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EFFECT OF REPLACEMENT OF SOYBEAN MEAL WITH RICE GLUTEN MEAL ON *in vitro* FERMENTATION CHARACTERISTICS OF TOTAL MIXED RATION

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ABSTRACT

An *in vitro* study was conducted to assess the effect of using rice gluten meal as a substitute of soybean meal (SBM) in ruminant diet. The SBM comprising 12.5% whole diet [total mixed ration (TMR)] was substituted by rice gluten meal (RGM) on w/w basis at 0, 25, 50, 75 and 100% level. *In vitro* dry matter disappearance (IVDMD), organic matter disappearance (IVOMD), net gas production, methane production and fermentation efficiency of TMRs was assessed. The chemical composition of TMRs revealed decrease in total ash, acid detergent fiber and total carbohydrate contents and increase in organic matter, crude protein, ether extract, neutral detergent fiber, acid detergent lignin, hemicellulose, neutral detergent insoluble crude protein and acid detergent insoluble crude protein contents with increase in RGM in TMRs. No significant difference in net gas production, IVDMD and IVOMD was observed between the TMR treatments having graded levels of RGM and control. The NH_3-N in TMRs declined with increase in RGM and was highest ($P<0.05$) in control. The relative proportion of propionic acid and isovaleric acid was highest in TMR 3 (50% RGM replacing SBM); whereas relative proportion of butyric acid was lowest in TMR 3. CH_4 production showed decline with increase in RGM in TMRs beyond 25% level replacing SBM. The H-recovery was lowest in control TMR. The hydrogen consumed via CH_4 as compared to the hydrogen consumed via VFA was lowest in TMR 3. The fermentation efficiency was highest in TMR 3. Thus, RGM could replace up to 100% of soybean meal in TMR of ruminants without any adverse effect on nutrient digestibility which is equivalent to 12.5% of whole dietary dry matter.

Keywords: *In vitro* digestibility, methane, rice gluten meal, ruminants, soybean meal, total mixed ration

INTRODUCTION

A variety of widely available agro-industrial byproducts serve as valuable animal feed-stuffs, because of their utilizable nutritional worth. In India, concentrate feed resources for ruminants are limited to the extent of 47% (NIANP, 2013) and concomitantly, the export of protein-rich oilseed meals in large quantity (as high as 5 million tonnes) further aggravates the nation's feed balance (Rao *et al.*, 2016). Soybean meal is used 40% towards poultry and livestock feeding and its net annual shortage during 2014-2025 is to the tune of about 2.5 million metric tonnes. As there is scarcity of soybean meal, there is need to utilize locally available alternate protein sources (Mandal, 2017). Thus, to combat the increasing feed costs and nutritional crisis, there is urgent need to search viable alternative feed resources which do not compete with human feed and are economical. In this respect, rice gluten meal (RGM) may serve as an excellent alternative for animal feeds which can substitute the expensive feed resources like soybean meal, thereby reduce the feed costs of livestock. RGM is dried residue of rice, left after the removal of starch, and separation of bran during wet milling manufacture of rice starch or glucose or syrup. It is a brown coarse powder available in appreciable quantities in northern India. RGM has good amino acid profile with comparatively higher methionine content than ground nut cake (Kumar *et al.,* 2016). Scanty information is available on the utilization of RGM in animal feeds. The present study was planned to assess the *in vitro* nutritional digestibility and methane production potential of the total mixed ration (TMR) containing graded levels of RGM.

MATERIALS AND METHODS

The present *in vitro* study was conducted in the year 2019 in the Department of Animal Nutrition, Guru Angad Dev Veterinary & Animal Sciences University (GADVASU), Ludhiana, Punjab, India.

Sample collection and preparation

Feedstuffs for concentrate preparation were procured from the Livestock Farm, GADVASU, Ludhiana (India). Soybean meal comprising of 12.5% of total mixed ration (TMR) was replaced by RGM at 0, 25, 50, 75 and 100% levels on N basis. The ingredient composition of TMRs containing the graded levels of RGM (%) replacing soybean meal on w/w basis was as under:

Ingredients	TMR 1 (control)	TMR 2 (25% RGM)	TMR 3 (50% RGM)	TMR 4 (75% RGM)	TMR 5 (100% RGM)
Bajra fodder	50.00	50.00	50.00	50.00	50.00
Maize	19.00	19.00	19.00	19.00	19.00
Soybean	12.50	9.37	6.25	3.12	0.00
Rice gluten meal	0.00	3.12	6.25	9.37	12.50
Wheat bran	7.50	7.00	7.00	7.00	7.00
Deoiled rice bran	5.75	6.50	8.00	10.00	10.00
Rice polish	3.75	3.50	2.00	0.00	0.00
Mineral mixture	1.00	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50	0.50

The roughage: concentrate ratio in TMRs was 50:50 on dry matter basis. All the TMRs contained dried and finely grounded bajra fodder as roughage.

