



# *In vitro* Evaluation and Methane Production Potential of Rice Gluten Meal based Concentrate Feeds in Buffalo Inoculum

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## ABSTRACT

**Background:** There is shortage of conventional feedstuffs to feed livestock in the country. Thus, this study was intended to evaluate the chemical composition and *in vitro* nutrient utilization of concentrate feeds containing graded levels of rice gluten meal (RGM).

**Methods:** Five concentrate feeds containing graded levels of RGM at 0, 6.25, 12.50, 18.75 and 25% replacing soybean meal @ 0, 25, 50, 75 and 100% on w/w basis were prepared and designated as conc-1, conc-2, conc-3, conc-4 and conc-5. *In vitro* evaluation of feeds was carried out by *in vitro* gas production technique. Data were analysed by one way ANOVA using SPSS (2012) version 21.

**Result:** The total ash, acid detergent fibre (ADF) and acid detergent lignin (ADL) content decreased while organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), hemicellulose, acid detergent insoluble crude protein (ADICP), neutral detergent insoluble crude protein (NDICP) and total carbohydrate (TCHO) content increased with increase in the inclusion of RGM in the concentrate feeds. The OM digestibility was lower ( $P < 0.05$ ) in concentrate feeds 3, 4 and 5 than concentrate feed 1 and 2. Microbial mass production (MMP), efficiency of MMP and ME availability were not affected by including RGM in feeds.  $\text{NH}_3\text{-N}$  showed a declining trend with increasing level of RGM in concentrate feeds. The relative proportion of acetate decreased ( $P < 0.05$ ) while that of propionate increased ( $P < 0.05$ ) with increasing level of RGM in the concentrate feeds. Methane production varied non-significantly among the concentrate feeds. The fermentation efficiency was higher ( $P < 0.05$ ) in concentrate 4 and 5 than other concentrates.

**Key words:** *In vitro* evaluation, Methane, Rice gluten meal, Volatile fatty acids.

## INTRODUCTION

Livestock is known to have the most appreciable role in income and employment generation. Agriculture and livestock production are internally connected and reliant on each other. Both of these are extremely important as a whole for food security. India is a developing country in which effective exploitation of previously existing feedstuffs is required as agricultural areas are decreasing day by day. Effective method to serve nutrition to livestock along with economic benefits is the utilization of agro-industrial byproducts. The rice production in whole world was 503.9 million tonnes out of which India contributed about 169.5 million tonnes (FAO, 2018). About 92% of total rice production is used for human food and about 8% is used for livestock and poultry feed in the form of rice bran, deoiled rice bran, rice polish and broken rice. Nowadays, certain newer rice by-products are available in appreciable quantities and cheaper price that can be utilized as protein sources from rice processing industries such as rice gluten meal.

Rice gluten meal (RGM) is a byproduct of wet milling of rice obtained during starch extraction and syrup preparation. It is prepared by centrifuging, filtering and drying of the slurry. Rice gluten meal consists of abundant amount of proteins, amino acids (especially methionine) and vitamins. It is relatively a new feedstuff having brownish coloured and coarse powdery texture. Commercial traders categorise RGM as a high crude protein and energy ingredient which is priced lower than soybean. Most of the research works were limited to only corn gluten meal. As there is shortage

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of conventional feedstuffs for livestock in the country and the cost of conventional ingredients is also high. Thus, the present study was conducted to explore the chemical composition and *in vitro* nutritional worth of concentrate mixtures containing graded levels of RGM replacing conventional protein source, viz. soybean meal.

## MATERIALS AND METHODS

This study was conducted in the month of November-December, 2019 in the Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab.

### Chemical analysis

Five concentrate mixtures were prepared by mixing dried and finely grounded feed ingredients in appropriate

proportion (Table 1). Concentrate mixture 1, 2, 3, 4 and 5 contained 0, 6.25, 12.50, 18.75 and 25% RGM replacing SBM @ 0, 25, 50, 75 and 100% on w/w basis. The concentrate mixtures were analysed for proximate (AOAC, 2005) and cell wall constituents (Van Soest *et al.*, 1991).

