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In Vitro Nutrient Digestibility and Methane Production Potential of Concentrate Mixtures containing Graded Levels of *Phalaris minor* Seeds

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ABSTRACT

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 concentrate mixture on nutrient digestibility and rumen fermentation pattern. Maize grain based conventional concentrate mixture was prepared and maize grains in the concentrate mixture were replaced by *P. minor* seeds at graded levels of 25, 50, 75 and 100%. The nutritional worth of various concentrate mixtures 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% in the concentrate mixture on *in vitro* dry matter and organic matter digestibility. 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 concentrate mixture without any adverse effect on nutrient digestibility.

Keywords: Concentrate, *In vitro* digestibility, fermentation, *Phalaris minor* seeds

Currently, India is facing shortage of conventional feedstuffs to the tune of 61.1% of green fodder, 21.9% dry-crop residues and 64% concentrate feed ingredients (Datta, 2013). 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 an annual fast spreading weed in the entire north-western belt of India, where rice-wheat cropping system is followed. 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 at graded levels in the concentrate mixture on *in vitro* nutrient digestibility, net gas production and methane production 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 lestimations.

Proximate and cell wall constituents

Samples were analyzed for dry matter (DM), Kjeldahl N, ether extract (EE) and ash content using the standard procedures (AOAC, 2005). CP content of samples was determined as Kjeldahl N × 6.25 by digestion in sulphuric acid and digestion mixture (consisting of sodium/potassium sulphate and copper sulphate in 10:1 ratio) using semi auto-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). Heat stable α-amylase (Sigma A3306, Sigma-Aldrich, USA) was used in NDF estimation. NDF and ADF were expressed inclusive of residual ash. Lignin was determined by solublization of cellulose with 72% sulphuric acid.

In vitro evaluation

Maize based conventional concentrate mixture (CCM) 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 (Table 2). Maize grains in the CCM were replaced by *P. minor* seeds at graded levels of 25, 50, 75 and 100%. The nutritional worth of various concentrate mixtures 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 buffalo fed 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 2nd set, total gas production was recorded after 24 h of incubation. From the head space 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 to one-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).

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

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) 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 hemi cellulose 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 concentrate mixtures

The maize grains in the conventional concentrate mixture were replaced by *P. minor* seeds at 25, 50, 75 and 100% levels (Table 2).

Table 2: Ingredient composition (kg/100 kg) of experimental concentrate mixtures

Feed Ingredients	Level of *P. minor* seeds (%)				
	0	25	50	75	100
Maize grains	38	28.5	19	9.5	-
P. minor seeds	-	9.5	19	28.5	38
Mustard cake	10	10	10	10	10
Groundnut cake	23	23	23	23	23
Deoiled rice bran	6	6	6	6	6
Wheat bran	20	20	20	20	20
Mineral mixture	2	2	2	2	2
Common salt	1	1	1	1	1

Various concentrate mixtures formulated, were analysed for proximate composition and fibre fractions and the data obtained are presented in Table 3. The CP and EE content of concentrate mixtures increased slightly with the increasing level of *P. minor* seeds, because of greater amount of protein and fat 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 concentrate mixture. The OM content decreased with increasing level of *P. minor* seeds, because of higher total ash content of *P. minor* seeds.

In vitro evaluation

The net gas production was depressed (P<0.05) when the maize grains were replaced by more than 25% *P. minor* seeds (Table 4). The net gas production from concentrate mixtures containing 25, 50 and 75% *P. minor* was similar, viz. 204.33, 200.00 and 198.67 L/kg DM/24h, respectively, but it was higher (P<0.05) than that produced from concentrate mixture where maize grains were completely replaced by *P. minor* seeds (189.33 L/kg DM/24h). This indicated that the gas production was depressed (P<0.05) when maize grains were completely replaced with *P. minor* seeds. Kaur *et al*. (2006) also observed similar trend in net gas production on replacing cereal grains in the concentrate mixture with *P. minor* seeds, where net gas production was depressed (P<0.05) when cereal grains were completely replaced by *P. minor* seeds. Methane production showed a declining trend with increasing level of *P. minor* seeds, similar to that of net gas production (Table 4). Methane production was depressed (P<0.05), when maize grains were completely replaced by *P. minor* seeds. Methane production was the highest (P<0.05) in CCM and the lowest (P<0.05) in the concentrate mixture where maize grains were completely replaced by *P. minor* seeds. However, the methane production was similar among the concentrate mixtures containing 25, 50 and 75% *P. minor* seeds.

Table 3: Chemical composition (% DM basis) of concentrate mixtures containing graded levels of *Phalaris minor* seeds

Parameters	Level of *P. minor* seeds (%)				
	0	25	50	75	100
DM	92.8	93.3	94.2	93.8	93.8
OM	93.3	93.0	92.4	92.1	91.9
CP	21.1	21.4	21.7	22.1	22.4
EE	4.6	4.9	5.1	5.2	5.5
Total ash	6.7	7.0	7.6	7.9	8.1
NDF	32.0	32.5	33.0	33.5	34.0
ADF	14.4	14.9	15.5	16.1	16.7
Hemicellulose	17.6	17.6	17.5	17.4	17.3
Cellulose	8.6	8.8	9.3	9.2	9.0
ADL	4.4	4.7	4.8	5.0	5.2

