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Research Article

Effects of Partial Replacement of Maize Stovers with Potato Culls on *In Situ* Digestion Kinetics of Cannulated Buffalo Bull

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ABSTRACT

This *in situ* study was conducted to determine the impact of replacing maize stovers with potato culls on the digestion kinetics of dry matter (DM) as well as neutral detergent fiber (NDF) and rumen characteristics. Four diets; A, B, C and D were formulated. A ruminally cannulated Nili-Ravi buffalo bull was used in a completely randomized design to study *in situ* digestion kinetics of diets. The diet A contained maize stovers alone and diet B, C and D contained maize stovers and potato culls in ratio; 95:05; 90:10 and 85:15, respectively on dry matter basis. Two weeks were offered as adaptation period for each experimental diet while 3rd week for data collection. The bull was kept in separate pen and fed for four periods. The bull was fed the respective diet *ad libitum*. The nylon bags containing experimental diets were incubated in rumen for 0, 1, 2, 4, 6, 10, 16, 24, 36, 48 and 96 hours (h), respectively. The residual of bags was weighed and analyzed for DM and NDF. The collected data were analyzed in a completely randomized design by analysis of variance and means were compared by Duncan's multiple range test. The digestibility of DM and NDF was increased with increasing the level of potato culls. Likewise, extent of digestion of DM and NDF was also improved with increasing the level of potato culls. The lag time was reduced while rate of digestion was increased with increasing the replacement of maize stovers with potato culls. There was decrease in rumen pH at 6 h post feeding. The rumen ammonia N decreased with increasing the level of potato culls at 6 h post feeding. Total bacterial count was increased with increasing the level of potato culls at 6 h post feeding. In conclusion, maize stovers DM and NDF digestion kinetics can be improved by partial replacement with potato culls resulting to enhance the utilization of maize stovers.

Keywords: Maize Stovers, Potato Culls, Digestibility, Buffalo, Bull.



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INTRODUCTION

Forage is significant portion of ruminants diet (Rudel et al., 2015). Forage resources are deficient in the country and cannot fulfill the requirement of animals (Habib et al., 2016). One of the options is to increase the forage production by cultivating more land, which is difficult to achieve due to competition with cash crops. This situation leaves the current forage resources under increasing pressure to meet the demand for the growing animal population.

Therefore, it is need of the time for efficient utilization of available feed resources to enhance the animal productivity in the country. Crop residues are major part of animal feed in the country and contribute 58.8% of total feed supply to animal feeding (Habib et al., 2016). Maize stovers are residue of maize crop and rich in fiber, which is basic need of ruminant animals, however, the maize stovers are deficient in nonstructural carbohydrates (instant source of energy for ruminal microbes) resulting in low digestibility of dry matter and fiber.

A vegetable waste is another nonconventional source of feed for ruminant animals (Wadhwa and Bakshi, 2013). Potato culls are the residue of vegetable market. Potato culls are rich in nonstructural carbohydrates and can be fed along with maize stovers for better utilization of maize stovers, which are deficient in nonstructural carbohydrates. The potatoes and potato byproducts are being used as an alternative feed for ruminant animals (Drake et al., 1994; Radunz et al. 2003; Priedniece et al., 2017). The potato is rich in starch (Torres et al., 2020) and can stimulate the rumen bacteria to increase the digestibility of maize stovers. Previously, Beetal male goats gained higher body weight when fed a total mixed ration containing 7% potatoes as compared to goats fed total mix ration without potatoes (Khan et al., 2018) without any negative effects on the performance of goats. Maize stovers and potato culls are both agriculture wastes and their combined inclusion in ruminant feed can result in efficient utilization of both wastes. We hypothesized that maize stovers digestibility can be improved by partial replacement with potato culls. Keeping in view this observation, the current study was planned to evaluate the effects of replacing maize stovers with potato culls on *in situ* digestion kinetics of maize stovers and ruminal characteristics in buffalo bull.

