutral detergent fiber (NDF), and acid detergent fiber (ADF).[23] To determine the dry matter content, samples were dried in an oven at a temperature of 105°C for a duration of 24 hours, after which they were allowed to stabilize in desiccators to reach equilibrium. For crude protein content, the calculation involved multiplying the percentage of nitrogen present in the samples by a factor of 6.25. The assessments of NDF and ADF were carried out following established procedures; for NDF determination, sodium sulfate was utilized, and alpha amylase was deliberately excluded from the analysis. It is important to note that both NDF and ADF values were reported without any residual ash, ensuring the accuracy of the fiber content measurements.

2.5 | Blood Sample Analysis

To analyze the blood samples, plasma was first separated by centrifugation at 1,500×g for 40 minutes. The resulting plasma was stored at -4°C until further analysis for BUN. For the determination of urea nitrogen levels, 0.25 mL of thawed plasma was initially brought to room temperature. It was then incubated with a urease solution (specific activity: 4.03 U/mL) for 10 minutes to convert urea into ammonia.[24] The urease conversion reaction was subsequently stopped by adding 0.15 mL of 65% TCA to the mixture. The solution was further incubated on ice for 30 minutes to allow complete precipitation of the reaction products. Following a centrifugation process conducted at a force of 21,000×g for a duration of 10 minutes, the supernatant was analyzed for urea concentration. This analysis was performed utilizing a method that had previously undergone rigorous validation, as outlined by Broderick and Kang. The method relies, ensuring that the quantification of urea is both accurate and reliable.[25]

2.6 | Milk Sample Analysis

A comprehensive analysis of milk samples was conducted to determine their nutritional composition. The study utilized the advanced Milko-Scan technology from Foss Electric based in Hillerod, Denmark, to examine the levels of various components present in the milk. Specifically, the fat content, lactose, proteins, solids not fat, and total solids were assessed to gain a deeper understanding of the milk's overall composition and nutritional value. To calculate the fat-corrected milk (FCM) at 4% fat, a formula was employed, taking into account the following factors: the weight of the milk in kilograms per day, the percentage of milk fat, and a predetermined set of constants to provide an accurate calculation. The formula used for this calculation is as follows: Fat-corrected milk (4% FCM) is computed by multiplying the daily milk production in kilograms by a constant of 44.01 and then by the milk fat percentage. This product is then added to a constant value of 163.56 and divided by a divisor of 339.60 to obtain the fat-corrected milk value.

2.7 | Statistical Analysis

The data that were gathered underwent analysis through the application of analysis of variance (ANOVA), specifically employing a randomized complete block design. This statistical methodology was executed utilizing the capabilities of the general linear model available in SPSS version 25.0. To assess the differences between the means of the groups studied, the least significant difference (LSD) test was employed as a means of post-hoc analysis. This comprehensive approach ensured a thorough examination of the data and allowed for precise comparisons among the different treatment means.

3 | RESULTS

The dry matter DM content of fermented wheat straw FWS decreased after 21 days of ensiling. Notably, the crude protein CP level of urea-molasses treated wheat straw that was ensiled with rumen digesta RD rose from 3.25% to 12.5%, in contrast to the non-fermented wheat straw. Furthermore, the true protein concentration in FWS increased from 0.23% to 7.65% when compared to the original wheat straw. Additionally, the levels of neutral detergent fiber NDF and acid detergent fiber ADF were lower in the wheat straw that was ensiled with RD.

Table 1 Ingredients and chemical composition of experimental diets

Ingredients	FWS-0	FWS-50	FWS-70
Fermented wheat straw	0	50	70
Non-fermented wheat straw	50	0	0
Maize grains	8	16	10
Wheat bran	5	8	4
Vegetable oil	1	1	1
Cotton seed meal	8	4	3
Maize gluten meal (30%)	10	5	4
Rice polish	9	7	3
Cane molasses	5	6	2
Mineral mixture	2	2	2
Urea	2	1	1
Total	100	100	100

The experimental diets were labeled FWS-0, FWS-50, and FWS-70, consisting of non-fermented wheat straw and concentrate in various ratios: FWS-0 had a 50:50 blend of wheat straw and concentrate, FWS-50 featured a 30:70 ratio, and FWS-70 included a composition of 70% concentrate to 30% wheat straw, all on a dry matter basis.