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

The *in vitro* DM digestibility was not affected on replacing maize grains with *P. minor* seeds up to 75% level and it was comparable to CCM (Table 4). However, beyond that level, the DM digestibility was depressed (P<0.05), when maize grains were completely replaced by *P. minor* seeds. *In vitro* OM digestibility also followed the similar trend. The OM digestibility of CCM was comparable with the concentrate mixtures containing up to 75% *P. minor* seeds, but beyond that level, it was depressed (P<0.05) when maize grains were completely replaced by *P. minor* seeds. The availability of ME (MJ/kg DM) from concentrate mixtures containing *P. minor* seeds ranged between 9.48 to 9.65, whereas in CCM it was 9.74. The data conclusively revealed that maize grains could be replaced, without affecting the digestibility and availability of nutrients, by *P. minor* seeds up to 75%. These findings are in line with the results of Kaur *et al*. (2006) who found similar trend in the digestibility of nutrients and ME availability on replacing cereal grains with *P. minor* seeds in the concentrate mixture.

Table 4: Effect of level of *Phalaris minor* seeds in the concentrate mixture on *in vitro* gas production and digestibility of nutrients

Level of *P. minor* seeds (%)	Net gas production (L/kg DM/24 h)	Methane (L/kg DM/24 h)	IVDMD (%)	IVOMD (%)	ME (MJ/kg DM)
0	211.17^{c} ± 2.24	40.46^{d} ± 0.90	74.17^{b} ± 0.20	76.40^{b} ± 0.14	9.74^{b} ± 0.06
25	204.33^{bc} ± 1.86	38.69^{cd} ± 0.75	74.30^{b} ± 0.15	76.29^{b} ± 0.02	9.65^{ab} ± 0.05
50	200.00^{b} ± 1.15	37.31^{bc} ± 0.56	73.90^{b} ± 0.21	76.31^{b} ± 0.42	9.61^{ab} ± 0.03
75	198.67^{b} ± 1.86	35.29^{b} ± 0.55	74.10^{b} ± 0.42	75.86^{b} ± 0.28	9.62^{ab} ± 0.05
100	189.33^{a} ± 1.76	32.76^{a} ± 0.28	70.27^{a} ± 0.19	72.01^{a} ± 0.03	9.48^{a} ± 0.05

Means bearing different superscripts in a column differ significantly (P<0.05); IVDMD=*In vitro* dry matter digestibility; IVOMD=*In vitro* organic matter digestibility; ME=Metabolizable energy.

In vitro fermentation characteristics

In vitro fermentation characteristics revealed that the TVFA production was the highest (P<0.05) from CCM containing maize grains, however, it was similar to the concentrate mixtures having 25 and 50% maize grains replaced with *P. minor* seeds (Table 5). The TVFA production was lower (P<0.05) in the concentrate mixture where maize grains were completely replaced with *P. minor* seeds (7.80 meq/100 ml), as compared to CCM (8.75 meq/100 ml), however, it was similar to the concentrate mixture containing 75% *P. minor* seeds (8.03 meq/100 ml) replacing maize grains.

Table 5: Effect of level of *Phalaris minor* seeds replacing maize grains on *in vitro* ruminal fermentation pattern of concentrate mixtures

Level of *P. minor* seeds (%)	TVFA (meq/100 ml incubation media)	NH_3-N (mg/100ml incubation media)	IVFA (molar %)		
			Acetate	Propionate	Butyrate
0	8.75^{c} ± 0.03	19.54^{a} ± 0.12	61.83 ± 0.77	26.87 ± 1.24	11.30 ± 0.48
25	8.45^{bc} ± 0.03	20.16^{b} ± 0.10	62.40 ± 1.19	26.04 ± 1.00	11.56 ± 0.36
50	8.43^{bc} ± 0.02	20.70^{c} ± 0.13	62.69 ± 1.34	25.57 ± 0.89	11.74 ± 0.45
75	8.03^{ab} ± 0.03	21.89^{d} ± 0.12	63.29 ± 0.88	24.85 ± 0.39	11.86 ± 0.50
100	7.80^{a} ± 0.25	22.29^{d} ± 0.12	63.46 ± 0.27	24.59 ± 0.05	11.95 ± 0.22

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.

The NH_3-N levels were lower (P<0.05) in CCM and increased (P<0.05) as the level of *P. minor* seeds in the concentrate mixture increased (Table 5). The reason may the higher CP content of *P. minor* seeds as compared to that of maize grains. Dung *et al.* (2014) also reported that NH_3-N concentration and microbial CP production increased (P<0.05) on increasing CP levels in the diet. The perusal of relative proportion of individual VFAs (acetate, propionate and butyrate) produced on fermentation of CCM (without *P. minor* seeds) and by the concentrate mixtures containing graded levels of *P. minor* seeds revealed that the molar percentage of acetate ranged from 61.83% in CCM to 63.46% in concentrate mixture having maize grains completely replaced with *P. minor* seeds. The molar percentage of butyrate ranged from 11.30% in CCM to 11.95% in *P. minor* based concentrate mixture. Though statistically non significant, both acetate and butyrate showed an increasing trend, with increasing level of *P. minor* seeds in the concentrate mixture. This increase may be due to comparatively higher fibre content of *P. minor* seeds as compared to maize grains. The molar percentage of propionate followed a reverse trend with the highest production (26.87%) from maize based CCM and the lowest (24.59%) from *P. minor* based concentrate mixture (maize grains completely replaced with *P. minor* seeds). However, no difference (P<0.05) was observed in the relative proportion of acetate, propionate and butyrate among the concentrate mixtures evaluated.

CONCLUSION

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

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