



Use of Potato Waste and its Effect on Ruminal Characteristics and *In-Situ* Digestion Kinetics in Cannulated Nili Ravi Buffalo Bulls

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ABSTRACT

The aim of this study was to evaluate the nutritional profile of dried potato powder (DPP) and its effect on ruminal characteristics, and *in-situ* digestion kinetics in 3 cannulated buffalo bulls. DPP samples were analyzed for different nutrients and gross energy. Three different iso-caloric (ME=2700 kcal/kg) and iso-nitrogenous (CP=17 %) concentrate rations were formulated using 10, 20 and 30 % of DPP. The trial was conducted in three periods. Each period was of 3 weeks with first 2 weeks for adaptation period and remaining 1 week for sample collection. Ruminal parameters including pH and NH₃-N were measured. Nylon bags were incubated in the rumen for 0, 1, 2, 4, 6, 10, 16, 24, 36, 48, 72 and 96 h intervals in reverse order. Fresh as well as rumen incubated samples were analyzed for DM, CP, NDF and ADF. The results of this experiment showed that DPP had non-significant effect on nutrient intake, nutrient digestibility, ruminal pH, NH₃-N and digestion kinetics. It can be concluded that dried potato powder can be used successfully in ruminant diet without any adverse effect even at 30 % of the diet for cheaper ration formulation.

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Authors' Contribution

MS and FA visualize the idea and methodology. SK and MLZ, executed the research and lab analysis. MMAH and WA performed statistical analysis and wrote the results and discussion. MKB and AA prepared first draft. SH did correspondence.

Key words

Potato culls, Cannulated buffalo bulls, Nylon bags, Digestion kinetics, Ruminal characteristics, Nilli ravi

INTRODUCTION

There is a significant gap between supply and demand of feed resources for livestock in Pakistan. This gap for energy and protein is 38.0 and 37.2 %, respectively (Habib *et al.*, 2016). Non-conventional feed resources (NCFR) refer to all those feeds that have not been traditionally used for animal feeding either by farmers or by feed manufactures in commercial feeds and it is the solution to fulfill animals nutrient requirements. Feedstuffs such as citrus pulp, sugar beet pulp, poultry litter, kitchen waste and cull potato can be used as NCFR. Many of these NCFR are high in protein, energy and minerals. That's why it can

be used to fulfill the supply and demand gap (Amata, 2014). Potato is known as a root vegetable and starchy tuber. It contains 65-75 % starch and 9.5-10 % crude protein (CP), high in potassium and vitamin C (Bakshi *et al.*, 2016). The ME value for potato is 17 MJ/Kg on dry matter DM basis but this value alters with storage time and quality of potato (Charmley *et al.*, 2006). There are various forms of potato byproducts used as livestock feed resources like potato vine and leaf mixed silage for fattening Angus bull (Deng *et al.*, 2023), potato pulp silage (Raina *et al.*, 2023; Senyuz and Karsli, 2021) and potato culls concentrates in present study. The potato culls (due to small size and damage, not fit for marketing) or processing waste from different industries like French fries or potato chips are available in bulk form which can be used in animal feed after drying. Washing, peeling, trimming, slicing, blanching, drying, frying, de-oiling, and packing are some of the primary steps in potato processing (Zobel, 2006). The utilization of alternate local sources of energetic feed, such as the starchy tubers in temperate and tropical regions, is encouraged by the high cost of certain feeds.

Dried potato powder (DPP) is an economical energy source compared to other grains and it can be added to

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substitute grains in ruminant diet (Murphy, 1997). Due to the high cost of yellow corn, DPP have been identified as a low-cost alternative and it can easily replace the maize (Omer *et al.*, 2010). The DPP has shown no effect on dry matters intake (DMI), DM and NDF digestibility but it decreased digestibility of CP and ADF (Sugimoto *et al.*, 2007). Whereas in another study, the inclusion of DPP in diets of animals improved the digestibility of ADF that may be due to fiber of DPP which is easier to degrade than other fiber sources (Pen *et al.*, 2006). In the digestion trial with increase potato waste level, the DM digestibility decreased and ruminal pH increased (Radunz *et al.*, 2003). The ruminal $\text{NH}_3\text{-N}$ increased by using DPP in the diet of steers (Radunz *et al.*, 2003). The potato vine silage could replace whole-plant corn silage in fattening Angus bull at the same dry matter status and did not affect the weight gain (Deng *et al.*, 2023). It is hypothesized that DPP can be used as cheaper energy source without any adverse effect on nutrient intakes and ruminal characteristics in buffalo bulls. This research's goal is to determine the dietary profile of DPP and its use as an energy source on *in-situ* degradation kinetics using cannulated buffalo bulls.