### ***In vitro* evaluation**

Before the morning feeding, rumen fluid was collected from 4 male rumen fistulated buffaloes maintained on 2 kg conventional concentrate mixture (maize-38, mustard cake-15, SBM-15, deoiled rice bran-12, wheat bran-10, rice polish-7, mineral mixture-2, common salt-1part), 17 kg green fodder, 3 kg wheat straw and *ad lib* urea molasses mineral block (UMMB). Rumen liquor was collected in pre-warmed (39°C) thermos after straining through 4 layered muslin cloth and transferred to the laboratory. Culture medium was prepared by mixing distilled water (960 ml), micro mineral solution (0.16 ml), buffer (660 ml), macro mineral solution (330 ml), resazurine (1.6 ml) and reducing solution (50 ml) (Menke *et al.*, 1979; Menke and Steingass, 1988).

Rumen liquor was diluted with culture medium in 1:2 ratios. Medium was flushed with CO<sub>2</sub> in a water bath at 39°C until the last syringe was filled. Test feed (375 mg) filled glass syringes were dispensed with buffered rumen liquor (30 ml). Glass syringes were placed in water bath for 24 h at 39°C. Blanks and standard hay in triplicate were also run with each set of incubation. Rise in the volume of each syringe was recorded after 24 h. OM and NDF digestibility was estimated by boiling the contents of syringes with neutral detergent solution in spoutless beakers (Van Soest and Robertson, 1988). The ME value of the substrate was calculated by using the equation developed by Menke *et al.* (1979). The PF was calculated by dividing substrate truly degraded (mg) to the volume of gas (ml) produced (France *et al.*, 1993).

### **Estimation of volatile fatty acids**

Netchrom 9100 gas chromatograph (Netel, New Delhi, India) equipped with flame ionization detector was used to estimate the volatile fatty acids production (Cottyn and Boucque, 1968). The gas column (6 ft length and 1/8-inch diameter) packed with chromosorb 101 was used for the estimation of VFA. The gas flows for nitrogen, hydrogen and zero air were 15, 30 and 300 ml/min, respectively. Temperature was

250°C, 175°C and 270°C for injector oven, column oven and detector, respectively. 0.2 ml of 25% metaphosphoric acid per ml of content of *in vitro* syringe was added. It was allowed to stand for 2 h. Supernatant was collected after centrifugation @4000 rpm for 7 min for VFA estimation. Standard VFA mixture was prepared by mixing stock solutions (each of 25 mg/ml concentration) of standard VFAs and distilled water in the proportion of acetic acid 1.68 ml, propionic acid 0.48 ml, isobutyric acid 0.12 ml, butyric acid 0.24 ml, isovaleric acid 0.12 ml, valeric acid 0.12 ml and made the volume to 10 ml to obtain final concentration of acetic acid, 7.0, propionic acid, 1.62; isobutyric acid, 0.34; butyric acid 0.68; isovaleric acid 0.29 and valeric acid 0.29 mM/100 ml. The standard was stored in deep freeze until further use.

### **Estimation of methane**

Methane was estimated in GLC (Netchrom 9100) equipped with stainless steel column packed with porapak Q and flame ionization detector. Standard calibration gas (Sigma gases, New Delhi) consisted of 50% methane and 50% carbon dioxide. The gas flow rates for nitrogen, hydrogen and zero air were 30, 30 and 320 ml/min, respectively. From the head space of each syringe (containing 200 mg of test feed sample buffered with rumen liquor, incubated at 39°C for 24 h), 100 µl gas was collected by puncturing the silicon tube and injected in gas chromatograph for the estimation of methane.

### **Determination of hydrogen balance**

Hydrogen recovery (%) was estimated as  $(4M+2P+2B)/(2A+P+4B) \times 100$ , the ratio of hydrogen consumed via CH<sub>4</sub>/VFA was estimated as  $4M/(2P+2B)$ , where acetate (A), propionate (P), butyrate (B) and methane (M) production was expressed in mmol by Demeyer (1991).

