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In Vitro Methane Production Potential, Nutrient Digestibility and Fermentation Pattern of Total Mixed Rations containing Incremental Levels of Novel Feedstuff - *Phalaris minor* Seeds in Buffalo Inoculum

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A B S T R A C T

The main objective of the present study was to investigate the effect of incorporation of graded levels of *Phalaris minor* seeds (a weed in wheat fields) in the total mixed ration on *in vitro* nutrient digestibility and rumen fermentation pattern using buffalo inoculum. Maize grain based conventional concentrate mixture was prepared and maize grains in the concentrate mixture were replaced by *Phalaris minor* seeds at graded levels of 25, 50, 75 and 100 per cent. Maize based concentrate mixtures were then mixed with oven dried oat fodder and wheat straw in 40:40:20 ratio, respectively to formulate five total mixed rations. The roughage to concentrate ratio of diet was kept at 60:40.The nutritional worth of various total mixed rations formulated was assessed by *in vitro* gas production technique. It was observed that net gas production and methane production was depressed ($P<0.05$), when maize grains in the concentrate mixture were completely replaced by *P. minor* seeds. Similar trend was observed in the *in vitro* digestibility of nutrients. However, there was no adverse effect of replacing maize grains with *P. minor* seeds up to 75 per cent in the concentrate mixture on *in vitro* dry matter and organic matter digestibility. The inclusion of *P. minor* seeds in total mixed ration did not have any adverse effect on fermentation parameters. Hence, it was concluded that *P. minor* seeds could be considered as promising energy supplement for livestock and can replace conventional cereal grains viz., maize upto 75 % in the total mixed ration without any adverse effect on nutrient digestibility and fermentation pattern.

Keywords

Total mixed ration, *In vitro* digestibility, Fermentation, *Phalaris minor* seeds.

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Introduction

India has the highest livestock's population in the world and the country is also the highest milk producer, but is facing a major green fodder crisis in absence of adequate grassland due to growing pressure of population on land. In 1947, the country had 70 million hectares under grassland which is now reduced to 38 million hectares. India may have to import milk in four years, if it cannot increase fodder supply, as rising pressure on land is reducing pastures nationwide. To boost milk yield, India would need to generate 1,764 million tonnes of fodder by 2020, according to an India Spend analysis of government data. But existing sources can only manage about 900 million tonnes of fodder. Currently, feed and fodder are in short supply to the tune of 63% green fodder, 24% dry fodder and 76% concentrates. Only 4% of total cultivable land in India is used for fodder production, a proportion that has remained stagnant for the last four decades (Patil, 2017). Thus, to meet the nutrient requirements of animals, we need to improve either the efficiency of utilization of already existing feed ingredients/nutrients or need to tap new non-conventional feed resources.

Phalaris minor is one such non-conventional feedstuff. It is an annual fast spreading weed in the entire north-western belt of India, where rice-wheat cropping system is followed. *P. minor* belongs to Graminae family. It is commonly known as *Gulli Danda*, *Mandusi* and *Gehunka Mama*. Its seeds are widely available from fields in Punjab, Haryana, Uttar Pradesh, Madhya Pradesh, parts of Bihar and Himachal Pradesh. The present work examines the effect of replacement of maize grains with *P. minor* seeds in the total mixed ration (TMR) at graded levels on *in vitro* nutrient digestibility, methane production and rumen fermentation pattern in buffalo inoculum.

