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In Vitro Evaluation of Rice and Maize Gluten Meals in the Concentrate Mixture of Ruminants

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ABSTRACT

Concentrate mixtures formulated to contain graded levels (25, 50, 75 and 100%) of rice gluten meal (RGM) and maize gluten meal (MGM), isonitrogenously replacing groundnut cake (GNC) were evaluated for chemical composition, protein fractions, *in vitro* gas production and rumen fermentation characteristics. The concentrate mixtures contained an average of 22.1% crude protein (CP). The range of values obtained for various CNCPS protein fractions (% CP) was: 6.9-8.9, 12.7-24.1, 46.3-55.1, 15.3-17.6, 5.4-11.1 for A, B_1, B_2, B_3 and C, respectively across various treatments. Furthermore, rumen undegradable protein was maximum (P<0.01) at 100% MGM inclusion, whilst it was minimum (P<0.01) with GNC-based control. However, intestinal protein digestibility did not differ across treatments. The gas volume (GV) was highest (P<0.01) in control and lowest (P<0.01) in 100% MGM inclusion. Both *in vitro* dry matter and organic matter digestibility were greatest (P<0.01) at 100% MGM and lowest (P<0.01) at 100% RGM level. Methane, expressed as ml/g was maximum (P<0.01) in control and minimum (P<0.01) at 100% MGM inclusion. In addition, partitioning factor, microbial biomass production (MBP) and MBP: GV were maximum (P<0.01) for 100% MGM and minimum (P<0.01) for the control group. While pH of fermentation fluid did not vary, ammonia nitrogen and total volatile fatty acids were found maximum (P<0.01) in control and minimum (P<0.01) at 100% MGM. Moreover, the molar percentage of acetic acid was highest (P<0.01) at 100% MGM but lowest (P<0.01) with 100% RGM diet, whereas the opposite results were true for propionate and butyric acid. These preliminary results suggested 50-75% substitution of GNC by RGM and a similar level of MGM in the concentrate mixture for feeding ruminant animals.

Key words: Alternative feedstuffs, Digestibility, Gluten meals, Protein fractions, Rumen fermentation

INTRODUCTION

Incorporating sustainability strategies has become a standard norm in the global agricultural systems including dairying (Eisler *et al.,* 2014). However, factors such as fluctuating availability and price volatility of commodity feed raw materials, among others, pose a continuous threat for sustainable and profitable dairy production in India. This has compelled a strong research impetus to explore untapped ingredients for use in ruminant diets.

Gluten meals are the by-products of the wet-milling manufacture of starch from cereal grains. When compared with maize gluten meal (MGM) that is used traditionally as a high protein-energy ingredient and as a source of high escape protein (Heuzé *et al.,* 2018), rice gluten meal (RGM) is relatively a new ingredient with limited past research (Kumar *et al.,* 2016; Mahesh *et al.,* 2017; Malik *et al.,* 2017; Mahesh and Thakur, 2018). Considering its continuous availability, rich crude protein (CP) content of >40% within a practically feasible price, it becomes prudent to evaluate concentrate mixtures incorporating various levels of RGM. Hence, the objectives of the present experiment were to evaluate concentrate mixtures with graded inclusions of RGM and MGM substituting GNC isonitrogenously on protein fractions, gas production and associated parameters, digestibility as well as rumen fermentation variables *in vitro.*

MATERIALS AND METHODS

Concentrate mixtures (n=9) were formulated to contain RGM and MGM at levels of 25, 50, 75 and 100% substitution of GNC (Control) isonitrogenously. The

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chemical composition like CP, ether extract (EE) and ash were estimated as per AOAC (2005). Cell wall constituents like neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the procedures delineated by Van Soest *et al.* (1991) with heat-stable α-amylase (A-3306, Sigma-Aldrich, USA) and expressed inclusive of residual ash. Acid detergent lignin (ADL) was analysed upon solubilisation of cellulose by 720 g/kg H_2SO_4 (Van Soest *et al.,* 1991). Hemicellulose and cellulose were calculated as NDF–ADF, and ADF–ADL, respectively. Protein fractions were calculated as per Cornell Net Carbohydrate and Protein System (CNCPS) as detailed by Sniffen *et al.*(1991), rumen undegradable protein (RUP) by treating samples with *Streptomyces griseus* protease (Krishnamoorthy *et al.,* 1983) and the residue was subjected to pepsin-pancreatin treatment to arrive at intestinal protein digestion (IPD; Calsamiglia and Stern, 1995).

The different concentrate mixtures were analysed for *in vitro* gas production using the Hohenheim gas test (Menke and Steingass, 1988) with buffalo rumen liquor and 200 mg of air-dried substrates. After 24 h, blank-corrected gas volume (GV) was noted and the contents of the syringes were quantitatively transferred to 100 ml flasks and refluxed with neutral detergent fibre for an hour to get *in vitro* dry matter and organic matter digestibility (IVDMD and IVOMD; Goering and Van Soest, 1970). Partitioning factor (PF) and microbial biomass production (MBP) were estimated in accordance with the equations proposed by Blümmel *et al.* (1997), and the ratio of MBP to GV was calculated.