Table 2 Nutritive value of wheat straw, fermented wheat straw, molasses and rumen digesta

Nutrients %	WS	FWS	Molasses	RD
Dry matter	92.35	49.55	76.9	14.5
Crude protein	3.25	12.5	3.15	12.11
True protein	-	7.65	-	-
Neutral detergent fiber	84.9	56.0	-	57.5
Acid detergent fiber	51.5	30.2	-	36.5
Ash	9.5	10.0	-	10.7

WS: wheat straw, FWS: fermented wheat straw, RD: rumen digesta

3.1 | Nutrient's Intake

When comparing the nutrient intake of buffaloes fed wheat straw with those not fed it, a significant difference was observed ($P<0.05$). The data showed that adding wheat straw to their diet increased the overall nutrient intake of the buffaloes. Among the buffaloes fed diets containing wheat straw, those receiving the highest concentration of wheat straw (FWS-70) had the lowest dry matter (DM) and crude protein (CP) intake, while those receiving the intermediate concentration (FWS-

50) had the highest intake of these nutrients. Notably, the highest intake of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was observed in the buffaloes fed the highest concentration of FWS (FWS-70), followed by those fed the intermediate concentration (FWS-50). However, an interesting contradiction was observed when analyzing the data. The intake of DM and CP decreased as the level of wheat straw in the diet increased from 50 to 70%, while the intake of NDF and ADF increased during the same period, following an opposite trend.

Table 3 Impact of varying levels of FWS on nutrients intake and digestibility in early lactating buffaloes

	Diets[1]			
Intake, Kg/day	**FWS-0**	**FWS-50**	**FWS-70**	**SE[2]**
Dry matter	10.61[c]	13.63[a]	12.34[b]	0.119
Crude protein	1.51[c]	2.15[a]	1.96[b]	0.151
Neutral detergent fiber	4.55[c]	5.02[b]	5.37[a]	0.504
Acid detergent fiber	2.27[c]	2.47[b]	2.79[a]	0.339
Digestibility %				
Dry matter	58.30[c]	69.33[a]	62.16[b]	0.339
Crude protein	73.36[c]	81.63[a]	74.86[b]	0.151
Neutral detergent fiber	50.46[c]	64.30[a]	56.56[b]	0.504
Acid detergent fiber	50.20[c]	62.83[a]	53.93[b]	0.339

Diets FWS-0, FWS-50, and FWS-70 consisted of 0%, 50%, and 70% dry matter (DM) from FWS, respectively, substituting varying portions of concentrate

Buffaloes that were fed diets incorporating fruit and vegetable waste (FWS) demonstrated improved nutrient digestibility compared to those that received a diet without FWS. The buffaloes exhibited the most favorable digestibility rates for dry matter, crude protein, neutral detergent fiber, and acid detergent fiber when they were fed a diet that consisted of 50% Fermented Whole Silage (FWS). This specific dietary composition resulted in the highest levels of nutrient absorption and utilization, optimizing the overall health and performance of the animals. The findings suggest that incorporating a significant proportion of FWS into the buffaloes' diet enhances the digestibility of essential nutrients, which is crucial for their growth and well-being., followed by those on diets with 70% FWS. Conversely, the lowest nutrient digestibility was recorded in buffaloes that did not consume any FWS. Notably, as the percentage of FWS in the diet increased from 50% to 70%, the digestibility of these nutrients showed a decline.