### **Determination of fermentation efficiency**

This was calculated on the basis of the equation worked out by Orskov (1975) and modified by Baran and Zithan (2002).

$$FE = (0.622a + 1.092p + 1.56b) 100/(a+p+2b)$$

Where

a, p and b express the concentration (µmol) of acetic, propionic and butyric acids respectively in the total concentration of VFA produced. The final results of this equation were expressed in percentage.

**Table 1:** Ingredient composition of concentrates (parts/100 parts).

Ingredient	CONC-1 (0% RGM)	CONC-2 (25% RGM)	CONC-3 (50% RGM)	CONC-4 (75% RGM)	CONC-5 (100% RGM)
Maize	38	38	38	38	38
Soybean	25	18.75	12.5	6.25	0
Rice gluten meal	0	6.25	12.5	18.75	25
Wheat bran	15	14	14	14	14
Deoiled rice bran	11.5	13	16	20	20
Rice polish	7.5	7	4	0	0
Mineral mixture	2	2	2	2	2
Salt	1	1	1	1	1

### Determination of VFAs utilization index

This was expressed by non-glucogenic VFAs/glucogenic VFAs ratio (NGGR) according to Orskov (1975).

$$\text{NGGR} = (A + 2B + V)/(P+V)$$

Where

A, P, B and V express the concentrations (μmol) of acetic, propionic, butyric and valeric acids, respectively. Valeric acid is classified as both glucogenic and non-glucogenic VFA because its oxidation creates 1 mole of acetic acid and 1 mole of the propionic acid. Too high NGGR indicates high loss of energy in the form of gases.

### Statistical analysis

Data were analysed by one way ANOVA, as described by Snedecor and Cochran (1994), by using SPSS (2012) version 21. The differences in means were tested by Tukey's b.

## RESULTS AND DISCUSSION

### Chemical composition of concentrate mixture

As the level of RGM increased in concentrate mixture 1 to 5, OM also showed increasing trend (Table 2). The results of the present study are in agreement with those of Kumar (2015) who also reported increasing trend in OM with increased RGM content in concentrate mixtures (50% and 75% of RGM replacing groundnut cake). All the concentrates mixtures were iso-nitrogenous as the CP content of concentrate mixtures varied from 22.10% to 23.05%. The EE content in concentrate mixture 1 (control) having 0% RGM level was 4.72% while in RGM containing concentrate mixtures it varied from 4.75% to 5.14%. Total ash content decreased with increase in the inclusion of rice gluten meal level in the concentrate mixtures. Kumar (2015) also reported a decline in total ash content in concentrate mixtures with increased RGM level.

The NDF showed an increasing trend as the RGM level increased. Mahesh (2016) also reported increased NDF

concentration in concentrate mixtures having graded levels of RGM (0, 25, 50, 75 and 100%). Kumar (2015) reported increased ADF content of concentrate mixtures with increased RGM level (50% and 75%) replacing groundnut cake (GNC). Both NDICP and ADICP increased with increasing RGM level in concentrate mixtures. Concentrate 1 (control) was having 64.98% of total carbohydrate content while it ranged from 65.12% to 65.68% in RGM containing concentrate mixtures.

### *In vitro* evaluation

No significant difference was observed in NGP among the concentrate mixtures (Table 3). Kumar (2015) also reported that NGP (ml/200mg DM) in concentrate mixture 1 (0% RGM), 2 (50% RGM) and 3 (75% RGM replacing GNC) was 43.53±0.20, 43.28±0.33 and 43.15±0.22, respectively with no significant difference. Our results are contrary to those of Mahesh (2016) who reported decreased ( $P < 0.01$ ) NGP with increased RGM and maize gluten meal levels (replacing GNC) in the concentrate mixtures.

The PF varied non-significantly among concentrate feeds. Mahesh (2016), however, reported that RGM inclusion above 25% in the concentrate mixtures linearly increased PF. The partitioning factor (PF) is the ratio of organic matter degraded (mg) *in vitro* to the volume of gas (ml) produced. A higher PF means proportionally more of degraded matter is incorporated into microbial mass *i.e.* the efficiency of microbial protein synthesis is higher. The PF of ruminant diets should be in the range of 2.71- 4.4 (Blummel *et al.* 1997). The PF in the present study is within the suggested range.