MATERIALS AND METHODS

Present trial has been performed at Raja Muhammad Akram Animal Nutrition Research Center, University of Agriculture, Faisalabad. All the procedures adopted in this research were approved by Graduate Studies and Research Board of the University (DGS No. 2892).

Preparation of potato powder

Potato culls were collected from the market. First potato culls were washed in washer. Then it was passed in automatic hammer slicer with 0.5mm blade size. The moisture content was reduced up to 12-15% through dehydration in polythene tunnel at 45-50 °C. The dried potato was collected and passed from grinder to get fine powder.

Experimental design

Three bulls in 3×3 having 420±20 kg average body weight and 5-6 years age. Animals were weighed before the start of trial to determine the feeding requirements. Experimental bulls were reared in separate and well-aerated pens with a concrete floor and open yard. Animals were dewormed before the start of the experiment. The diet consisted of 50 % roughages and 50 % concentrate. Three different iso-caloric (ME: 2700 Kcal/Kg) and iso-nitrogenous (CP: 17 %) concentrate ration were formulated using 10, 20 and 30 % of DPP and denoted as A, B and C, respectively (Table I). Animals were free access to clean

and fresh water. Experiment was divided into three periods. Each experimental period was of 21 days. First 14 days of each experimental period were used for dietary adjustment while remaining seven days for collection period. Overall experimental duration was 63 days.

Table I. Ingredients inclusion level (%) and its substitution with varying level of dried potato powder (DPP).

Ingredients	A	B	C
Potato peel powder (DPP)	10	20	30
Corn	23	20	16
Rice polishing	16	12	10
Wheat bran	11	13	12
Cotton seed meal	9	6	8
Canola meal	10	9	8
Corn gluten 30%	12	12	7
Dicalcium phosphate	1.5	1.5	1.5
NaCl	0.5	0.5	0.5
Molasses	7	6	7
Total	100	100	100

Data collection and chemical analysis

The DPP was analyzed for dry matter (DM), organic matter OM, CP, NDF and ADF according to the reported official methods of AOAC (2012). The essential minerals such as Na and K were determined by using flame photometer (JENWAY Ltd. Model No. PEF-7) while Ca and Mg by titration method (Tucker and Kurtz, 1961) and P by spectrophotometer after wet digestion. Gross energy was determined by Oxygen PARR Bomb Calorimeter (Model No. A50MEE). Data on feed offered, feed refusal and orts were collected daily. For first two days of each collection period, rumen liquor was taken from four different locations in the rumen at 3, 6, 9 and 12h post feeding. The samples were squeezed through four layers of cheesecloth and 50mL of the liquid was acidified with 3mL of 6N HCl to terminate fermentation and these samples were frozen till analysis. After thawing, these samples were used to determine the ruminal NH_3 (Chaney and Marbach, 1962). Rumen pH was measured immediately after sample collection by portable pH meter. The DPP was ground to 2mm through willey mill. Triplicate nylon bags measuring 10×23cm (50µ pore size) were used: Two bags containing 5g sample each while third bag were served as a blank. The bags were closed and tied with nylon thread and were soaked in the distilled water at 39°C for 15 min just before placing into the rumen to remove soluble or

50µm filterable material. These bags were incubated in the rumen of buffalo bulls for 0, 1, 2, 4, 6, 10, 16, 24, 36, 48, 72 and 96 h intervals, in reverse order and were removed at one time. The bags were washed in running tap water till the rinse was clear and then they were dried in a hot air oven at 55°C. *In-situ* digestion kinetics parameter, i.e., rate, lag and extent of DM, CP, NDF and ADF were analyzed for each period individually. Degradation rates were calculated by subtracting the residue (96 h) from the amount in the bag for each interval and then regression natural logarithm of that value against time after correction for lag time. Samples of feed samples and rumen residues were analyzed for DM, NDF, ADF and CP (AOAC, 2012; Van Soest *et al.*, 1991).

