

Nutritional elucidation of rice- and maize gluten meal-based diets: *in vitro* gas production, digestibility, methane and rumen fermentation

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Abstract: This experiment intends to elucidate the nutritional potential of alternative ingredient – rice gluten meal (RGM) with groundnut cake (GNC) and maize gluten meal (MGM) at incremental levels in the complete diets under *in vitro* system. Nine complete diets were formulated using concentrate mixture, green maize and wheat straw in the ratio of 40:40:20 on a dry basis. The dietary treatments differed in the source of major protein sources used in three concentrate mixtures, i.e., GNC in control, while incremental levels of RGM and MGM – both at 25, 50, 75 and 100% substituted GNC on an isonitrogenous basis. These diets were characterised for chemical composition, protein fractions, *in vitro* gas production profile as well as rumen fermentation attributes. The chemical composition of diets showed a mean crude protein (CP) and total digestible nutrients values of 12.9 and 63.6%, respectively. The range of values obtained for various CNCPS protein fractions (% CP) was 11.3-12.4, 9.42-14.0, 40.9-44.4, 19.1-20.1 and 13.6-14.9 for P_A, P_{B1}, P_{B2}, P_{B3} and P_C, respectively across the diets. Furthermore, rumen undegradable protein was maximum at 100% MGM inclusion and least for the control. However, intestinal protein digestibility did not differ due to treatments. While the GV₂₄ was maximum (P<0.01) in the control, it was lowest at 100% MGM. Additionally, *in vitro* dry matter and organic matter digestibilities were higher (P<0.01) for 100% MGM diet and lowest (P<0.01) at 100% RGM level.

Moreover, the control diet generated maximum (P<0.01) CH₄ but was minimum (P<0.01) for 100% MGM diet. In addition, values of PF, MBP and MBP:GV₂₄ were highest (P<0.01) for 100% MGM and lowest (P<0.01) for control diet. The pH of rumen fluid did not differ; however, concentrations of ammonia nitrogen and total volatile fatty acids were recorded to be maximum (P<0.01) in control and minimum (P<0.01) at 100% MGM. Further, the molar concentration of acetic acid was higher (P<0.01) with MGM-based diets than that of control and RGM diets. Lastly, the proportions of both propionic and butyric acids were minimum with MGM-based diets than with either control or RGM-based diets. Therefore, it could be recommended that RGM could substitute 50-75% of GNC, corresponding to 5-8% inclusion in the complete diet of ruminants. In addition, MGM-based diets excelled in terms of greater digestibility, reduced CH₄ emission as well as beneficially influenced rumen fermentation.

Keywords: Alternative ingredients, *In vitro* fermentation, Maize gluten meal, Protein fractions, Rice gluten meal, Rumen

Introduction

The world is facing the formidable challenge of feeding an ever-increasing human populace, which is projected to reach 9.7 billion by 2070, with the finite available resources (Adam, 2021). Simultaneously, the developing countries are poised to have a greater exigency for livestock-source foods including dairy (OECD/FAO 2021). Under Indian scenario, there is an existing disequilibrium between demand and the availability of feed resources for dairy production. On top of that, driven by commodity inflation in the recent past, most of the traditional ingredients are becoming expensive at farms. Hence, to circumvent the dairy sector incurring economic loss due to high input (feed) costs, there has been a burgeoning research impetus on alternative feed ingredients in ration formulations (Manpreet et al. 2022; Singh et al. 2022). Moreover, alternative ingredients with poor human-edible value may further contribute to sustainable animal diets (Singh et al. 2022). In addition, dietary strategies addressing the reduction of enteric methane (CH₄, a potent greenhouse gas) would be desirable from the viewpoint of cleaner ruminant production since about 40% of global agricultural CH₄

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emission is directly linked with the livestock sector (Pal et al. 2015).