The OM digestibility (%) was less ( $P < 0.05$ ) in concentrate mixture 3 (77.69), 4 (77.16) and 5 (75.00) than concentrate mixture 1 (81.48) and 2 (80.42). No significant difference was observed in OM digestibility of concentrate mixtures 3, 4 and 5. Mahesh (2016) reported decreased OM digestibility in concentrate mixtures with increased RGM inclusion (0, 25, 50, 75 and 100%). Our results are contrary

**Table 2:** Chemical composition of concentrates containing graded levels of RGM, % DM basis.

Parameter	CONC-1 (0% RGM)	CONC-2 (25% RGM)	CONC-3 (50% RGM)	CONC-4 (75% RGM)	CONC-5 (100% RGM)
OM	91.80	92.28	92.85	93.13	94.47
CP	22.10	22.40	22.70	22.90	23.05
EE	4.72	4.75	4.77	5.05	5.14
Total ash	8.20	7.73	7.15	6.88	6.13
NDF	32.40	33.80	34.70	34.80	35.40
ADF	12.10	11.45	10.80	10.30	9.20
Hemicellulose	20.30	22.35	23.90	24.50	26.20
ADL	2.05	1.86	1.60	1.45	1.00
ADICP	6.59	7.42	10.41	11.76	15.66
NDICP	9.42	11.82	13.99	17.28	18.04
TCHO	64.98	65.12	65.38	65.17	65.68

OM: Organic matter; CP: Crude protein; EE: Ether extract; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin; TCHO: Total carbohydrates; ADICP: Acid detergent insoluble crude protein; NDICP: Neutral detergent insoluble crude protein.

**Table 3:** *In vitro* utilization of concentrates containing graded levels of RGM (24 h).

Parameter	CONC-1 (0% RGM)	CONC-2 (25% RGM)	CONC-3 (50% RGM)	CONC-4 (75% RGM)	CONC-5 (100% RGM)	SEM
NGP, ml/g DM/24h	222.20	217.67	214.67	215.33	206.00	2.81
PF, mg/ml	3.37	3.41	3.38	3.34	3.42	0.04
OMD, %	81.48 <sup>b</sup>	80.42 <sup>b</sup>	77.69 <sup>a</sup>	77.16 <sup>a</sup>	75.00 <sup>a</sup>	0.61
NDFD, %	51.98 <sup>c</sup>	44.24 <sup>b</sup>	38.72 <sup>ab</sup>	38.89 <sup>ab</sup>	32.37 <sup>a</sup>	1.71
MMP, mg	97.19	98.69	93.42	91.80	94.06	2.15
EMMP, %	34.60	35.47	34.56	34.09	35.64	0.75
DMD, %	82.07 <sup>c</sup>	81.00 <sup>bc</sup>	78.49 <sup>ab</sup>	78.73 <sup>ab</sup>	76.40 <sup>a</sup>	0.55
SCFA, mmole	0.98	0.96	0.95	0.95	0.91	0.01
ME, MJ/kg DM	9.94	9.85	9.79	9.95	9.78	0.08
NH <sub>3</sub> -N, mg/dl	25.80 <sup>c</sup>	23.65 <sup>bc</sup>	22.60 <sup>abc</sup>	20.15 <sup>ab</sup>	18.75 <sup>a</sup>	0.87
Fer CO <sub>2</sub> , mmol	56.48 <sup>c</sup>	52.51 <sup>b</sup>	53.10 <sup>b</sup>	52.93 <sup>b</sup>	51.26 <sup>a</sup>	0.58
Fer CH <sub>4</sub> , mmol	28.89 <sup>d</sup>	26.94 <sup>c</sup>	27.29 <sup>c</sup>	24.72 <sup>b</sup>	23.27 <sup>a</sup>	0.67

NGP: Net gas production; PF: Partitioning factor; D: Digestibility; OM: Organic matter; NDF: Neutral detergent fibre; MMP: Microbial mass production; EMMP: Efficiency of microbial mass production; DM: Dry matter; SCFA: Short chain fatty acids; NH<sub>3</sub>-N: Ammoniacal nitrogen; Fer CO<sub>2</sub>: Fermentable carbon dioxide; Fer CH<sub>4</sub>: Fermentable methane.