MATERIALS AND METHODS

Animals, experimental material and dietary treatments

The study was conducted at Dairy Animal Teaching and Research Center at Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan. A ruminally cannulated Nili-Ravi buffalo bull (weight of 357 kg) was used to study *in situ* digestion kinetics of diets. The bull was housed in a separate pen at the farm. Maize stovers were procured from field on daily basis for the feeding of bull. Culled potatoes were procured at the start of experiment, from vegetable market Bahawalpur. The maize stovers and potato culls samples were chopped, shadow dried and then ground to 2 mm screen. The dried and ground samples were thoroughly mixed and stored in the polyethylene bags for *in situ* study and laboratory analysis. The dried maize stovers and potato culls were analyzed for DM, ether extract and ash (Table 1) following AOAC (2000). The NDF was analyzed following Van Soest et al. (1991).

Table 1. Chemical composition of maize stovers and potato culls on dry matter basis

Parameters (%)	Maize stovers	Potato culls
Dry matter	30.12	15.27
Neutral detergent fiber	68.30	18.62
Ether extract	0.81	0.63
Ash	12.65	7.74

Four diets; A, B, C and D were formulated. The diet A contained maize stovers alone. The diet B, C and D contained maize stovers and potato culls in a ratio as; 95:05; 90:10 and 85:15, respectively. The bull was fed for four periods. For each period, the animal was fed for three weeks, two weeks for adaptation of each diet and third week for data collection. The animal was fed *ad libitum* twice a day in equal portions for each diet.

In situ digestion experiment

The nylon bags with an average pore size of 60 μ m were used in this experiment. Bag size was 15 cm x 10 cm. All the nylon bags were weighed before and after the material added. Approximately 10 grams of sample on dry matter basis of each treatment was used for *in situ* study. Nylon bags (triplicate) were incubated in rumen for 0, 1, 2, 4, 6, 10, 16, 24, 36, 48 and 96 h for each diet to determine the digestion kinetics of DM and NDF. The bags were soaked in the 39°C distilled water for 15 minutes before placing into the rumen. All the bags were placed in reverse sequence and were retrieved all at the same time to reduce variation associated with washing procedure (Grigsby et al., 1992). After removal from the rumen, bags were washed under tap water until the rinse was clear. The residuals of the bags were weighed and analyzed for DM and NDF contents.

The indigestible residue that is, the residue after 96 h was subtracted from the total amount in the bag at each time to calculate the rate of disappearance of DM and NDF. The natural log (Ln) of that value was then regressed against time. The amount of DM and NDF digestion as well as the lag time were then ascertained. The digestibility of DM and NDF was assessed at 48 h. Lag time was calculated according to equation by Sarwar et al. (1999).

Lag time (h) = (Ln 100) – Intercept / Rate of disappearance

Extent of DM and NDF disappearance were determined at 96 h of incubation.

Rumen characteristics

The rumen specimens were collected from four different locations after 6 h morning feeding to measure rumen pH, rumen ammonia N and total bacterial count. Portable pH meter (Milwaukee MW150 MAX) was used to measure the rumen pH. The rumen specimen was passed through four layers of cheesecloth to collect the rumen liquor. The rumen liquor was frozen and around 50 mL of it was acidified with 3 mL of 6 N HCl to stop the fermentation. The rumen liquor was steam distilled in Kjeldahl apparatus then titrated against sulfuric acid to determine the rumen ammonia N (Giri et al., 2005). Rumen liquor was store in sterile plastic bottles to determine the total bacterial count. Total bacterial count determined was deter following the modified version of Knaysi and Ford's (1938) method.

Total bacterial count / mL of rumen liquor = $N \times DF \times MF \times 100$

N for average number of bacteria counted per field, DF for dilution factor and MF for microscopic factor

Statistical analysis

The *in situ* digestion kinetics data (digestibility, lag time, rate of degradation and extent of digestion DM and NDF) with three replicates were analyzed using analysis of variance (ANOVA) in a completely randomized design using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used to compare the means (Steel et al., 1997).