With the increased wet milling of rice, rice gluten meal (RGM) has become available in India as a by-product thereof. Due to its rich crude protein (CP) of approximately 45%, it was investigated recently as the protein alternative for conventional oilseed meals in cattle (Kumar et al. 2016; Malik et al. 2017), buffalo (Mahesh and Thakur 2018) as well as monogastrics (Wani et al. 2021). Whereas, maize gluten meal (MGM) is a similar co-product of corn wet milling with CP value of >60% has been used conventionally in animal feeding (Heuzé et al. 2018). In our previous *in vitro* experiment, it was observed that concentrate mixtures containing 50-75% substitution of groundnut cake (GNC) by RGM and MGM could be possible without affecting digestibility and rumen fermentation. Recently, Manpreet et al. (2022) concluded from an *in vitro* study that soya bean meal (SBM) protein could be completely (up to 100%) replaced by RGM in the concentrate mixture for ruminants. Nonetheless, it becomes imperative to know the effect of inclusion of any dietary treatment in the complete diet of ruminants to gauge its practical utility, as ruminant diets are typically a combination of forages and concentrates at farm conditions. Therefore, in the present experiment, our objective was to evaluate the incremental inclusions of RGM and MGM, isonitrogenously-substituting GNC in the diet on protein fractions, gas production and associated parameters, methane (CH₄), digestibility and rumen fermentation attributes under *in vitro* conditions.

Materials and Methods

Dietary treatments

Nine complete diets were formulated using concentrate mixture, green maize (*Zea mays*) and wheat (*Triticum aestivum*) straw in the ratio of 40:40:20 on a dry basis. The concentrate mixtures were prepared in mash form, while chopped green and dry forages were oven-dried and ground prior to constituting test diets in the laboratory. The dietary treatments differed in the source of major protein ingredients used in three types of concentrate mixtures, i.e., GNC in control, while incremental levels of RGM and MGM – both at 25, 50, 75 and 100% substituted GNC on an isonitrogenous basis. On a physical basis, the range of inclusion of RGM and MGM across various diets was 2.8-11% and 2-8%, respectively (Table 1).

Compositional characterisation of diets

All the diets were analysed for chemical composition like dry matter (DM), organic matter (OM), crude protein (CP; Kjeldahl N × 6.25) and ether extract (EE) as per standard procedures of AOAC (2005). Cell wall fibre fractions like neutral detergent fibre (NDF), acid detergent fibre (ADF) as well as 72% (w/w) H₂SO₄ soluble lignin were estimated by the methods outlined by Van Soest et al.

(1991). Heat-stable α-amylase (A-3306, Sigma-Aldrich, USA) was used for NDF estimation, and the values of both NDF and ADF were expressed inclusive of residual ash.

Feed protein fractionation (P_A, P_{B1}, P_{B2}, P_{B3} and C) was performed in accordance with the Cornell Net Carbohydrate and Protein System as delineated by Sniffen et al. (1992) (Table 2). Furthermore, feed protein resistant to commercial broad-spectrum protease of *Streptomyces griseus* (type XIV, Sigma P-5147, St. Louis, MO, USA) was regarded as rumen undegraded protein (RUP; Krishnamoorthy et al. 1983), and subsequent to its pepsin-pancreatin digestion, intestinal protein digestion (IPD) was assayed (Calsamiglia and Stern, 1995).

In vitro Hohenheim gas test

A 24 h *in vitro* gas production profile (GV₂₄) of various treatment diets was carried out as per Menke and Steingass (1988). About 200 mg of air-equilibrated substrates of various diets were taken in the glass syringes in triplicate and mixed with the buffered rumen liquor of buffalo steers. These donor animals were fed on maintenance ration with seasonally available forages and concentrate mixture. The DM and OM digestibilities were determined in accordance with Goering and Van Soest (1970) by treating the whole syringe contents with neutral detergent solution (Van Soest et al. 1991). Other parameters such as gas chromatographic measurement of CH₄, partitioning factor (PF; Blümmel et al. 1997), microbial biomass production (MBP) as well as MBP:GV₂₄ were calculated as detailed in our previous experiment (Mahesh et al. 2021). Furthermore, rumen fermentation variables, namely pH, ammonia nitrogen (NH₃-N) and volatile fatty acids (VFA) were determined by the standard procedures as described in Mahesh and Mohini (2015).