Proximate chemical analysis

TMRs were analyzed for dry matter, Kjeldahl N, ether extract and ash content using the standard procedures (AOAC, 2007). Cell wall fractions *viz.*, neutral detergent fibre (NDF), detergent fibre (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 determined by solubilization of cellulose with 72% sulphuric acid. Acid detergent insoluble protein and neutral detergent insoluble protein were determined as per Licitra *et al.* (1996).

In vitro evaluation

Before morning feeding, the rumen fluid was collected from rumen fistulated four male buffaloes (age approximately 5 years) maintained on 2 kg conventional concentrate mixture (maize - 38 parts, mustard cake - 15 parts, SBM - 15 parts, deoiled rice bran - 12 parts, wheat bran - 10 parts, rice polish - 7 parts, mineral mixture - 2 parts and common salt - 1 part), 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. The nutritional value of various TMRs was assessed by *in vitro* gas production technique (Menke *et al*., 1979; Menke and Steingass, 1988). Two sets of samples were incubated in triplicates. In 1st set, 375 mg ground sample (dry matter basis) was incubated at 39°C for 24 h in triplicate in 100 mL calibrated glass syringes with buffered rumen fluid for assessing the net gas production, digestibility of nutrients and metabolizable energy (ME) availability. In 2nd set, 200 mg ground sample (on dry matter basis) was incubated at 39°C for 24 h in triplicate in 100 mL calibrated glass syringes with buffered rumen fluid for assessing the methane production. The 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 (Cottyn and Boucque, 1968). Supernatant was used for the estimation of individual volatile fatty acids. The ME value of substrate was calculated as per the equation of Menke *et al.* (1979). The partitioning factor (PF) was calculated by dividing the substrate truly degraded (mg) to the volume of gas (mL) produced (France *et al.,* 1993).

Estimation of methane

Methane (CH_4) was estimated in a gas liquid chromatograph (Netchrom 9100) equipped with stainless steel column packed with Porapak Q and flame ionization detector. Standard calibration gas (Sigma gases, New Delhi, India) consisted each of 50% CH_4 and CO_2. The gas flow rates for nitrogen, hydrogen and zero air were 30, 30 and 320 mL min^{-1}, respectively. From the head space of each syringe (containing 200 mg 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 into gas chromatograph for the estimation of CH_4.

Determination of hydrogen balance and fermentation efficiency

Hydrogen recovery was estimated as per Demeyer (1991) using the following equation:

$$\text{Hydrogen recovery (\%)} = \frac{4M + 2P + 2B)}{(2A + P + 4B)} \times 100$$

The ratio of hydrogen consumed via CH_4/VFA was estimated as 4M/(2P + 2B), where acetate (A), propionate (P), butyrate (B) and methane (M) production were expressed in m mol. Fermentation efficiency (FE) was calculated as per the equation of Orskov (1975) modified by Baran and Zitnan (2002).

$$FE = \frac{(0.622a + 1.092p + 1.56b)}{(a + p + 2b)} \times 100$$

Where a, p, and b are the concentration (µ mol) of acetic, propionic and butyric acids, respectively, in total concentration of VFA produced. VFAs utilization index was expressed by non-glucogenic VFAs/glucogenic VFAs ratio (NGGR) was worked out as per Orskov (1975).

Statistical analysis

The data were analyzed by using simple ANOVA as described by Snedecor and Cochran (1994) using SPSS (2012) version 21. The differences in means were tested by Tukey's b at 0.05% level of significance.