Statistical analysis

The recorded data were analyzed using general linear model procedure of statistical package for the social sciences (SPSS, 1999) using Latin square design and means were compared using Tukeys test (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

The average values of DM, CP, NDF, ADF, ash were 92.5, 9.51, 16.6, 6.66, 5.41 %, respectively. The Na, K, P, Ca and Mg values were 0.18, 8.46, 2.26, 2.39 and 1.31mg/Kg, respectively (Table II) and the average gross energy was 3734Kcal/Kg. Results shown that lowest price was seen when DPP was used 30% in concentrate ration which was Rs 37.45/kg as against Rs 41.20/kg for 10% DPP and Rs 38.95/kg for 20% DPP.

Table II. Nutrient composition of dried potato powder (DPP).

Nutrition profile	10% DPP	20% DPP	30% DPP	Aver- age
Dry matter (%)	93.2	91.7	92.6	92.5
Organic matter (%)	94.56	94.62	94.58	94.59
Protein (%)	9.46	9.58	9.51	9.51
Neutral detergent fiber (%)	16.6	16.5	16.9	16.6
Acid detergent fiber (%)	6.80	6.52	6.67	6.66
Ash (%)	5.44	5.38	5.42	5.41
Sodium (mg/kg)	0.18	0.20	0.17	0.18
Potassium (mg/kg)	8.42	8.51	8.46	8.46
Phosphorous(mg/kg)	2.24	2.28	2.26	2.26
Calcium (mg/kg)	2.43	2.37	2.40	2.39
Magnesium (mg/kg)	1.32	1.33	1.30	1.31
Gross energy (kcal/kg)	3785	3680	3735	3734

Results of this study revealed the nutritional profile of dried potato powder in which average DM and CP were 92.5 and 9.51 %, respectively. The result showed that NDF and ADF were low and its value 16.6 and 6.80 %, respectively. These finding were similar to Bakshi *et al.* (2016) who reported that culled potato from cold storage contained 65-75 % starch, 0.4 % ether extract (EE) and 9.5-10 % CP while waste potatoes had, 13-14 % CP. It contained less quantity of NDF and ADF. So, potatoes should not be used as a forage substitute rather as a grain substitute due to their low fiber content. According to NRC (1989) potatoes had around 80 % TDN, high ME (3.16 Mcal/kg DM), and net energy (1.87 Mcal/kg DM). According to Nelson *et al.* (2000), potato by-product contains 55.9, 11.6, and 1.7 % of starch, CP and total fatty acid on DM basis, respectively. The CP content of potato by product mainly consists of non-protein nitrogen which contains 60 % of rumen degradable protein (Bradshaw *et al.*, 2002). Ncobela *et al.* (2017) showed the protein content of a potato is about 10 %, similar to the protein content of wheat flour, and higher than that of rice and corn (Bartova *et al.*, 2015). The current study showed the mineral estimation in which sodium, potassium, phosphorous, calcium and magnesium values were 0.18, 8.46, 2.26, 2.39 and 1.31 (mg/kg), respectively which were similar to Bakshi *et al.* (2016) who reported that potato is high in potassium and low in other minerals. Our finding had revealed that the DPP was used as an economical energy source because as the inclusion level of dried potato powder increased in the concentrate rations the per kg cost of ration decreased. The per kg cost of concentrate rations were 41.20, 38.95 and 37.45 rupees with the inclusion level of 10, 20 and 30 % DPP. These finding were similar to Murphy (1997) who reported that potato by-products were used an economical energy source compared to grains and it can be added to substitute grains in ruminant diet. Omer *et al.* (2010) also reported that due to the high cost of yellow corn, DPP has been identified as a low-cost alternative and it can easily replace the maize.

Result indicated that there were no significant differences ($P>0.05$) among various treatments regarding DM, CP, ADF and NDF intake in Nili Ravi buffalo bulls. Result indicated that there were no significant ($P>0.05$) differences for DM, CP, NDF and ADF digestibility when Nili Ravi buffalo bulls were fed various levels of DPP (Table III).