Statistical analysis

The data generated in the experiment were presented as means along with a pooled standard error of means. Statistical analysis was performed using one-way ANOVA utilising software package of SAS (2012).

Results and Discussion

Chemical composition and protein fractions

The control diet had 12% GNC, whilst the levels of RGM and MGM across diets were in the range of 2.8-11% and 2-8%, respectively (Table 1). Since all the diets were isonitrogenous and MGM had a greater CP (65.2%; Mahesh et al. 2017), its inclusion was relatively less compared with GNC and RGM diets. The chemical composition of diets showed a mean CP and calculated total digestible nutrients values of 12.9 and 63.6%, respectively (Table 3). This nutrient profile is adequate to support the milk production of cows yielding ~ 12-15 kg/d (ICAR, 2013) enabling the results to be applicable for *in vivo* conditions. The

range of values obtained for various CNCPS protein fractions (% CP) was 11.3-12.4, 9.42-14.0, 40.9-44.4, 19.1-20.1 and 13.6-14.9 for P_A, P_{B1}, P_{B2}, P_{B3} and P_C, respectively across diets. It is apparent that since the protein of GNC is highly rumen degradable in nature (NASEM 2021), the corresponding value of P_A was greatest in the control. By contrast, fractions P_{B2} and P_{B3} were greater in diets containing RGM and MGM, which are reported to be less rumen degradable (Mahesh et al. 2017; Mahesh and Thakur 2018).

Furthermore, this has been justified by the actual RUP values obtained in the present study, showing maximum (P<0.01) at 100% MGM inclusion and least (P<0.01) for the control. However, IPD did not differ due to treatments; the value averaged 75.0%. The three major test protein feeds employed in the study, i.e., GNC, RGM and MGM contained 43.1, 47.5 and 65.2% CP, respectively (Mahesh et al. 2017). It could be deduced that the variation in the compositional parameters such as EE, fibre fractions, CHO and NFC was a function of inherent characteristics of the test

Table 1 Ingredient composition of complete diets (% DM) used in the experiment

Component	Control	Level of RGM (%)				Level of MGM (%)			
		25	50	75	100	25	50	75	100
Forage									
Green maize	40	40	40	40	40	40	40	40	40
Wheat straw	20	20	20	20	20	20	20	20	20
Concentrate mixture									
Maize	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
Groundnut cake	12	8.4	6.0	3.2	-	9.0	6.0	3.6	-
Rice gluten meal	-	2.8	5.5	8.2	11	-	-	-	-
Maize gluten meal	-	-	-	-	-	2.0	4.0	6.0	8.0
De-oiled rice bran	7.2	8.0	7.6	7.6	8.0	8.0	9.0	9.4	11
Wheat bran	6.4	6.4	6.5	6.6	6.6	6.6	6.6	6.6	6.6
Mineral mixture ^a	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Salt (as NaCl)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4

^aBureau of Indian Standards-compliant type-II mixture comprising of macrominerals, trace minerals and vitamins

DM: dry matter; RGM: rice gluten meal; MGM: maize gluten meal

Table 2 CNCPS protein fractionation scheme employed for diet characterization

Fraction	Constituent	Degradation pattern	Calculation
P _A	NPN compounds: ammonia, amino acids, nitrates and peptides	Instantaneously degradable in the rumen and none reaches intestine	$P_A = \text{NPN (\% SP)} \times 0.01 \times \text{SP (\% CP)}$
P _{B1}	Globulins and some albumins	Rapidly degradable in the rumen and completely digestible at intestine	$P_{B1} = \text{SP (\% CP)} - P_A (\% \text{ CP})$
P _{B2}	Most albumins and glutelins	Intermediately degradable in the rumen and completely digestible at intestine	$P_{B2} = 100 - P_A (\% \text{ CP}) - P_{B1} (\% \text{ CP}) - P_{B3} (\% \text{ CP}) - P_C (\% \text{ CP})$
P _{B3}	Prolamins, cell wall (extension) proteins and denatured proteins	Slowly degradable in the rumen	$P_{B3} = \text{NDICP (\% CP)} - \text{ADICP (\% CP)}$
P _C	Maillard products, N bound to lignin/tannins	Undegradable in the rumen and unavailable at intestine	$P_C = \text{ADICP (\% CP)}$