RESULTS AND DISCUSSION

Chemical composition of TMRs containing graded levels of RGM

The organic matter content in TMRs varied from 91.73 to 92.40% (Table 1). The organic matter content showed increase with the increasing level of rice gluten meal in TMRs. The crude protein

Table 1: Chemical composition of TMRs containing graded levels of RGM replacing soybean meal (% DM basis)

Chemical constituents	Treatments				
	TMR 1 (control)	TMR 2 (25% RGM)	TMR 3 (50% RGM)	TMR 4 (75% RGM)	TMR 5 (100% RGM)
Organic matter	91.73	91.83	92.13	92.25	92.40
Crude protein	14.49	14.69	15.00	15.49	15.50
Ether extract	3.33	3.45	3.48	3.77	4.10
Total ash	8.28	8.18	7.88	7.75	7.60
Neutral detergent fibre	53.70	55.40	55.00	56.00	56.40
Acid detergent fibre	26.15	25.20	24.70	24.30	23.55
Hemicellulose	27.55	30.20	30.30	31.70	32.85
Acid detergent lignin	3.75	3.80	4.10	4.95	6.40
ADICP	3.17	3.69	3.92	4.21	5.34
NDICP	7.67	9.46	10.90	12.86	14.47
Total carbohydrates (TCHO)	73.90	73.68	73.64	72.99	72.80

ADICP = Acid detergent insoluble crude protein; NDICP = Neutral detergent insoluble crude protein

content in TMRs varied from 14.49 to 15.50%. All the TMRs prepared were iso-nitrogenous. The ether extract content in TMRs ranged from 3.33 to 4.10% while NDF content varied from 53.70 to 56.40%. Both the ether extract and NDF exhibited increase with increase in the level of RGM in TMRs. The total ash content varied between 7.60 to 8.28% while ADF, hemicellulose and ADL (acid detergent lignin) contents varied from 23.55 to 26.15, 27.55 to 32.85 and 3.75 to 6.40%, respectively. From TMR 1 to TMR 5, the ADL content showed increase with increase in TMR level. The ADICP (acid detergent insoluble crude protein) and NDICP (neutral detergent insoluble crude protein) contents varied from 3.17 to 5.34 and 7.67 to 14.47%, respectively. There was increase in ADICP and NDICP values with increase in RGM in TMR. The total carbohydrate content in TMRs was 72.80 to 73.90%. Experimental TMRs had slightly less total carbohydrate content as compared to the control TMR. This could be due to less total carbohydrate content in RGM as compared to SBM.

In vitro evaluation of TMRs containing graded levels of RGM

The net gas production (NGP, mL g^{-1} DM 24 h^{-1}) in TMR 1, 2, 3, 4 and 5 containing 0, 25, 50, 75 and 100% RGM replacing SBM in the concentrate was 204.7, 208.0, 209.3, 211.3 and 208.0, respectively (Table 2). No significant difference in NGP was observed among TMR treatments. This may be due to the similar nutrient digestibility among the TMRs. Contrary to our observations, Mahesh (2016) noticed reduction in NGP in TMRs having graded levels (25 to 100%) of RGM. The partitioning factor (PF, mg mL^{-1}) was 3.47, 3.39, 3.36, 3.44 and 3.41 in TMR 1, 2, 3, 4 and 5, respectively and varied non-significantly. The PF in the present study was within the suggested range.

The organic matter digestibility of TMRs varied non-significantly from 76.3 to 78.7%. Mahesh (2016) showed decreased OM digestibility when RGM was included in TMRs at 50% level. The reason for contrary results to the supporting literature might be the difference in the chemical composition of RGM used and the difference of oilseed cakes used in the concentrate feed. The NDF digestibility of TMRs ranged from 60.2 to 65.0%. The NDF digestibility was similar among the TMRs. The microbial mass production (MMP, mg) in TMR 1 (97.60), TMR 2 (92.73), TMR 3 (90.75), TMR 4 (98.09) and TMR 5 (94.40) was almost similar. Our results are in agreement with those of Mahesh (2016) who reported non-significant difference in MMP among the TMRs having graded levels of RGM (0, 25, 50, 75 and 100%). The efficiency of microbial mass production was also similar among the TMRs. TMR 1, 2, 3, 4 and 5 had 36.6, 35.1, 34.4, 36.0 and 35.5% EMMP, respectively.