A separate incubation was carried out to estimate methane (CH_4) from gas sample drawn from the tip of the syringe and analysed using a gas chromatograph (Nucon 5700, India) fitted with stainless steel column packed with Porapak-N and flame ionisation detector. Syringe contents were centrifuged (Heraeus, Germany) at 4500 rpm for 20 minutes. The supernatant was used for recording pH using digital pH meter, and ammonia nitrogen (NH_3-N), total volatile fatty acids (TVFA) and individual VFA (IVFA) were determined by the procedures described previously (Mahesh and Mohini, 2015).

The data were presented as means with a pooled standard error of means. Statistical analysis was carried out using one-way analysis of variance using the software package of SAS (2012). The difference among means were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The chemical composition of concentrate mixtures revealed an average CP content of 22.1% (Table 1). The range of values obtained for various CNCPS protein fractions (% CP) was: 6.9-8.9, 12.7-24.1, 46.3-55.1, 15.3-17.6, 5.4-11.1 for A, B_1, B_2, B_3 and C, respectively across various treatments. Furthermore, rumen undegradable protein was maximum ($P<0.01$) at 100% MGM inclusion, whilst it was minimum ($P<0.01$) with GNC-based control. However, intestinal protein digestibility did not differ across treatments. The test protein meals used in the study *i.e.,* GNC, RGM and MGM contained 43.1, 47.5 and 65.2% CP, respectively (Mahesh *et al.,* 2017). Variation in other parameters like EE, NDF, ADF, carbohydrates as well as CNCPS protein fractions, RUP and IPD across various concentrate mixtures closely reflect the actual values of three protein supplements (Mahesh and Thakur, 2018).

The GV was highest ($P<0.01$) in the control and lowest ($P<0.01$) in 100% MGM inclusion (Table 2). Both IVDMD and IVOMD were greatest ($P<0.01$) at 100% MGM and lowest ($P<0.01$) at 100% RGM level. CH_4 expressed as ml/g was maximum ($P<0.01$) in control and minimum ($P<0.01$) at 100% MGM inclusion. In addition, PF, MBP and MBP:GV were maximum ($P<0.01$) for 100% MGM and minimum ($P<0.01$) for the control group (Table 2). The varied range of GV obtained depicts the inherent difference in the rumen fermentability of different substrates. The gas production is the result of the fermentation of feed components in the rumen (Menke and Steingass, 1988; Pal *et al.,* 2015). Previously, Kumar *et al.* (2016) noted that RGM yielded ~5% less gas than GNC, and MGM produced lower gas and CH_4 when compared with several conventional oilseed cakes/meals (Lamba *et al.,*

Table 1. Chemical composition (% DM) and protein fractions (% CP) of various concentrate mixtures

Components	Control	Level of RGM (%)				Level of MGM (%)				SEM
		25	50	75	100	25	50	75	100	
CP	22.0	22.0	22.0	22.1	22.3	22.0	22.2	22.2	22.1	-
EE	5.08	4.62	4.36	4.02	3.63	4.80	4.50	4.30	3.91	-
Ash	5.47	5.60	5.52	5.50	5.58	5.44	5.44	5.34	5.44	-
NDF	24.5	25.6	26.7	27.7	28.9	24.3	24.1	23.8	23.6	-
ADF	10.3	10.4	10.5	10.6	10.7	9.95	9.60	9.32	8.91	-
Hemicellulose	14.2	15.3	16.2	17.1	18.2	14.4	14.5	14.5	14.6	-
Cellulose	6.92	7.14	7.45	7.71	7.98	6.82	6.73	6.70	6.54	-
ADL	3.38	3.22	3.07	2.90	2.75	3.12	2.87	2.63	2.37	-
CHO	64.9	65.4	65.3	65.2	65.7	64.8	64.6	64.1	64.4	-
Protein fractions*										
A	8.97	8.75	8.76	8.68	8.56	8.27	7.55	6.94	6.10	-
B_1	24.1	20.4	17.7	14.6	11.3	21.2	18.4	16.0	12.7	-
B_2	46.3	48.1	49.8	51.3	53.2	48.6	50.8	52.9	55.1	-
B_3	15.3	15.7	15.6	15.6	15.9	15.9	16.4	16.7	17.6	-
C or ADICP	5.36	7.00	8.22	9.90	11.1	6.10	6.90	7.51	8.50	-
RUP (% CP)	41.1^e	44.8^d	47.5^c	50.6^b	53.8^a	44.4^d	47.7^c	50.7^b	54.7^a	0.49
IPD (% RUP)	85.8	85.3	85.0	84.7	84.3	85.5	85.4	84.6	84.1	0.53

Means bearing different superscripts in a row differ significantly (P<0.01); *Expressed as rounded percentage, hence cumulative fractions may not add exactly to 100; CHO (%)=100–CP (%)–EE (%)–ash (%); RGM: rice gluten meal; MGM: maize gluten meal; DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; CHO: carbohydrates; A: instantaneously degradable non-protein nitrogen compounds (ammonia, nitrates, amino acids and peptides); B_1: rapidly degradable globulin and few albumins; B_2: intermediately degradable albumin and glutelin; B_3: slowly degradable prolamin, extensin and denatured proteins; C: undegradable and indigestible Maillard proteins as well as proteins bound with lignin and tannin; RUP: rumen undegradable protein; IPD: intestinal protein digestibility