Source: Sniffen et al. (1992) and Licitra et al. (1996)

CNCPS: Cornell net carbohydrate and protein system; CP: crude protein (N×6.25); NPN: non-protein nitrogen, determined as the difference between total crude protein and trichloroacetic acid-precipitable crude protein; SP: borate-phosphate buffer soluble protein; NDICP: neutral detergent-insoluble crude protein; ADICP: acid detergent-insoluble crude protein

Table 3 Chemical composition and protein fractions of various dietary treatments

	Control	Level of RGM (%)				Level of MGM (%)				SEM
		25	50	75	100	25	50	75	100	
Chemical composition (% DM)										
CP	12.9	12.8	12.9	12.9	12.9	12.9	12.9	12.9	12.9	-
EE	3.07	2.89	2.78	2.65	2.49	2.96	2.84	2.76	2.61	-
Ash	8.22	8.27	8.24	8.23	8.26	8.21	8.21	8.17	8.21	-
NDF	51.2	51.7	52.1	52.5	53.0	51.1	51.0	50.9	50.8	-
ADF	33.1	33.1	33.2	33.2	33.2	32.9	32.8	32.7	32.5	-
Hemicellulose ^a	18.1	18.6	18.9	19.3	19.8	18.2	18.2	18.2	18.3	-
Cellulose ^b	27.2	27.2	27.4	27.4	27.5	27.1	27.1	27.1	27.0	-
ADL	5.95	5.88	5.82	5.76	5.70	5.85	5.75	5.65	5.54	-
CHO ^c	74.8	75.1	75.0	75.0	75.2	74.8	74.7	74.5	74.6	-
NFC ^d	23.6	23.4	22.9	22.5	22.2	23.7	23.7	23.6	23.8	-
TDN ^e	63.5	63.5	63.4	63.4	63.4	63.7	63.8	63.9	64.1	-
Protein fraction ^f (% CP)										
P _A	12.4	12.3	12.3	12.3	12.2	12.1	11.8	11.6	11.3	-
P _{B1}	14.0	12.5	11.4	10.2	8.84	12.8	11.7	10.7	9.42	-
P _{B2}	40.9	41.6	42.3	42.9	43.7	41.8	42.7	43.5	44.4	-
P _{B3}	19.1	19.3	19.2	19.2	19.4	19.3	19.6	19.7	20.1	-
P _C or ADICP	13.6	14.3	14.7	15.4	15.9	13.9	14.2	14.5	14.9	-
RUP (% CP)	36.4 ^d	37.8 ^{cd}	38.9 ^{bc}	40.1 ^{ab}	41.5 ^a	37.7 ^{cd}	39.1 ^{bc}	40.2 ^{ab}	41.8 ^a	0.60
IPD (% RUP)	75.4	75.2	75.1	75.0	74.8	75.2	75.0	74.9	74.6	0.41

^aNDF-ADF; ^bADF-ADL; ^c100-CP-EE-ash; ^dCHO-NDF; ^e92-0.86×ADF (Owens et al. 2010); ^fExpressed as rounded percentage, and hence cumulative fractions (P_A to P_C) may not add exactly to 100

Means bearing different superscripts in a row differ significantly (P<0.01)

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; NFC: non-fibrous carbohydrates; TDN: total digestible nutrients; RUP: rumen undegradable protein; IPD: intestinal protein digestibility

Table 4 *In vitro* gas production and associated parameters of various dietary treatments