Table 2: Effect of different levels of RGM on *in vitro* gas production and digestibility of nutrients in TMRs (24 h)

Chemical constituents	Treatments					SEM
	TMR 1 (control)	TMR 2 (25% RGM)	TMR 3 (50% RGM)	TMR 4 (75% RGM)	TMR 5 (100% RGM)	
Net gas production (mL g^{-1} DM 24 h^{-1})	204.67	208.00	209.33	211.33	208.00	1.17
Partitioning factor (mg mL^{-1})	3.47	3.39	3.36	3.44	3.41	0.03
Organic matter digestibility (%)	77.47	76.77	76.26	78.75	76.77	0.38
Neutral detergent fibre digestibility (%)	61.51	61.49	60.24	65.00	61.94	0.67
Microbial mass production [MMP} (mg)	97.60	92.73	90.75	98.09	94.40	1.66
Efficiency of MMP (%)	36.63	35.08	34.44	35.96	35.49	0.48
Dry matter digestibility (%)	77.47	78.00	77.07	79.60	78.00	0.38
Short chain fatty acids (m mole)	0.90	0.92	0.93	0.93	0.92	0.01
Metabolizable energy (MJ kg^{-1} DM)	8.77	8.92	9.02	9.20	9.18	0.06
Ammoniacal nitrogen [NH_3-N] (mg dL^{-1})	27.50^{c}	25.40^{bc}	24.00^{abc}	21.20^{ab}	20.15^{a}	0.94
Fermentable CO_2 (m moles)	51.35^{b}	51.09^{b}	47.75^{a}	50.56^{b}	50.70^{b}	0.44
Fermentable CH_4 (m moles)	27.58^{b}	26.77^{b}	24.64^{a}	26.43^{b}	26.71^{b}	0.35

The roughage to concentrate ratio in TMRs was 50:50 on dry matter basis, Means bearing different superscripts in a row differ significantly ($P < 0.05$); SEM = Standard error of means

The DM digestibility of TMRs did not vary significantly and ranged from 77.1 to 79.6% with non-significant difference among TMRs. However, Mahesh (2016) reported decline in DM digestibility with inclusion of RGM at above 50% level in TMRs. The net gas production and digestibility have not been affected by the inclusion of RGM at graded levels.

The short chain fatty acids (SCFA) varied from 0.90 to 0.93 m mol among the TMRs. No significant difference was observed in SCFAs among TMRs. The metabolizable energy (ME) did not vary significantly among the TMRs. The ammonia nitrogen was highest ($P < 0.05$) in TMR 1 (27.50 mg dL^{-1}) and lowest ($P < 0.05$) in TMR 5 (20.15 mg dL^{-1}). TMR 2, TMR 3 and TMR 4 had 25.4, 24.0 and 21.2 mg dL^{-1} NH_3-N concentration, respectively. Our results are in agreement with those of Mahesh (2016) who reported a linear decline in NH_3-N in TMRs when ground nut cake was replaced with RGM. This might be due to high rumen escape potential of gluten proteins already documented by Heuze *et al.* (2015).

The fermentable CO_2 (m mol) was lowest ($P<0.05$) in TMR 3 (47.75) and similar in rest of TMR treatments (1, 2, 4 and 5). The fermentable CH_4 (m mol) was lowest ($P < 0.05$) in TMR 3 (24.6) and similar in TMR 1 (27.6), TMR 2 (26.8), TMR 4 (26.4) and TMR 5 (26.7).

Individual and total VFA production

The effect of different levels of rice gluten meal in total mixed rations on individual and total volatile fatty acids is presented in Table 3. The acetic acid (mM dL^{-1}) production was higher ($P < 0.05$) in TMR 1 (3.08) as compared to the other TMRs tested. The acetic acid production (mM dL^{-1}) was similar ($P < 0.05$) in TMR 2 (2.72), TMR 4 (2.90) and TMR 5 (2.75). The acetic acid production was lowest in TMR 3 (2.64 mM dL^{-1}). The propionic acid (mM dL^{-1}) production was higher in TMR 4 (1.44) and lower in TMR 2 (1.32). The propionic acid production (mM dL^{-1}) was similar in TMR 1 (1.42) and TMR 3 (1.42). The butyric acid (mM dL^{-1}) production was highest in TMR 1 (0.55), lowest in TMR 3 (0.37) but statistically similar in TMR 2 (0.50), TMR 4 (0.50) and TMR 5 (0.48). The isovaleric acid production varied non-significantly among TMRs tested and its value ranged from 0.220 to 0.243 mM dL^{-1}. The total VFA (mM dL^{-1}) production was highest in TMR 1 (5.30) and lowest ($P < 0.01$) in TMR 3 (4.67). The total VFA (mM dL^{-1}) production was statistically similar in TMR 2 (4.79) and TMR 5 (4.79). The acetate: propionate ratio was higher in