Materials and Methods

Sample collection and preparation

Samples of common energy feeds fed to livestock, viz. maize (*Zea mays*), wheat (*Triticum aestivum*), pearl millet (*Pennisetum typhoides*), barley (*Hordeum vulgare*) and unconventional energy supplement, viz. *Phalaris minor* seeds were collected from various places (Farm section of National Dairy Research Institute, Karnal and local market, Karnal). The samples were dried in hot air oven (60°C, 24 h) and ground to pass through 1.0 mm sieve. Grounded samples were stored in plastic containers for chemical estimations. Maize based conventional concentrate mixture containing maize grains 38, mustard cake 10, groundnut cake 23, deoiled rice bran 6, wheat bran 20, mineral mixture 2 and common salt 1 part each was prepared. Maize grains in the conventional concentrate mixture were replaced by *P. minor* seeds at graded levels of 25, 50, 75 and 100 per cent on w/w basis. Maize based concentrate mixtures were then mixed with oven dried oatfodder and wheat strawin 40:40:20 ratio, respectively to formulate five TMRs. The roughage to concentrate ratio of TMR was kept at 60:40.

Proximate and cell wall constituents

Energy feeds and TMRs were analysed for dry matter (DM), Kjeldahl N, ether extract (EE) and ash content using the standard procedures (AOAC, 2005). Crude protein (CP) content of samples was determined as Kjeldahl N × 6.25 by digesting in sulphuric acid and digestion mixture (consisting of sodium/potassium sulphate and copper sulphate in 10:1 ratio) using semiauto-analyser (Kel plus Classic-DX, Pelican). Cell wall fractions, viz. NDF, ADF, cellulose and lignin were estimated sequentially using the standard procedure (Van Soest *et al.*, 1991). NDF and ADF were expressed inclusive of residual ash. Lignin was determinedbysolublizationofcellulosewith72 per cent sulphuric acid.

In vitro evaluation

The nutritional worth of various TMRs formulated was assessed by *in vitro* gas production technique (Menke *et al.*, 1979; Menke and Steingass, 1988). Rumen contents were collected from fistulated adult male buffalofed concentrate mixture (maize 32, groundnut cake 25, wheat bran 40, mineral mixture 2 and salt 1 part each), wheat straw and chopped green oat fodder as per the requirements (ICAR, 2013).Two sets of samples were incubated in triplicates. In the Ist set, about 375 mg of the ground sample (dry matter basis) was incubated at 39°C for 24h in triplicate in 100 ml calibrated glass syringes with buffered rumen fluid for assessing the net gas production, digestibility of nutrients, total volatile fatty acid (TVFA) production (Barnett and Reid, 1957) and metabolizable energy (ME) availability. Individual volatile fatty acids were determined by using GLC equipped with a glass column (6 ft length and 1/8 inch diameter) packed with chromosorb 101. Samples were prepared by adding 0.2 ml of 25% metaphosphoric acid per ml of rumen liquor, allowing it to stand for 2 h followed by centrifugation at 4000 rpm for 7 min. Supernatant was used for estimation of individual volatile fatty acids (IVFA).

In the 2^{nd} set, total gas production was recorded after 24 h of incubation. From the headspace of each syringe, 100 ml gas was collected by puncturing the silicon tube and injected in gas chromatograph for the estimation of methane. Standard calibration gas (Sigma gases, New Delhi) consisted of equal proportion of methane and carbon dioxide. The flow rates for nitrogen, hydrogen and zero air were 30, 30, 320 ml/min respectively. Blank and standard hay (berseem hay) were run in triplicate with each set.

Statistical analysis

The data were subjected toone-way analysis of variance procedure of SAS (2003), using the linear model. The post-hoc comparison of means was done for the significant difference by Tukey's b. Significant differences of treatments were considered at P<0.05 level.

Results and Discussion

Chemical composition of *Phalaris minor* seeds and conventional energy sources

Organic matter content in *P. minor* seeds was lower than conventional cereal grains (Table 1). This might be attributed to the higher total ash content in *P. minor* seeds. CP content of *P. minor* seeds was similar to that of wheat and was higher than other conventional cereal grains evaluated.These findings are in accordance with those of Kaur *et al.*, (2006, 2017) and Kaur and Thakur (2016). *P. minor* seeds recorded highest EE and total ash. *P. minor* seeds had higher NDF content than maize and wheat, however, it was lower than pearl millet and barley. The hemicellulose content of *P. minor* seeds was similar to that of maize, though it was lower than pearl millet and barley.