RESULTS

The DMI and *In situ* digestion kinetics of dry matter

The DM intake of Buffalo bull was similar across all diets. The DM degradability after nylon bag incubation for 48 h (an estimate of *in vivo* digestibility) was highest ($P < 0.05$) in diet D than other diets (Table 2). The digestibility of DM was increased with increasing the level of potato culls. The DM lag time was shorter ($P < 0.05$) in diet C and D compared to diet A and B (Table 2). Rate of digestion was highest ($P < 0.05$) in diet D among the experimental diets and increased with increasing the level of potato culls. Highest ($P < 0.05$) extent of DM digestion was observed in diet D among the experimental diets. The extent of digestion of DM was increased with increasing the level of potato culls.

Table 2. Effect of replacement of maize stovers with potato culls on *in situ* digestion kinetics of dry matter.

Parameters	Diets ¹				SE	P value
	A	B	C	D		
Dry matter intake (kg/day)	7.05	7.05	7.05	7.08	0.03	0.99
Digestibility (%) at 48 hours	46.75 ^d	49.68 ^c	58.20 ^b	67.41 ^a	2.43	<0.05
Lag time (h)	4.73 ^a	4.22 ^a	3.73 ^b	3.11 ^b	0.20	<0.05
Rate (%)/h	4.21 ^c	4.14 ^c	4.45 ^b	5.15 ^a	0.12	<0.05
Extent of digestion (%)	60.75 ^c	61.97 ^c	66.51 ^b	69.14 ^a	1.04	<0.05

^{a, b, c, d} Means within row with different superscripts differ ($P < 0.05$).

¹A diet contains 0 % potato culls; B diet contains 5 % potato culls; C diet contains 10 % potato culls; D diet contains 15 % potato culls.

In situ digestion kinetics of neutral detergent fiber

The NDF degradability at 48 h (an estimate of *in vivo* digestibility) was highest ($P < 0.05$) in diet D as compared to all other diets (Table 3). The NDF digestibility was similar in diet A and B, however, it was lower than diet C. The NDF digestibility was increased with increasing the replacing level of potato culls. The NDF lag time was shorter for diet C and D than diet A and B. The rate of disappearance was highest ($P < 0.05$) in diet D as compared to other diets. Lowest ($P < 0.05$) rate of disappearance was noted in diet A and B. The higher degradability rate might be due to addition of potato culls containing nonstructural carbohydrates which stimulate the rumen bacteria with provision of early energy to digest the feed early at higher rate. The extent of NDF disappearance for 96 h was highest ($P < 0.05$) in diet D. The lowest ($P < 0.05$) extent of NDF digestion was noticed in diet A and B. The extent of NDF digestion was increased with supplementation of potato culls and increased with increasing the level of potato culls.

Rumen parameters and blood glucose

The rumen pH at 6 h post-feeding was similar in diet A and B; however, it was higher than diet C and D (Table 4). The rumen ammonia N at 6 h post-feeding decreased with increasing the level of potato culls in diet of bull. Total bacterial count in bull at 6 h post feeding was significantly different across all treatments. The total bacterial count was highest (7.90×10^{10} cells/mL) in diet D than other diets.

Blood glucose was higher ($p < 0.05$) in bull when fed diet D than fed other diets (Table 4). Highest ($p < 0.05$) blood glucose

concentration (68.67 mg/dL) was observed in diet D. Similar glucose concentration was noted with diet A, B and C.

Table 3. Effect of replacement of maize stovers with potato culls on *in situ* digestion kinetics of neutral detergent fiber.

Parameters	Diets ¹				SE	P value
	A	B	C	D		
Digestibility at 48 hours (%)	45.54 ^c	47.58 ^c	52.45 ^b	57.95 ^a	1.49	<0.05
Lag time (h)	5.53 ^a	4.65 ^a	3.67 ^b	2.77 ^b	0.33	<0.05
Rate (% / h)	4.74	4.77	4.85	5.00	0.04	0.052
Extent of digestion (%)	53.38 ^c	53.29 ^c	56.45 ^b	62.47 ^a	1.13	<0.05

a, b, c, d Means within row with different superscripts differ ($P < 0.05$).

¹A diet contains 0 % potato culls; B diet contains 5 % potato culls; C diet contains 10 % potato culls; D diet contains 15 % potato culls

Table 4. Effect of replacement of maize stovers with potato culls on rumen characteristics and blood glucose.