Table 3: Effect of different levels of RGM on *in vitro* VFA production (mM dL^{-1}) in TMRs (24 h)

Volatile fatty acid (VFA)	TMR 1 (control)	TMR 2 (25% RGM)	TMR 3 (50% RGM)	TMR 4 (75% RGM)	TMR 5 (100% RGM)	SEM
Acetate (A)	3.08^{b}	2.72^{ab}	2.64^{a}	2.90^{ab}	2.75^{ab}	0.06
Propionate (P)	1.42^{bc}	1.32^{a}	1.42^{bc}	1.44^{c}	1.34^{ab}	0.02
Isobutyrate	0.00	0.00	0.00	0.00	0.00	0.00
Butyrate	0.55^{c}	0.50^{b}	0.37^{a}	0.50^{b}	0.48^{b}	0.02
Isovalerate	0.234	0.239	0.243	0.232	0.220	0.003
Valerate	0.00	0.00	0.00	0.00	0.00	0.00
Total VFA	5.30^{c}	4.79^{ab}	4.67^{a}	5.07^{bc}	4.79^{ab}	0.08
A:P ratio	2.18^{b}	2.06^{ab}	1.86^{a}	2.01^{ab}	2.05^{ab}	0.04
Relative proportion (%)						
Acetate	58.11	56.85	56.51	57.12	57.42	0.29
Propionate	26.73^{a}	27.65^{a}	30.34^{b}	28.38^{a}	27.99^{a}	0.42
Isobutyrate	0.00	0.00	0.00	0.00	0.00	0.00
Butyrate	10.41^{b}	10.50^{b}	7.94^{a}	9.94^{b}	10.00^{b}	0.32
Isovalerate	4.42^{a}	5.00^{bc}	5.21^{c}	4.57^{ab}	4.59^{ab}	0.11
Valerate	0.00	0.00	0.00	0.00	0.00	0.00

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

TMR 1 (2.18) and lower ($P < 0.05$) in TMR 3 (1.86). The A:P ratio was similar in TMR 2 (2.06), TMR 4 (2.01) and TMR 5 (2.05). The higher ($P <0.05$) A:P ratio in TMR 1 is due to the higher ($P < 0.05$) acetic acid production in TMR 1.

The relative proportion of acetic acid varied non-significantly among TMRs. The relative proportion of propionic acid was highest in TMR 3 (30.34) and was similar in the rest of TMRs. The relative proportion of butyric acid was lower in TMR 3 (7.94) than other TMRs tested. The relative proportion of isovaleric acid was higher in TMR 3 (5.21) and lower ($P < 0.05$) in TMR 1 (4.42). TMR 2, TMR 4 and TMR 5 was having similar relative proportion (%) of isovaleric acid.

Methane production

The CH_4 production was higher ($P < 0.05$) in TMR 1 (13.52) and TMR 2 (13.97) than other TMRs evaluated (Table 5). TMR 4 (11.34 mL) and TMR 5 (11.33 mL) had similar CH_4 production. CH_4 production followed a declining trend with RGM inclusion in TMRs beyond 25% level replacing SBM. However, Mahesh (2016) reported decreased CH_4 production with RGM inclusion in TMRs at 75% or above. The results indicated that incorporation of RGM in the diet showed promising effect by decreasing the *in vitro* methane production.

Hydrogen balance

The H-recovery was lowest ($P < 0.05$) in TMR 1 (90.61%) [Table 6]. TMR 2, TMR 3 and TMR 5 had similar H-recovery. The hydrogen consumed via methane as compared to the hydrogen consumed via VFA was lowest ($P < 0.05$) in TMR 3 (3.86), highest ($P < 0.05$) in TMR 1 (4.59) and similar in TMR 2 (4.17) and TMR 5 (4.16).