Table 4 Impact of varying levels of FWS on N balance in early lactating buffaloes

	Diets[1]			
Items Kg/day	**FWS-0**	**FWS-50**	**FWS-70**	**SE[2]**
N intake	0.242[c]	0.344[a]	0.315[b]	0.004
Fecal N	0.069[b]	0.062[c]	0.080[a]	0.001
Milk N	0.038	0.044	0.035	0.004
Urinary N	0.107[b]	0.157[a]	0.112[b]	0.004
Total N excretion	0.215[b]	0.264[a]	0.228[b]	0.005
N balance	0.026[b]	0.079[a]	0.087[a]	0.003

Buffaloes consuming the FWS-50 diet had the highest nitrogen N intake $P<0.05$, while those on the FWS-0 diet exhibited the lowest intake refer to Table 4. The fecal nitrogen levels were highest $P<0.05$ in buffaloes on the FWS-70 diet and lowest in the FWS-50 group. Milk nitrogen content remained stable across all dietary variations. In a study examining the effect of dietary composition on urinary nitrogen levels in buffaloes, it was observed that animals fed with the FWS-50 diet exhibited substantially elevated levels of urinary nitrogen compared to those on the FWS-70 and FWS-0 diets, a statistically significant difference confirmed at $P<0.05$. This disparity extended to total nitrogen excretion, where the trend was similarly consistent. Moreover, an analysis of nitrogen balance revealed that buffaloes fed the FWS-50 and FWS-70 diets demonstrated a significantly enhanced nitrogen balance compared to the FWS-0 diet group. This notable distinction in nitrogen metabolism underscores the potential importance of dietary formulation and composition on nitrogen excretion and balance in these animals.

Table 5: Impact of varying levels of FWS on milk production and its composition in early Lactating buffaloes

	Diets[1]			
Items	FWS-0	FWS-50	FWS-70	SE[2]
Milk production, kg/day	6.16	8.21	6.24	0.778
4% FCM Production, kg/day	9.68	11.09	9.87	0.869
Milk fat %	6.58^b	6.99^a	7.10^a	0.006
Milk protein %	4.09^b	4.25^a	4.18^a	0.007
Milk lactose %	6.1	6.17	6.3	0.028
Solid not fat %	11.52	11.77	11.2	0.04
Total solids %	18.1	18.76	18.3	0.33
Blood urea nitrogen, mg/dL	30.33	31.06	33.83	2.38

The milk production and 4% fat-corrected milk levels were comparable across all dietary groups. However, a tendency for increased milk yield was noted in buffaloes consuming diets that included FWS compared to those on the control diet without FWS. The analysis revealed that the percentages of both milk fat and protein were markedly elevated ($P<0.05$) in buffaloes that were fed the FWS-50 and FWS-70 diets, especially when compared to those on the FWS-0 diet, as indicated in Table 5. However, when examining other components of milk, such as lactose, solids not fat, and total solids, there were no statistically significant differences observed across the different dietary treatments. This suggests that while certain diets may enhance the fat and protein content in buffalo milk, they do not appear to affect the lactose levels or the overall solids content. Regarding blood urea nitrogen BUN levels, concentrations did not vary significantly between the diet groups. The highest BUN levels were recorded in buffaloes on the FWS-70 diet, followed by those on the FWS-50 and FWS-0 diets.

4 | DISCUSSION

The observed reduction in dry matter DM of fermented wheat straw FWS in this study may be attributed to the action of anaerobic bacteria that break down DM for their growth during the 21-day ensiling period. These findings align with the observations made by Singh et al. [26], who noted that the DM content of roughages decreases with prolonged ensiling. The loss in DM could also be partially justified by the emission of carbon dioxide accompanying the conversion of lactic acid into acetic acid. [27] The increase in crude protein CP content of FWS post 21 days of fermentation could be due to the ample time and nutrient availability for microbial growth. In similar studies, Khan et al. [28] documented a rise in the content of crude protein (CP) in sugarcane bagasse increased from 18.4% to 22.2% following fermentation. cattle manure over a 60-day period, attributing this increase to enhanced ammonia nitrogen N availability stemming from urea breakdown during ensiling. The improved CP levels in FWS could also be boosted by the addition of molasses, which supplies easily fermentable carbohydrates that create an optimal environment for bacterial growth, leading to better retention of ammonia N. [29] Energy scarcity is a primary limiting factor for anaerobic microbial proliferation, but the incorporation of urea-molasses likely provides both ammonia and energy, resulting in increased microbial counts and subsequent improvements in both CP and true protein contents. [30] Previous research [31, 32] has corroborated the enhancement of CP levels in corn silage following ammonia treatment. The present results are consistent with the research conducted by Hassan et al. [33], which indicated a reduction in dry matter (DM), neutral detergent fiber (NDF), and acid detergent fiber (ADF). In contrast, their study found an increase in crude protein (CP) and true protein levels when wheat straw (WS) was treated with 4% urea, 6% molasses, and cattle manure during the ensiling process.