Means bearing different superscripts in a row differ significantly (P<0.05).

to those of Kumar (2015) who reported non-significant difference in OM digestibility among the concentrate mixtures having GNC replaced with RGM at 50 (67.16±0.33 g/kg) and 75% (67.17±0.22 g/kg) inclusion level.

The NDF digestibility (%) was higher (P<0.05) in control concentrate mixture (51.98) followed by concentrate mixture 2 (44.24) and lower (P<0.05) in concentrate mixture 5 (32.37). Concentrate mixture 3 (38.72) and 4 (38.89) had similar NDF digestibility.

No significant difference was seen in MMP and EMMP among all the concentrate mixtures. Our results are contrary to those of Mahesh (2016) who reported linearly increased MMP in concentrate mixtures having graded levels of RGM (0, 25, 50, 75 and 100%).

The DM digestibility (%) was highest (P<0.05) in control concentrate mixture (82.07) and lowest (P<0.05) in concentrate mixture 5 (76.40). Concentrate mixtures 3 (78.49%) and 4 (78.73%) had similar DM digestibility. Mahesh (2016) also reported decrease in DM digestibility of concentrate mixtures with inclusion of RGM at 25% inclusion level or above.

The short chain fatty acids (SCFA) production and ME availability were similar among all the concentrate mixtures. The results of present study are in agreement with those of Kumar (2015) who also reported non-significant difference in ME among concentrate mixtures having different inclusion levels of RGM (50 and 75%) replacing GNC.

The ammonia nitrogen (NH<sub>3</sub>-N, mg/dl) was higher (P<0.05) in concentrate mixture 1 (25.80) and lower (P<0.05) in concentrate mixture 5 (18.75). Ammonia-N showed a declining trend with increased level of RGM in concentrate mixtures. Mahesh (2016) also observed linear decrease in NH<sub>3</sub>-N concentration when GNC was replaced by RGM and MGM in the concentrate mixtures at graded levels.

The fermentable carbon dioxide (Fer CO<sub>2</sub>, mmol) was highest (P<0.05) in concentrate mixture 1 (56.48) and lowest

(P<0.05) in concentrate mixture 5 (51.26). Concentrate mixture 2 (52.51), 3 (53.10) and 4 (52.93) had similar values of Fer CO<sub>2</sub>. The fermentable methane (Fer CH<sub>4</sub>, mmol) was also highest (P<0.05) in concentrate mixture 1 and lowest (P<0.05) in concentrate mixture 5.

#### ***In vitro* volatile fatty acids production**

The acetic acid content (mM/dl) was higher (P<0.05) in concentrate 1 (3.11), concentrate 2 (3.00) and concentrate 3 (3.11) followed by concentrate mixture 4 (2.26) and concentrate mixture 5 (1.79) (Table 4). Concentrate 5 had lowest (P<0.05) acetic acid content. The propionic acid content (mM/dl) was similar in concentrate mixture 1 (1.42) and 2 (1.46). Concentrate 5 (1.11 mM/dl) had lowest (P<0.05) propionic acid content. The propionic acid content was highest (P<0.05) in concentrate mixture 3 (1.49 mM/dl). Our results are contrary to those of Mahesh (2016) who reported non-significant difference in propionic acid production among the concentrate mixtures having different levels of RGM (0, 25, 50, 75 and 100%).

There was no significant difference in isobutyric acid content among the concentrate mixtures. The butyric acid concentration (mM/dl) was higher (P<0.05) in concentrate 1 (0.83) followed by concentrate 3 (0.71), concentrate 2 (0.66) and concentrate 4 (0.58). Concentrate 5 (0.46 mM/dl) had lowest (P<0.05) butyric acid content. The isovalerate content was lowest (P<0.05) in concentrate 1 and highest (P<0.05) in concentrates 2 and 3. Concentrate mixtures 4 and 5 had similar isovaleric acid content.