ADF, cellulose and lignin levels were the highest in *P. minor* seeds among the energy sources evaluated. Starch content of *P. minor* seeds was similar to that of barley, but it was lower than other conventional cereal grains evaluated. The values obtained for chemical composition of conventional cereal grains were within the normal range (Ranjhan, 1998) and similar to those reported by earlier workers (Kamble *et al.*, 2010; Lamba *et al.*, 2014). Our findings regarding chemical constituents of *P. minor* seeds are also in close agreement with those of Gupta *et al.*, (1989).

Chemical composition of experimental total mixed rations

The chemical composition of various TMRs formulated is presented in table 2. The crude protein, ether extract and total ash content of TMRs increased slightly with the increasing level of *P. minor* seeds because of greater amount of protein, fat and ash in *P. minor* seeds as compared to maize grains. Similar trend was observed in total ash, NDF, ADF and ADL levels which showed increase, with increasing level of *P. minor* seeds in the TMR. The OM content decreased with increasing level of *P. minor* seeds, because of higher total ash content of *P. minor* seeds.

In vitro evaluation

Net gas production

The net gas production from TMR 2, TMR 3 and TMR 4 containing 25, 50 and 75 per cent *P. minor* seeds in the concentrate mixture, respectively was similar to that from TMR 1 (without *P. minor* seeds) (Table 3).

However, the net gas production was depressed (P<0.05) in TMR 5 (171.84 L/kg DM/24h) containing concentrate mixture where maize grains were completely replaced with *P. minor* seeds. The above findings are in close agreement with those of Kaur *et al.,* (2006), who observed similar trend in net gas production on replacing cereal grains in the concentrate mixture with *P. minor* seeds.

Methane production

The methane production in TMRs followed a trend similar to that of net gas production (Table 3). The methane production (L/kg DM/24h) was the highest (P<0.05) from TMR 1 (54.58) and lowest (P<0.05) from TMR 5 (50.33), containing concentrate mixture with complete replacement of maize grains by *P. minor* seeds.

Table.1 Chemical composition (% DM basis) of *Phalaris minor* seeds and Conventional energy sources

Parameter	*Phalaris minor*	Maize	Wheat	Pearl millet	Barley
Organic matter	92.46	98.67	98.77	98.03	97.53
Crude protein	12.33	9.03	12.03	9.40	10.90
Ether extract	6.27	4.23	2.00	3.00	2.10
Total ash	7.53	1.30	1.17	2.00	2.50
NDF	24.47	19.93	17.80	38.20	40.07
ADF	10.23	5.47	4.47	5.43	9.00
Hemicellulose	14.33	14.47	13.33	32.77	31.07
Cellulose	5.50	1.83	1.47	1.77	4.67
ADL	2.03	0.20	0.57	0.40	0.77
Starch	50.00	65.00	62.00	54.00	50.67

NDF=Neutral detergent fibre; ADF=Acid detergent fibre; ADL=Acid detergent lignin

Table.2 Chemical composition (% DM basis) of total mixed rations containing incremental Levels of *Phalaris minor* seeds

Parameters	Level of *P. minor* seeds in the concentrate mixture (%)				
	0	**25**	**50**	**75**	**100**
DM	93.2	93.3	93.7	93.5	93.5
OM	91.3	91.2	90.5	90.3	90.2
CP	12.6	12.8	12.9	13.0	13.2
EE	3.0	3.3	3.4	3.6	3.7
Total ash	8.7	8.8	9.5	9.7	9.8
NDF	53.5	54.0	54.5	54.0	54.5
ADF	38.0	38.0	40.0	41.0	41.0
Hemicellulose	15.5	16.0	14.5	13.0	13.5
Cellulose	24.1	24.5	25.0	25.6	26.0
ADL	4.3	4.5	4.6	4.8	4.9

DM=Dry matter; OM=Organic matter; CP=Crude protein; EE=Ether extract; NDF=Neutral detergent fibre; ADF=Acid detergent fibre; ADL=Acid detergent lignin.