Treatment	GV ₂₄ (mL/g)	IVDMD (%)	IVOMD (%)	CH ₄ (mL/g)	PF	MBP (mg)	MBP:GV ₂₄
Control	178 ^a	65.6 ^{cd}	67.0 ^c	52.8 ^a	3.77 ^c	279 ^d	1.57 ^c
Level of RGM (%)							
25	172 ^b	65.4 ^{cd}	67.0 ^c	52.4 ^a	3.90 ^{cd}	293 ^{cd}	1.70 ^{cd}
50	169 ^{bc}	64.5 ^d	66.1 ^c	51.3 ^{ab}	3.90 ^{de}	289 ^d	1.70 ^{de}
75	163 ^{def}	63.2 ^e	64.8 ^d	49.3 ^{bc}	3.98 ^{cd}	289 ^{cd}	1.78 ^{cd}
100	159 ^{ef}	62.5 ^e	64.0 ^d	48.3 ^c	4.03 ^{cd}	290 ^{cd}	1.83 ^{cd}
Level of MGM (%)							
25	166 ^{cd}	66.0 ^{bc}	67.3 ^{bc}	51.1 ^{ab}	4.05 ^c	307 ^c	1.85 ^c
50	163 ^{de}	66.9 ^{ab}	68.6 ^{ab}	50.5 ^{ab}	4.20 ^b	327 ^b	2.00 ^b
75	159 ^f	67.1 ^{ab}	68.9 ^a	49.6 ^{ab}	4.34 ^b	339 ^b	2.14 ^b
100	150 ^f	67.9 ^a	69.4 ^a	48.2 ^c	4.63 ^a	364 ^a	2.43 ^a
SEM	1.28	0.37	0.38	0.67	0.04	5.19	0.04

Means bearing different superscripts in a column differ significantly (P<0.01)

RGM: rice gluten meal; MGM: maize gluten meal; GV₂₄: gas produced after 24 h of incubation

IVDMD: *in vitro* dry matter digestibility; IVOMD: *in vitro* organic matter digestibility; CH₄: methane; PF: partitioning factor; MBP: microbial biomass production

ingredients since the forage level remained constant. Such a trend has been noted previously for concentrate mixtures prepared with the incremental levels of these ingredients (Mahesh et al. 2021).

In vitro gas production and associated parameters

While the GV_{24} was maximum ($P<0.01$) in the control diet, it was gradually decreased ($P<0.01$) as the level of RGM and MGM increased, and found lowest at 100% MGM (Table 4). *In vitro* digestibilities of DM and OM were higher ($P<0.01$) for 100% MGM diet and lowest ($P<0.01$) at 100% RGM level. Furthermore, the control diet produced the most ($P<0.01$) CH_4 , whereas the 100% MGM diet produced the least ($P<0.01$). In addition, values of PF, MBP and MBP:GV₂₄ were highest ($P<0.01$) for 100% MGM and lowest ($P<0.01$) for control diet (Table 4).

The gas production in the rumen is a reflection of the fermentability of feed substrates. A relatively greater GV_{24} obtained for GNC-based control depicts its rapid rate of fermentation compared with RGM- and MGM-based diets. This is in general agreement with the previous reports for RGM (Kumar et al. 2016) and MGM (Lamba et al. 2016). Although GV_{24} reflects OM digestion and thus energetic feed value (Menke and Steingass 1988; Pal et al. 2015), diets containing MGM showed maximum digestibility despite producing low GV_{24} , as has already been reported by earlier researchers (Lamba et al. 2016; Prusty et al. 2017; Mahesh et al. 2021).

Ruminal production of CH_4 is a result of the fermentative digestion of complex feed nutrients. Previously, when a range of protein ingredients were evaluated, it was evident that MGM produced low GV_{24} as well as CH_4 (Lamba et al. 2016; Prusty et al. 2017) as seen with MGM-based diets in the present study. Additionally, our data on RGM is consistent with that of Manpreet et al. (2022),

who noticed a lower *in vitro* CH_4 formation when RGM was substituted for 100% of SBM in the concentrate mixture.

Being an index of the efficiency of microbial protein synthesis, the values for PF across various diets fall closely within the theoretical range of 2.74-4.41 (Blümmel et al. 1997). Moreover, it is reasonable that owing to a greater digestibility per unit of GV_{24} , diets based on MGM yielded greater values for MBP:GV₂₄.