Parameters	Diets ¹				SE	P value
	A	B	C	D		
Rumen pH	7.05 ^a	7.05 ^a	6.91 ^b	6.42 ^c	0.08	<0.05
Rumen Ammonia N (mg/dL)	11.97 ^a	8.43 ^b	8.20 ^b	5.33 ^c	0.71	<0.05
Total bacterial count (cells/mL) $\times 10^{10}$	0.68 ^d	2.61 ^c	4.20 ^b	7.40 ^a	0.75	<0.05
Blood glucose (mg/dL)	57.00 ^b	57.33 ^b	56.33 ^b	68.67 ^a	1.66	<0.05

a, b, c, d Means within row with different superscripts differ ($P < 0.05$).

¹A diet contains 0 % potato culls; B diet contains 5 % potato culls; C diet contains 10 % potato culls; D diet contains 15 % potato culls.

DISCUSSION

Highest DM digestibility (67.41%) was observed in diet D among the experimental diets. The digestibility of DM was increased linearly with increasing the replacing level of potato culls (nonstructural carbohydrates). The DM digestibility was lowest (46.75%) in diet A containing only maize stovers which was deficient in nonstructural carbohydrates. Rumen microbes responsible to degrade structural carbohydrates require instant energy and diet A contained less instant source of energy for microbes that's why digestibility of DM was lower in diet A. Ma et al. (2015) reported that organic matter digestibility was increased with increasing the level of nonstructural carbohydrates in diet of lambs. Likewise, Orumich et al. (2017) fed different levels of nonstructural carbohydrate (33, 36, 39, 42%) to lactating dairy cows and reported that increasing the level of nonstructural carbohydrates, significantly increased the DM digestibility in dairy cows. The increase in DM digestibility might be due to associative effect of maize stovers and potato culls. There was linear decrease in rumen ammonia N with increasing the level of potato culls which showed that rumen microbes utilized the ammonia N for microbial protein synthesis resulting to increase rumen microbes and consequently increased the digestibility of DM. The improvement in DM digestibility with potato culls supplementation might be due to the early supply of energy by nonstructural carbohydrate, so more suitable environment for the growth and metabolism of rumen microbes that increase the rate of digestion of DM. Souza et al. (2010) reported that organic matter digestibility of the forage 48 h of *in vitro* digestion was increased by starch supplementation. The increase in DM digestibility of maize stovers might be due to decrease in lag time and increase in rate of disappearance, so early and fast digestion increased the digestibility of DM. The extent of DM digestion significantly increased by replacing maize stovers with potato culls. Similarly, Heldt et al. (1999) evaluated the impact of supplementation of sugar and starch in ruminally fistulated steers and reported that organic matter digestibility of low-quality tallgrass-prairie hay was increased with supplementation of sugar or starch.

In contrast, an *in situ* study was conducted by Niepes et al. (2023) who supplement 20% and 40% concentrate with Napier silage and urea treated straw in ruminally fistulated Brahman bull. They reported that DM digestibility was decreased when bull fed high concentrate. High concentrate might had resulted in decrease in the rumen pH resulting to decrease the fiber with is main part of diet. In another study, Filho et al. (2023) reported that NDF digestibility was decreased linearly with increasing level (0, 180, 360, 570 g/Kg) of wheat bulgars (high in nonstructural carbohydrates). Heldt et al. (1999) evaluated the impact of supplementation of sugar and starch in ruminally fistulated steers and reported that NDF digestibility of low-quality tallgrass-prairie hay was increased with supplementation of sugar or starch. Niepes et al. (2023) reported that NDF digestibility was decreased when