The total volatile fatty acid production (TVFA, mM/dl) was highest (P<0.05) in concentrate 1 (5.59), concentrate 2 (5.43) and concentrate 3 (5.63). The TVFA production was lowest (P<0.05) in concentrate 5 (3.64 mM/dl) followed by concentrate 4 (4.36 mM/dl). The value of acetate: propionate (A: P) ratio was higher (P<0.05) in concentrate 1 (2.19),

**Table 4:** *In vitro* volatile fatty acids production (mM/dl) in concentrates containing graded levels of RGM (24 h).

Parameter	CONC-1 (0% RGM)	CONC-2 (25% RGM)	CONC-3 (50% RGM)	CONC-4 (75% RGM)	CONC-5 (100% RGM)	SEM
Acetate	3.11 <sup>c</sup>	3.00 <sup>c</sup>	3.11 <sup>c</sup>	2.26 <sup>b</sup>	1.79 <sup>a</sup>	0.18
Propionate	1.42 <sup>bc</sup>	1.46 <sup>bc</sup>	1.49 <sup>c</sup>	1.30 <sup>b</sup>	1.11 <sup>a</sup>	0.05
Isobutyrate	0.00	0.04	0.05	0.00	0.02	0.00
Butyrate	0.83 <sup>e</sup>	0.66 <sup>c</sup>	0.71 <sup>d</sup>	0.58 <sup>b</sup>	0.46 <sup>a</sup>	0.04
Isovalerate	0.23 <sup>a</sup>	0.28 <sup>b</sup>	0.27 <sup>b</sup>	0.25 <sup>ab</sup>	0.25 <sup>ab</sup>	0.01
Valerate	0.00	0.00	0.00	0.00	0.00	0.00
TVFA	5.59 <sup>c</sup>	5.43 <sup>c</sup>	5.63 <sup>c</sup>	4.36 <sup>b</sup>	3.64 <sup>a</sup>	0.27
A:P	2.19 <sup>b</sup>	2.05 <sup>b</sup>	2.09 <sup>b</sup>	1.71 <sup>a</sup>	1.61 <sup>a</sup>	0.08
<b>Relative proportion, %</b>						
Acetate	55.62 <sup>b</sup>	55.20 <sup>b</sup>	55.29 <sup>b</sup>	51.06 <sup>a</sup>	49.18 <sup>a</sup>	0.92
Propionate	25.43 <sup>a</sup>	26.89 <sup>b</sup>	26.51 <sup>ab</sup>	29.83 <sup>c</sup>	30.64 <sup>c</sup>	0.68
Isobutyrate	0.00	0.71	0.89	0.00	0.51	0.15
Butyrate	14.87 <sup>b</sup>	12.13 <sup>a</sup>	12.55 <sup>a</sup>	13.30 <sup>a</sup>	12.67 <sup>a</sup>	0.33
Isovalerate	4.07 <sup>a</sup>	5.07 <sup>b</sup>	4.76 <sup>b</sup>	5.81 <sup>c</sup>	6.99 <sup>d</sup>	0.34
Valerate	0.00	0.00	0.00	0.00	0.00	0.00

TVFA: Total volatile fatty acids; A:P: Acetate: Propionate.

Means bearing different superscripts in a row differ significantly (P<0.05).

**Table 5:** Methane production from fermentation of concentrates containing graded levels of RGM (24 h).

Parameter	CONC-1 (0% RGM)	CONC-2 (25% RGM)	CONC-3 (50% RGM)	CONC-4 (75% RGM)	CONC-5 (100% RGM)	SEM
CH <sub>4</sub> , ml	17.90 <sup>b</sup>	16.16 <sup>ab</sup>	15.56 <sup>ab</sup>	13.62 <sup>a</sup>	16.66 <sup>ab</sup>	0.53
CH <sub>4</sub> , ml/100 mg DM	8.95 <sup>b</sup>	8.08 <sup>ab</sup>	7.78 <sup>ab</sup>	6.81 <sup>a</sup>	8.33 <sup>ab</sup>	0.26
CH <sub>4</sub> , ml/100 mg DMD	10.90	9.98	9.91	8.65	10.90	0.31
CH <sub>4</sub> , ml/100 mg OMD	10.99	10.05	10.02	8.83	11.11	0.31

Means bearing different superscripts in a row differ significantly (P<0.05).