Results of this study indicated that there was non-significant ($P>0.05$) effect of various inclusion levels of DPP on feed intake of Nilli Ravi buffalo bulls. The results of this study were in accordance with Onwubuemeli *et al.* (1985) who also reported no significant changes in feed intake when potato by-product was added up to 40 %

Table III. Effect of varying level of dried potato powder (DPP) on nutrient intake and digestibility in cannulated buffalo bulls.

Parameters	10% DPP	20% DPP	30% DPP	SEM	*P value
Dry matter intake (kg/day)	9.95	9.77	9.85	0.21	0.96
Dry matter digestibility (%)	76.12	78.06	77.82	0.08	0.78
Crude protein intake (kg/day)	1.25	1.32	1.33	0.14	0.81
Crude protein digestibility (%)	69.58	67.42	67.62	0.01	0.46
Neutral detergent fiber intake (kg/day)	3.41	3.44	3.40	0.138	0.66
Neutral detergent fiber digestibility (%)	64.59	62.57	62.95	0.1	0.44
Acid detergent fiber intake (kg/day)	2.48	2.54	2.42	0.12	0.48
Acid detergent fiber digestibility (%)	57.77	56.64	56.74	0.09	0.16

*Means with no superscripts within row are statistically non-significant ($P > 0.05$).

in the diet. Similarly, [Omer *et al.* \(2010\)](#) also reported replacement of corn by potato waste up to 50 % in the diet of lambs did not show significant effect on feed intake. This might be due to that the potato waste had no adverse effect on palatability. Moreover, [Sadri *et al.* \(2018\)](#) observed non-significant effect of potato silage on feed intake of lamb when they replaced concentrate feed and alfalfa hay with different levels (0, 100, 200 or 300 g/ kg of diet DM) of potato silage. This was probably due to the lack of difference in the nutrients digestibility among treatments, particularly NDF digestibility. As outlined by [Van-Soest \(1994\)](#), NDF digestibility had a close association with intake in ruminants. However, DMI of South African Droper lambs was decreased by feeding the diet having 22 % potato silage ([Nkosi and Ratsaka, 2010](#)). [Pen *et al.* \(2006\)](#) reported that substituting concentrate with potato-based silage up to 27 % on DM basis in diets did not affect total digestible nutrients or CP intakes, but increased DM, OM, NDF and ADF intakes. These effects may be due to potato-based silage being higher in DM, OM, NDF and ADF, and lower in EE and hemicellulose) than concentrates. [Tawila *et al.* \(2008\)](#) stated that by replacing concentrate feed mixture with 50 % inclusion of PW did not change feed intake significantly. In contrast, apparent CP digestibility of the potato vine silage groups was higher than that of the corn silage ($p < 0.05$) ([Deng *et al.*, 2023](#)). Additionally, [Nelson *et al.* \(2000\)](#) concluded that when ensiled potato used with 10 % addition in diets then dry matter intake increased. These findings were also supported by [Hoover *et al.* \(1976\)](#) who found that potato-based silage diet increased the dry matter intake compared to maize silage when fed to ruminant animals. This increased in feed intake might be attributed to high palatability of rations containing potato waste.

Our findings revealed that there were no significance differences among all treatments when various inclusion

levels of DPP were offered to Nili Ravi buffalo bulls. [Pen *et al.* \(2006\)](#) reported that replacing concentrate feed mixture with potato-based silage at different inclusion levels of 0, 19 and 27 % did not affect on the digestibility except for CP and ADF in Holstein cattle. CP and ADF digestibility of the experimental diets having 19 % potato silage was increased compared with control. [Sugimoto *et al.* \(2007\)](#) reported that increasing the feeding level had no effect on the rate (k) of *in situ* forage dry matter disappearance in steers given the potato pulp silage diet, whereas the grain diet tended to decrease the rate of *in situ* disappearance as the feeding level increased. The treatment had no effect on the percentage of DM disappearances at 72 h. [Schneider *et al.* \(1985\)](#) reported that when DPP was used in diet of cow the digestion rate of NDF and OM decreased due to fermentation of starch but due to fat content of DPP it increased the digestibility of EE. [Onwubuemeli *et al.* \(1985\)](#) observed a decrease in ADF digestibility in steers fed 20 % potato waste diet. The depression in ADF digestibility might be associated with an increased rate of rumen fermentation. [Tawila *et al.* \(2008\)](#) observed that when concentrate feed mixture replaced with potato waste at different inclusion levels of 0, 25 and 50 % in sheep, decreased in CP digestibility was recorded for 50 % level of inclusion. While DM, OM, EE, CF and NFE were not affected by the treatment. The decreased CP digestibility might be related to low intake of protein by the animals that provided in 50 % inclusion level of treatment. Similarly, [Sadri *et al.* \(2018\)](#) they observed non-significant effect on the digestibility coefficient of DM, OM, EE and NDF. However, CP digestibility was increased. It can be attributed to starch that is available in potato containing more amylopectin which decreased the ruminal fermentation rate as compared to control diet. So, more energy was available for the growth of ruminal microbes which increased the digestion of crude