fistulated brahman bull fed high concentrate that might be due to decrease in rumen pH which depressed the fibrolytic bacteria. Lowest digestibility of NDF in diet A might be attributed to greater lag time and lower rate of disappearance. Reason for increase in the extent of digestion might be due to an improvement in the rumen environment by supplying deficient nutrients or readily fermentable substrate for cellulolytic bacteria supplied by potato culls. The low non-structural carbohydrates content of maize stovers might have restricted microbial activity and the addition of nonstructural carbohydrates content supplied by potato culls might have corrected this limitation. The replacement of maize stovers with potato culls had positive effect on increase NDF digestibility, reduction of lag time of NDF and enhance the rate of NDF digestion. Potato culls provided instant availability of energy for rumen microbes to digest the NDF at higher rate and rumen microbes started early digestion of fiber with reduction of lag time resulting to enhance the NDF digestibility. The maximum increase in NDF digestibility was observed when maize stovers were replaced 15 % with potato culls. Ma et al. (2015) reported that increasing the level of nonstructural carbohydrates, there was no effect on NDF digestibility. Replacing maize stovers with potato culls resulted in linear decrease in rumen pH at 6 h post-feeding and lowest rumen pH (6.42) was observed in diet D. The decreasing (7.05 to 6.42) trend of rumen pH might be attributed to increased rumen fermentation due to inclusion of more nonstructural carbohydrates supplied by potato culls resulting more volatile fatty acids production leading to linear drop in rumen pH. Zhang et al. (2015) reported that rumen pH was decreased when bulls were fed diets having high concentration of starch or nonstructural carbohydrates. Niepes et al. (2023) fed different levels of concentrate (20 and 40%) to ruminally cannulated brahman bull with Napier silage and urea treated straw and reported decline in rumen pH when bull fed high concentrate (high nonstructural carbohydrates). The decreasing trend of rumen ammonia N with increasing the replacing level of potato culls indicates that ammonia N was incorporated more into microbial protein with increase in rumen fermentation and acid production resulting to decrease in rumen pH. Rumen ammonia N decreased with increasing the level of nonstructural carbohydrates in lambs and bulls (Ma et al., 2015; Zhang et al., 2015). However, in another study, Filho et al. (2023) reported that the rumen ammonia N concentrations did not change when lambs fed varying level of wheat bulgars. The linear increase in total bacterial count indicated that rumen fermentation was improved by replacing maize stovers with potato culls. Total bacterial count in bull at 6 h post feeding was increased by replacing maize stovers with potato culls and maximum total bacterial count (7.90×10^{10} cells/mL) was observed in bull fed diet D when maize stovers were replaced with 15% potato culls. The rumen ammonia N at 6 h post-feeding decreased with increasing the level of replacing maize stovers with potato culls in diet of bull and lowest rumen ammonia N was noticed when bull fed diet D. Increasing the potato culls in the diet resulted in a linear increase in total bacterial count, while decrease in rumen ammonia N might be attributed to increased utilization of ammonia N in to microbial protein synthesis. Concentration of rumen ammonia N from 5 to 80 mg/dL is considered optimum for microbial protein synthesis (Satter & Roffler, 1975). In the current study, ammonia N concentration was 5.33 to 11.97 mg/dL which falls in the normal range. Blood glucose concentration was highest (68.67 mg/dL) in bull when fed diet D. The diet D contained high replacing level of potato culls (15%). Potato culls are high in nonstructural carbohydrates and fermented to increase the production of propionic acid in the rumen which is glucogenic, resulting in increased level of blood glucose in bull fed diet D.

CONCLUSION

The present study demonstrated that supplementation of potato culls with maize stovers had positive effects on *in situ* digestion of maize stovers, fermentation parameters, increasing total bacterial count and decreasing the rumen pH. Particularly, decreasing ammonia N and increasing total bacterial count when supplemented potato culls (high nonstructural carbohydrate) indicated better N utilization and microbial protein synthesis. Supplementation with potato culls was beneficial up to 15% of DM to enhance the utilization of maize stovers and N utilization *in situ*. *In vivo* trial is suggested to determine the impact potato culls on the utilization of maize stovers on large number of animals.

AUTHOR CONTRIBUTIONS

Conceptualization, MTA, AJ, ZMI; Data curation, MTA, AA; Formal analysis, IHR, MUA, FS; Investigation, MTA, AA, AJ; Methodology, AJ, IHR, FS; Resources, AJ, FS, ZMI; Software, MUA, AJ; Supervision, AJ; Validation, AJ, FS, ; Writing – original draft, MTA, AA; Writing – review & editing, AJ, MUA.

COMPETING OF INTEREST

The authors declare no competing interests.

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