4.1 | Nutrient Intake

The significant levels of DM, CP, NDF, and ADF found in diets based on fish waste may be attributed to.the improved digestibility of NDF and ADF compared to diets containing untreated WS. The fermentation of WS with roughage diet RD likely leads to enhanced breakdown of lignocellulosic bonds, thereby improving fiber digestibility. [14] The reductions that have been observed in the consumption of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) for the FWS-70 and FWS-0 diets, when compared with the FWS-50 diet, could potentially be attributed to the elevated levels of NDF present in these diets. Such increased NDF content may negatively influence the digestibility of the feed, resulting in a lower overall intake of these important nutritional components[14]. Supporting evidence from Sarwar et al. [34] indicated higher nutrient intakes buffalo bulls of the Nili-Ravi breed were provided with a diet that included urea-molasses treated wheat straw. Other studies by Allam et al. [35] and El-Ashry et al. [36] have reinforced these findings when treating WS with urea 3%, molasses 3%, and bacterial inoculants. Increased DM intake and digestibility can be ascribed to the initial fermentation of WS, which stimulates rumen microbes and enhances fiber digestion, leading to elevated DM intake. Similar observations were made by Hanafy et al. [38] when lactating buffaloes were offered urea-treated corn silage. Ensiling WS with urea has been found to improve digestibility and, consequently, increase DM intake; this has also been corroborated by Nisa et al. [6] in their research involving urea-treated wheat straw, both with and without the inclusion of corn steep liquor, the study focused on lactating buffaloes. Further, Khan et al. [39] reported greater nutrient intakes in lactating buffaloes fed urea-treated WS, both with and without molasses. Likewise, Wanapat et al. [40] documented a significant increase in DM intake in cows consuming roughage-based diets with urea-treated WS. Supplementation and ensiling of low-quality fibrous feed with non-protein nitrogen sources have been shown to enhance diet digestibility [41] and improve feed intakes. [42]

4.2 | Nutrient Digestibility

The enhanced digestibility of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) observed in buffaloes that are fed fermented whole crop silage (FWS) could be attributed to the inclusion of various components such as urea, molasses, and microbial inoculants, which are typically found in the raw diets (RD). These ingredients likely play a crucial role in improving the overall nutrient breakdown and utilization in the animals' digestive systems, leading to better overall nutritional outcomes. This combination likely facilitates a better degradation of the bonds between hemicelluloses and lignin. [43] Studies have demonstrated that urea and molasses have similar impacts on the nutrient digestibility of animals consuming diets based on unfermented wheat straw (WS). The enhanced digestibility of neutral detergent fiber (NDF) and acid detergent fiber (ADF) observed in fermented wheat straw (FWS) compared to unfermented WS is likely linked to the incorporation of urea in the diet. This suggests that urea supplementation could play a significant role in improving the overall digestibility of fibrous materials in the context of animal feed. This supplementation results in elevated levels of ammonia nitrogen in the rumen and an increase in pH, both of which enhance cellulolytic activity. Greater digestibility is linked to heightened cellulolytic activity, which in turn accelerates the rates of fermentation in the rumen [44]. Khattak et al. [14] also observed that the presence of fermentable carbohydrates heightened the fragility of urea-treated WS, increasing the surface area available for lignocellulose degradation and the dietary manipulation of Nili-Ravi buffaloes through the use of urea-molasses treated wheat straw (WS) and rice distillers dried grains with solubles (RD) significantly impacted the digestion and utilization of nutrients. In particular, the diet consisting of wheat straw treated with urea and molasses (FWS-50) exhibited the highest dry matter digestibility. This superior digestibility can be attributed to the optimal balance between the forage and concentrate components of the diet, which facilitated efficient nutrient breakdown and absorption. Nisa et al. [6] comparable results were noted with wet silage that had been treated using a combination of 5% urea and 9% corn steep liquor. The findings indicate that this particular treatment yielded effects similar to those observed in other studies. By utilizing these additives, the silage's overall quality and fermentation process may have been positively influenced, thereby enhancing its nutritional value and digestibility. The lower digestibilities of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) in buffaloes fed the FWS-70 diet, compared to those on the FWS-50 diet, can be attributed to the increased forage-to-concentrate ratio, which led to a higher fiber content in the diet.. Abouheif et al. [45] observed that higher fiber contents were inversely related to NDF and ADF digestibility in 100% RD diets in comparison to those containing 25% and 50% RD. The increased CP digestibility in FWS diets may be associated with the molasses treatment, which provides fermentable carbohydrates and enhances nitrogen utilization in the rumen. Sarwar et al. [12] and Hanafy et al. [38] also reported similar findings regarding nutrient digestibility when calf diets included urea and molasses-treated WS.