Table.3 Effect of level of *Phalaris minor* seeds on the *in vitro* gas production, methane and Digestibility of nutrients in the total mixed ration

TMR	Level of *P. minor* seeds in concentrate mixture (%)	Net gas production (L/kg DM/24 h)	Methane (L/ kg DM/24 h)	IVDMD (%)	IVOMD (%)
TMR-1	0	$181.62^{b}\pm0.64$	$54.58^{b}\pm0.52$	$57.76^{b}\pm1.17$	$64.49^{b}\pm1.10$
TMR-2	25	$180.16^{b}\pm0.80$	$53.78^{ab}\pm1.29$	$56.39^{b}+\pm0.57$	$63.22^{b}\pm0.55$
TMR-3	50	$178.38^{b}\pm0.85$	$52.15^{ab}\pm0.67$	$54.93^{b}\pm0.15$	$62.03^{b}\pm0.37$
TMR-4	75	$177.43^{b}\pm1.56$	$51.24^{ab}\pm0.68$	$54.52^{b}\pm1.20$	$61.46^{b}\pm1.66$
TMR-5	100	$171.84^{a}\pm0.79$	$50.33^{a}\pm0.33$	$48.96^{a}\pm0.93$	$55.46^{a}\pm0.58$

Means bearing different superscripts in a column differ significantly (P<0.05); Roughage: concentrate ratio in TMR 1, TMR 2, TMR 3, TMR 4 and TMR 5 was 60:40 on DM basis; All the 5 TMRs contained oats fodder and wheat straw in the ratio of 2:1; IVDMD=*In vitro* dry matter digestibility; IVOMD=*In vitro* organic matter digestibility; ME=Metabolizable energy.

Table.4 Effect of level of *Phalaris minor* seeds replacing maize grains on *in vitro* ruminal fermentation pattern of total mixed rations

TMR	Level of *P. minor* seeds in concentrate mixture (%)	TVFA (meq/100 ml incubation media)	NH_3-N (mg/100ml incubation media)	IVFA (molar %)		
				Acetate	Propionate	Butyrate
TMR-1	0	7.20±0.15	15.09±0.97	69.32±0.86	22.02±0.75	8.65±0.40
TMR-2	25	6.97±0.10	15.36±0.09	69.54±0.77	21.71±0.71	8.75±0.20
TMR-3	50	6.85±0.17	15.53±0.35	70.07±0.44	21.20±0.29	8.72±0.19
TMR-4	75	6.83±0.08	15.62±0.10	70.41±0.25	20.59±0.30	9.00±0.24
TMR-5	100	6.63±0.07	15.87±0.24	70.53±0.30	20.31±0.15	9.16±0.33

Means bearing different superscripts in a column differ significantly (P<0.05); TVFA=Total volatile fatty acids; NH_3-N= Ammonia-N; IVFA=Individual volatile fatty acids.

However, there was no difference in methane production among TMR 2, TMR 3 and TMR 4 containing concentrate mixtures with 25, 50 and 75 per cent *P. minor* seeds replacing maize grains, respectively. The results of the present study corroborate well with those of Ahmed (2013) who reported similar range of methane production from TMRs containing roughage to concentrate ratio of 60:40.

Nutrient digestibility

The *in vitro* DM digestibility was not affected on replacing cereal grains with *P. minor* seeds up to 75 per cent level in the concentrate mixture (Table 3). However, beyond that level, *in vitro* DM digestibility was depressed ($P<0.05$) when maize grains were completely replaced with *P. minor* seeds. The *in vitro* OM digestibility of TMRs also followed a similar trend to that of DM digestibility. The OM digestibility of TMR 2, TMR 3 and TMR 4 (25, 50 and 75 per cent *P. minor* seeds in the concentrate mixture, respectively) was comparable with that of TMR 1(without *P. minor*), but it was depressed ($P<0.05$) in TMR 5 having maize grains completely replaced with *P. minor* seeds. The data conclusively revealed that maize grains could be replaced by *P. minor* seeds up to 75 per cent in the diet without affecting the digestibility of nutrients. The findings of the current *in vitro* study of TMRs are also in good agreement with Kaur *et al.,* (2006) who reported similar trend in the digestibility of nutrients on replacing cereal grains by *P. minor* seeds.