2014; Prusty *et al.,* 2017). A similar trend has been noted in the present experiment. Furthermore, the higher PF, MBP and the ratio of MBP to GV observed in MGM-based concentrate mixtures is a direct consequence of low GV coupled with a greater digestibility. In other words, the test protein ingredients dictated the pattern

Table 2. *In vitro* gas production and associated parameters of various concentrate mixtures

Treatment	GV (ml/g)	IVDMD (%)	IVOMD (%)	CH_4 (ml/g)	PF	MBP(mg)	MBP: GV
Control	246^a	76.4^{ab}	78.1^{ab}	34.7^a	3.17^f	205^e	0.83^f
Level of RGM (%)							
25	242^a	75.29^{abc}	77.08^{abc}	34.18^{ab}	3.19^{ef}	205^e	0.85^{ef}
50	228^{bc}	74.17^{bc}	76.46^{bc}	32.70^{bc}	3.36^d	232^d	1.02^d
75	224^c	72.19^{cd}	74.42^{cd}	30.71^d	3.32^{de}	220^{de}	0.98^{de}
100	213^d	70.83^d	73.02^d	29.71^{de}	3.44^{cd}	233^d	1.10^{cd}
Level of MGM (%)							
25	234^b	76.9^{ab}	78.2^{ab}	33.1^{ab}	3.35^d	235^d	1.01^d
50	222^c	77.1^{ab}	79.2^{ab}	31.2^{cd}	3.57^c	272^c	1.23^c
75	211^d	77.6^a	79.5^a	28.1^{ef}	3.77^b	301^b	1.43^b
100	202^e	78.1^a	80.7^a	27.4^f	3.99^a	333^a	1.65^a
SEM	1.83	0.90	0.57	0.53	0.04	7.44	0.04

Means bearing different superscripts in a column differ significantly (P<0.01); RGM: rice gluten meal; MGM: maize gluten meal; GV: gas produced at 24 h of incubation; IVDMD: *in vitro* dry matter digestibility; IVOMD: *in vitro* organic matter digestibility; CH_4: methane; PF: partitioning factor; MBP: microbial biomass production

Table 3. ***In vitro*** **rumen fermentation characteristics of various concentrate mixtures**

Treatment	pH	NH_3-N (mg/dl)	Volatile fatty acid (%)			
			Total	Acetic acid	Propionic acid	Butyric acid
Control	6.53[a]	24.9[a]	92.1[a]	65.3[b]	23.2[cd]	11.5[a]
Level of RGM (%)						
25	6.53[a]	23.9[bc]	91.1[ab]	64.9[bc]	23.7[bcd]	11.5[a]
50	6.60[a]	22.8[d]	90.7[ab]	64.4[bc]	24.2[abc]	11.4[a]
75	6.60[a]	21.8[f]	90.3[ab]	64.3[bc]	24.4[ab]	11.3[a]
100	6.56[a]	20.8[g]	89.5[b]	63.8[c]	25.0[a]	11.2[a]
Level of MGM (%)						
25	6.63[a]	24.2[b]	83.7[c]	66.5[a]	23.9[bcd]	9.75[b]
50	6.63[a]	23.2[cd]	81.3[d]	66.8[a]	23.5[bcd]	9.66[b]
75	6.53[a]	22.6[de]	78.8[e]	67.3[a]	23.2[cd]	9.48[b]
100	6.63[a]	21.9[ef]	76.9[e]	67.4[a]	23.2[d]	9.41[b]
SEM	0.04	0.19	0.59	0.33	0.29	0.20

Means bearing different superscripts in a column differ significantly (P<0.01); RGM: rice gluten meal; MGM: maize gluten meal; NH_3-N: ammonia nitrogen

of *in vitro* GV and associated parameters in the concentrate mixtures. These results also deduce that RGM-based concentrate mixtures performed in between GNC- and MGM-based concentrate mixtures.

The pH of fermentation fluid did not vary; however, NH_3-N and TVFA were found maximum (P<0.01) in control and minimum (P<0.01) at 100% MGM. Moreover, the molar percentage of acetic acid was highest (P<0.01) at 100% MGM but lowest (P<0.01) with 100% RGM diet, whereas the opposite results were true for propionate and butyric acid (Table 3). No alteration in pH indicates absence of any deleterious effect on rumen fermentation. The protein degradability in rumen has been known to positively correlate with NH_3-N (Pal *et al.,* 2015) as well as GV, and IVOMD with TVFA (Getachew *et al.,* 2004, Mahesh and Mohini, 2015; Pal *et al.,* 2015) which justifies the pattern observed in the present study.

CONCLUSIONS

Based on the results of this *in vitro* study, 50-75% substitution level of GNC by RGM and a similar level of MGM can be recommended in the concentrate mixture for feeding ruminant animals.

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