4.3 | Nitrogen Balance

The higher fecal and urinary nitrogen losses in buffaloes fed FWS diets might be linked to their greater DM and CP intake than those consuming unfermented WSThe diets based on fermented wasted substrates (FWS) demonstrated improved nitrogen retention, likely attributed to increased dry matter (DM) and crude protein (CP) intake and digestibility. This aligns with studies involving growing calves that were given a diet comprising 4% urea, 4% molasses, and 30% cattle manure. Research by Hassan in 2004 indicated that buffalo bulls exhibited better nitrogen balance when fed urea-molasses treated wasted substrates that were fermented with cattle manure under controlled feeding conditions. Similar findings were reported by Nisa et al. regarding the nitrogen retention in Nili-Ravi buffalo bulls receiving this type of diet either urea-treated WS with or without corn steep liquor.

4.4 | Milk Yield and Composition

Although milk yield remained comparable, there was an observed trend towards increased milk production in buffaloes fed diets containing FWS compared to those consuming unfermented WS. This may be a result of the higher nutrient intake and digestibility associated with FWS. Several researchers have reported improvements in milk production linked to increased consumption of urea-molasses treated WS in lactating goats and cows. [43] Comparable findings were noted in

Maradi goats consuming both urea-treated and untreated straw. The increased milk fat and protein percentages can be attributed to enhanced intake and digestibility of NDF and CP. Wanapat et al. [43] similarly noted elevated milk fat content in goats fed diets containing urea-molasses treated rice straw. The higher milk fat content observed in this study may relate to superior utilization of cellulolytic fibers by rumen microbes in an environment optimized by urea-supplied ammonia. [44] Unchanged lactation performance has also been reported by Zhang et al. [27] in Holstein cows fed rice straw silage fermented with lactic acid bacteria.

4.5 | Blood Urea Nitrogen

The increased blood urea nitrogen BUN concentrations seen in FWS diets versus unfermented WS mainly stem from higher DM and CP intake and corresponding digestibility. This resulted in elevated ruminal ammonia nitrogen levels, which subsequently convert into urea nitrogen following absorption through the rumen wall. Wanapat et al. [43] found a direct relationship between BUN levels and ruminal ammonia concentrations. Another potential reason for higher BUN is the rapid release of ammonia nitrogen in the rumen, possibly exceeding the rumen microbes' ability to retain it and allowing more to enter systemic circulation. [46] Wanapat et al. [43] depicted that cows consuming rice straw treated with urea and calcium hydroxide, or straw ensiled with urea, showed an increase in BUN levels.

5 | CONCLUSION

In summary, the study concludes that urea-molasses treated wheat straw fermented with RD significantly enhances nutrient intake and digestibility. It is feasible to replace up to 20% of concentrates with FWS on a dry matter basis without adversely affecting milk production, milk constituents, and blood urea nitrogen levels. Additionally, the RD demonstrated no negative impact on buffalo health. Nevertheless, further feeding trials with larger animal groups are warranted before making definitive practical recommendations.

Acknowledgements
We acknowledge the agriculture farm, University of Agriculture Faisalabad and slaughter house, Faisalabad to provide WS and RD to conduct this study.

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