Table IV. Effect of varying levels of dried potato powder (DPP) on rumen pH, $\text{NH}_3\text{-N}$, *in-situ* DM digestibility, *in-situ* CP digestibility, *in-situ* NDF digestibility and *in-situ* ADF digestibility at various time interval in cannulated buffalo bulls.

	DPP				*P value
	10%	20%	30%	SEM	
pH at different intervals					
3 h	6.72	6.88	6.81	0.08	0.43
6 h	6.58	6.24	6.40	0.09	0.29
9 h	6.68	6.45	6.55	0.09	0.30
12 h	6.55	6.35	6.65	0.10	0.25
Ruminal NH₃-N at different					
3 h	2.84	3.15	3.02	0.54	0.21
6 h	3.92	3.58	3.76	0.67	0.74
9 h	2.47	2.61	2.32	0.71	0.62
12 h	3.12	2.89	3.56	3.7	0.35
<i>In-situ</i> DM digestibility					
Rate of digestion (%)	5.95	5.51	5.75	0.24	0.81
Lag time (h)	8.70	6.38	7.76	0.46	0.11
Extent of digestion (%)	91.50	92.26	91.06	0.54	0.21
<i>In-situ</i> CP digestibility					
Rate of digestion (%)	3.22	3.99	3.43	0.13	0.519
Lag time (h)	8.62	6.92	7.63	0.70	0.508
Extent of digestion (%)	73.40	74.35	72.82	0.65	0.155
<i>In-situ</i> NDF digestibility					
Rate of digestion (%)	2.92	3.48	3.12	0.54	0.21
Lag time (h)	3.01	2.53	2.33	0.27	0.165
Extent of digestion (%)	91.32	88.73	89.15	0.71	0.62
<i>In-situ</i> ADF digestibility					
Rate of digestion (%)	2.35	3.28	2.33	0.06	0.546
Lag time (h)	7.28	6.63	7.32	0.33	0.556
Extent of digestion (%)	62.18	58.15	59.33	0.97	0.50

DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein. *Means with no superscripts within row are statistically non-significant ($P > 0.05$)

protein in rumen. Nkosi and Meeske (2010) replaced potato silage with maize silage in lamb diets (at a 20 % dietary inclusion level) reduced DM, OM, CP, and NDF digestibility. In contrast, Omer *et al.* (2010) concluded that addition of DPP in the diet of lambs significantly improved digestibility coefficients of DM, OM and CP however, it had no effect on the digestibility of CF and nitrogen free extract. Similarly, Pen *et al.* (2006) showed that inclusion of DPP in diet of animals improved the digestibility of

ADF that might be due to fiber of DPP which is easier to degrade as compared to other fiber sources. Ncobela *et al.* (2017) observed that potato waste contained relatively high amount of fermentable carbohydrates such as potato peelings, culled potatoes, potato pulp, and the fermentation of these potato waste carbohydrates will allow ruminal microorganisms to digest the fiber more efficiently in steers.

Results of statistical analysis had shown non-significant ($P > 0.05$) differences for pH value and ruminal $\text{NH}_3\text{-N}$ at 3, 6, 9 and 12h among different inclusion levels of DPP (Table IV). Rate of digestion, extent of digestion and lag time for DM, CP, NDF and ADF remained unchanged ($P > 0.05$) among various treatments in Nili Ravi buffalo bulls (Table IV).