In vitro fermentation characteristics

In vitro fermentation characteristics revealed that the total volatile fatty acid (TVFA) production ranged from 6.63 in TMR 5 to 7.20 in TMR 1 (Table 4). The TVFA production showed a declining trend with increasing level of *P. minor* seeds, however, the decrease was statistically non-significant. The results are in line with those of Kaur *et al.,* (2006a, 2009) who carried out rumen studies in adult rumen fistulated male buffaloes fed TMRs containing different concentrate mixtures with graded levels of *P. minor* seeds. They reported that replacement of wheat grains by *P. minor* seeds up to 75% in concentrate mixture as well as inclusion of higher levels of *P. minor* seeds up to 60% in the concentrate mixture, showed no adverse effect on the TVFA concentration in the rumen. The TVFA levels in the present study were within the normal range as reported by Khan *et al.,* (2015) who carried out *in vitro* evaluation of TMRs having roughage to concentrate ratio of 60:40. However, the values of TVFAs in the present study were lower than those reported by Ahmed (2013) from TMRs with roughage to concentrate mixed in the ratio of 60:40. The difference may be because of the different green fodder used and also due to the variation in the ingredient composition of the concentrate mixture used for formulation of TMR.

The NH_3-N levels (mg/100ml) ranged from 15.09 (TMR 1) to 15.87 (TMR 5) (Table 4). The NH_3-N showed an increasing trend with increasing level of *P. minor* seeds in the TMR. The reason may be the higher CP content of *P. minor* seeds as compared to that of maize grains. However, the difference in NH_3-N production amongst various TMRs was statistically non-significant.

The results of the present study corroborate well with the findings of Kaur *et al.,* (2009) who studied the effect of TMRs containing concentrate mixtures with 0, 30, 40, 50 and 60 per cent *P. minor* seeds on the biochemical changes in the rumen of fistulated male buffaloes. They reported that the NH_3-N was higher ($P<0.05$) in the rumen liquor of the buffaloes fed *P. minor* seeds as compared to that in control group. The NH_3-N production, in the present study, is within the normal range as reported Khan *et al.*, (2015) for TMRs having roughage to concentrate ratio of 60:40.

The relative proportion of *in vitro* individual VFAs (acetate, propionate and butyrate) produced (molar % of TVFA) on the fermentation of TMR 1 (without *P. minor*) was compared with other TMRs containing graded levels of *P. minor* seeds. The molar percentage of acetate ranged from 69.32% in TMR 1 (without *P. minor*) to 70.53% in TMR 5 (containing concentrate mixture with maize grains completely replaced by *P. minor* seeds). The molar percentage of butyrate ranged from 8.65% in TMR 1 to 9.16% in TMR5.

Though statistically non-significant, both acetate and butyrate exhibited an increasing trend, with increasing level of *P. minor* seeds in the concentrate mixture. This increase may be because of comparatively higher fibre content of *P. minor* seeds as compared to that of maize grains.

The molar percentage of propionate followed a reverse trend with higher production (22.02%) from TMR 1 (without *P. minor*) and lower (20.31%) from TMR 5 (maize grains completely replaced with *P. minor* seeds). However, no significant difference was observed in the relative proportion of acetate, propionate and butyrate amongst the TMRs evaluated.

The results conclusively revealed that *P. minor* seeds can be safely used as a replacement of maize grains up to 75 percent in the diet without any adverse effect on the *in vitro* nutrient digestibility and rumen fermentation pattern.

Conflict of interest

The authors declare no conflict of interest.

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