**Table 6:** Hydrogen balance of nutrients of concentrates containing graded levels of RGM (24 h).

Parameter	CONC-1 (0% RGM)	CONC-2 (25% RGM)	CONC-3 (50% RGM)	CONC-4 (75% RGM)	CONC-5 (100% RGM)	SEM
H- recovery, %	88.16 <sup>a</sup>	89.65 <sup>a</sup>	87.96 <sup>a</sup>	101.34 <sup>b</sup>	111.85 <sup>c</sup>	3.20
H-consumed via CH <sub>4</sub>	5.33 <sup>c</sup>	4.83 <sup>c</sup>	5.05 <sup>c</sup>	4.03 <sup>b</sup>	3.24 <sup>a</sup>	0.26
FE, %	77.23 <sup>a</sup>	77.69 <sup>a</sup>	77.57 <sup>a</sup>	79.15 <sup>b</sup>	79.70 <sup>b</sup>	0.33
VFA UI	3.36	2.96	3.03	2.60	2.43	0.11

FE: Fermentation efficiency; H: Hydrogen; VFA UI: Volatile fatty acids utilization index.

Means bearing different superscripts in a row differ significantly.

concentrate 2 (2.05) and concentrate 3 (2.09) than concentrate 4 (1.71) and concentrate 5 (1.61).

The relative proportion of acetate decreased (P<0.05) whereas propionate increased (P<0.05) with increasing level of RGM in concentrate mixtures. The relative proportion of butyrate was higher (P<0.05) in concentrate 1 than other concentrates. However, Mahesh (2016) reported that relative proportion of butyrate in concentrates containing graded levels of RGM was similar. Concentrate 1 had lowest (P<0.05) and concentrate mixture 5 had highest (P<0.05) relative proportion of isovalerate. Concentrate mixture 2 and 3 showed no significant difference in isovalerate content.

### Methane production

The methane production (CH<sub>4</sub>, ml) was highest (P<0.05) in concentrate 1 (17.90) and lowest (P<0.05) in concentrate 4 (13.62) containing 75% RGM replacing SBM (Table 5). Concentrate 2 (16.16 ml), 3 (15.56 ml) and 5 (16.66 ml) had similar methane production. Mahesh (2016) reported decreased CH<sub>4</sub> production with RGM inclusion above 25% level (replacing GNC) in the concentrate mixtures. The methane production (ml/100 mg DM) also showed similar trend.

The methane production (ml/100 mg DM digested and ml/100 mg OM digested) varied non-significantly among the concentrates. Mahesh (2016) reported decreased CH<sub>4</sub> per g



of OM digested in the concentrate mixtures having RGM at above 25% level.

### Hydrogen balance

Concentrate 5 (complete replacement of SBM with RGM) had highest ( $P<0.05$ ) H- recovery followed by concentrate 4 (Table 6). The hydrogen consumed via  $\text{CH}_4$  was lowest ( $P<0.05$ ) in concentrate 5(3.24) and highest ( $P<0.05$ ) in concentrate 1(5.33), concentrate 2(4.83) and concentrate 3(5.05).

The fermentation efficiency (%) was lower ( $P<0.05$ ) in concentrate 1(77.23), concentrate 2(77.69) and concentrate 3(77.57) than concentrate 4(79.15) and concentrate 5(79.70). No significant difference was observed in the volatile fatty acids utilization index (VFA UI). VFA UI is non-glucogenic to glucogenic VFA ratio. The decrease in VFA UI correlates with the increase in the molar proportion of propionate with increasing level of RGM in the concentrate mixtures.

### CONCLUSION

It was concluded that soybean meal could be replaced with RGM upto 100% without any adverse effect on MMP, efficiency of MMP, ME availability and without increase in the methane production.

**Conflict of interest:** None.

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