Non-significant differences were observed for rumen pH after feeding different levels of DPP to Nili Ravi buffalo bulls. These results were in accordance with Onwubuemeli *et al.* (1985) who reported that the inclusion of potato by-products or potato waste up to 40 % did not affect ruminal pH. Similarly, Omer *et al.* (2010) also concluded that dietary inclusion of dried potato had no significant effect on ruminal pH. Moreover, Gado *et al.* (1998) reported that by feeding Baladi goats on diets replaced by 0, 25, 50, or 100 % of concentrate feed mixture by potato waste had no significant effect on ruminal pH. In contrast, Tawila *et al.* (2008) reported that by feeding sheep on 0, 25 and 50% potato processing waste diet significantly increased the ruminal pH for 25 % diet but as the inclusion level of potato waste increased to 50 % of the diet; the ruminal pH decreased due to increasing the concentration of VFA. Sugimoto *et al.* (2007) observed no differences in ruminal pH after changing feeding level. However, it changed gradually with time after feeding. They also reported that steers fed the potato pulp silage had a higher ($P < 0.1$) ruminal pH compared with steers fed the grain diet. It might be expected that a decreased in the amount of starch when fed the potato pulp silage-based diet would lead to higher ruminal pH. Ruminal pH was expected to reduce due to fermentation of available carbohydrate in potato (Nagaraja and Titgemeyert, 2007). When pH of the rumen was normal, a balance exists between the acids production and utilization. Low pH disrupts this balance and acids begin to accumulate in the rumen. Zebeli *et al.* (2008) also reported that ruminal pH is very important for the stability of the rumen microbial ecosystem. Maintaining rumen pH above 5.8 is helpful to prevent rumen disorders. Pen *et al.* (2006) showed the effect of increasing levels of potato-based silage on ruminal fermentation. They found that rapid fermentation of readily available carbohydrates in potato-based silage may reduce ruminal pH, however, ruminal pH was unaffected by potato-based silage

inclusion. In contrast, an increase in pH of rumen has been reported with the addition of potato waste in animal diets (Radunz *et al.*, 2003).

Results have revealed that there were non-significance effects of various levels of DPP on $\text{NH}_3\text{-N}$. The results are in accordance with Pen *et al.* (2006) who reported that inclusion of potato-based silage in the diet of steers did not affect ruminal $\text{NH}_3\text{-N}$ concentration in steers. However, Onwubuemeli *et al.* (1985) concluded that ruminal $\text{NH}_3\text{-N}$ decreased linearly when potato waste replaced high-moisture corn in lactating dairy cow diet. They further stated that potato starch fermentation rate was faster than corn starch, causing a decreased in ruminal ammonia. Tawila *et al.* (2008) reported that $\text{NH}_3\text{-N}$ concentration was not significantly decreased with increasing the level of potato processing waste in the diet. In contrast, Omer *et al.* (2010) recorded significant decreased in $\text{NH}_3\text{-N}$ concentrations while partially replacing yellow corn with potato waste. Similarly, Murphy (1994) found that ruminal ammonia concentrations decreased below the level before eating and then progressively increased back to pre-feeding levels. The decreased in $\text{NH}_3\text{-N}$ in the rumen fluid might be due to increase $\text{NH}_3\text{-N}$ incorporation into microbial protein. It could be due to increase microbial activity, while higher total volatile fatty acid could be linked to increase utilization of dietary energy and positive fermentation in the rumen. Radunz *et al.* (2003) reported an increase ruminal $\text{NH}_3\text{-N}$ concentration in steers fed diets with potato waste inclusion. Same findings were also reported by Sugimoto *et al.* (2007) who stated that ruminal $\text{NH}_3\text{-N}$ of steers fed the potato pulp silage diet was higher at 2 h post-feeding and became lower after 6 h from the time of feeding than that of steers fed the grain diet. This suggests that the protein present in the potato pulp silage diet went through a more rapid degradation than that present in the grain diet which increased the ruminal $\text{NH}_3\text{-N}$.

CONCLUSION

It can be concluded that nutrient intake, nutrient digestibility, ruminal characteristics and digestion kinetics remained unchanged across the treatments in buffalo bulls fed varying level of DPP which indicate that it can be used successfully in ruminant diet up to 30 % without any adverse effect for cheaper ration formulation.

DECLARATIONS

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Ethics approval

All methodology and procedures were approved by the Graduate Studies Research Board, University of Agriculture, Faisalabad-Pakistan.

Data availability

It does not apply to this article as no new data were created in this study

Statement of conflict of interest

The authors have declared no conflict of interest.

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