In general, the different *in vitro* variables tested in the current experiment are in line with previous studies that used a 60:40 ratio of forage:concentrate as substrates and evaluated using an *in vitro* gas technique (Getachew et al. 2004; Patra et al. 2006; Goswami et al. 2012; Kumar et al. 2013).

Rumen fermentation characteristics

There was no difference in the pH of the clarified rumen fluid across various diets (Table 5). However, concentrations of NH_3-N and total VFA were recorded to be maximum ($P<0.01$) in control and minimum ($P<0.01$) at 100% MGM. The fractionation of VFA revealed that the molar concentration of acetic acid was higher ($P<0.01$) with MGM-based diets than that of control and RGM diets. The proportions of both propionic and butyric acids were minimum with MGM-based diets than with either control or RGM-based diets (Table 5).

The pH is critical for optimum fibre digestion by rumen microbial consortia and all the nine diets recorded pH within the physiological range of 6.7-6.9. A drastic deviation of pH was not expected since the diets evaluated contained 60% of forage that is sufficient to maintain buffering, and the treatments differed only in the type of protein sources rather than energy. It appears that protein degradation in the rumen has influenced NH_3-N , whose concentration was lower with diets having relatively high

Table 5 *In vitro* rumen fermentation characteristics of various dietary treatments

Treatment	pH	NH_3-N (mg/dL)		Volatile fatty acid (mol/100 mol)		
				Total	Acetic acid	Propionic acid
Control	6.53	17.0 ^a	94.4 ^a	70.3 ^b	19.4 ^c	10.3 ^a
Level of RGM (%)						
25	6.56	16.3 ^{bc}	93.2 ^{ab}	69.8 ^{bc}	19.9 ^{bc}	10.3 ^a
50	6.53	15.5 ^d	92.7 ^{ab}	69.3 ^{bc}	20.4 ^{ab}	10.3 ^a
75	6.60	14.8 ^f	92.3 ^{ab}	69.3 ^{bc}	20.6 ^{ab}	10.2 ^a
100	6.56	14.1 ^g	91.4 ^b	68.8 ^c	21.1 ^a	10.1 ^a
Level of MGM (%)						
25	6.53	16.5 ^b	85.7 ^c	71.3 ^a	20.0 ^{bc}	8.67 ^b
50	6.50	15.8 ^{cd}	83.5 ^d	71.6 ^a	19.7 ^{bc}	8.74 ^b
75	6.53	15.4 ^{de}	81.1 ^e	72.1 ^a	19.5 ^c	8.49 ^b
100	6.56	14.9 ^{ef}	79.1 ^e	72.2 ^a	19.4 ^c	8.43 ^b
SEM	0.04	0.13	0.58	0.30	0.26	0.18

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

RUP (i.e., 100% MGM-diets). A greater concentration of total VFA in the control diet could be explained by the fact that GV_{24} and VFA production are highly positively correlated (Getachew et al. 2004; Singh et al. 2010). Indeed, the pattern of individual VFA produced in rumen could be closely related to the type of substrate being fermented. It is well established that lower propionic and butyric acids are associated with a low GV_{24} (Singh et al. 2010), which reaffirms the minimum GV_{24} exhibited by the MGM-based diets.

The overall results of the experiment infer that most of the studied parameters corroborated with the earlier report on concentrate mixtures formulated with graded levels (0, 25, 50, 75 and 100%) of RGM and MGM (Mahesh et al. 2021). As a result, forage components of diets, such as green maize and wheat straw, appeared to have a minimal associative effect on the studied response variables. However, this warrants *in vivo* substantiation through feeding trials.

Conclusions

The *in vitro* evaluation of diets containing incremental levels of gluten meals in this study revealed that RGM could be recommended at 50-75% substitution of GNC, corresponding to 5-8% inclusion in the complete diet of ruminants. In addition, MGM-based diets excelled in terms of greater digestibility, reduced CH_4 emission as well as beneficially influencing rumen fermentation.

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