Table 5: Methane production from the fermentation of TMRs containing graded levels of RGM (24 h)

Methane (CH_4)	TMR 1 (control)	TMR 2 (25% RGM)	TMR 3 (50% RGM)	TMR 4 (75% RGM)	TMR 5 (100% RGM)	SEM
CH_4, mL	13.52^{b}	13.97^{b}	12.11^{ab}	11.34^{a}	11.33^{a}	0.39
CH_4, mL 100 mg^{-1} DM	6.76^{b}	6.98^{b}	6.05^{ab}	5.67^{a}	5.66^{a}	0.20
CH_4, mL 100 mg^{-1} DMD	8.73^{b}	8.95^{b}	7.86^{ab}	7.12^{a}	7.26^{a}	0.26
CH_4 mL 100 mg^{-1} OMD	8.73^{b}	9.10^{b}	7.94^{ab}	7.20^{a}	7.38^{a}	0.26

DM = Dry matter, DMD = Dry matter digestibility, OMD = Organic matter digestibility

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

Table 6: Effect of different levels of RGM on the fermentation parameters and hydrogen balance in TMRs (24 h)

Parameters	TMR 1 (control)	TMR 2 (25% RGM)	TMR 3 (50% RGM)	TMR 4 (75% RGM)	TMR 5 (100% RGM)	SEM
Hydrogen recovery (%)	90.61[a]	96.09[b]	97.52[b]	93.07[ab]	95.99[b]	0.88
H consumed via CH_4/VFA	4.59[c]	4.17[b]	3.86[a]	4.40[bc]	4.16[b]	0.08
Fermentation efficiency (%)	77.20[a]	77.66[ab]	78.52[b]	77.84[ab]	77.68[ab]	0.16
VFA utilization index	2.95[b]	2.82[b]	2.39[a]	2.71[b]	2.77[b]	0.07

The means bearing different superscripts in a row differ significantly ($P < 0.05$)

The fermentation efficiency (%) was higher ($P < 0.05$) in TMR 3 (78.52) and lower ($P < 0.05$) in TMR 1 (77.20) than other TMRs evaluated. TMR 2, TMR 4 and TMR 5 were having similar fermentation efficiency. The VFA utilization index (VFA UI) was lowest in TMR 3 (2.39) and similar in TMR 1 (2.95), TMR 2 (2.82), TMR 4 (2.71) and TMR 5 (2.77). The non-glucogenic to glucogenic VFA ratio is associated with effects on methane production, milk composition and energy balance (Morvay *et al.*, 2011). The lowest ($P < 0.05$) VFA UI in TMR 3 correlates well with the highest ($P < 0.05$) proportion of propionate production in TMRs. Glucogenic propionate contributes to energy deposition in body tissues, whereas non-glucogenic acetate and butyrate are sources for long chain fatty acid synthesis.

Conclusion: The study revealed that soybean meal could be replaced by RGM upto 100% without affecting the nutrient digestibility and microbial biomass production. *In vitro* CH_4 production followed a declining trend with increase in the level of RGM beyond 25% replacement of SBM in concentrate mixture.

REFERENCES

AOAC. 2007. *Official Methods of Analysis* (18th edn.). Association of Official Analytical Chemists, Arlington, Virginia, USA.

Baran, M. and Zitnan, R. 2002. Effect of monensin sodium on fermentation efficiency in sheep rumen. *Archiv Tierzucht/ Archives Animal Breeding*, **45**: 181-185.

Cottyn, B.G. and Boucque, C.V. 1968. Rapid methods for the gas chromatographic determination of volatile fatty acids in rumen fluid. *Journal of Agricultural and Food Chemistry*, **16**: 105-107.

Demeyer, D.I. 1991. Quantitative aspects of microbial metabolism in the rumen and hind gut. pp. 217-237. **In**: *Rumen Microbial Metabolism and Ruminant Digestion* (ed. J.P. Journay). INRA, Paris, France.

France, J., Dhanoa, M.S., Theodorou, M.K., Lister, S.J., Davies, D.R. and Isac, D. 1993. A model to interpret gas accumulation profiles associated with *in vitro* degradation of ruminant feeds. *Journal of Theoretical Biology*, **163**(1): 99-111.

Heuze, V., Tran, G., Sauvant, D., Renaudeau, D., Lessire, M. and Lebas, F. 2015. *Corn Gluten Meal. Feedipedia*, A programme by INRA, CIRAD, AFZ and FAO. [*http://www.feedipedia.org/node/715*].

Kumar, R., Thakur, S.S. and Mahesh, M.S. 2016. Rice gluten meal as an alternative by-product feed for growing dairy calves. *Tropical Animal Health and Production*, **48**(3): 619-624.

Licitra G., Hernandez, T.M. and Van Soest P.J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology*, **57**: 347-358.

Mahesh, M.S. 2016. *Evaluation of Rice and Maize Gluten Meals as Protein Sources for Lactating Buffaloes*. Ph.D. dissertation. National Dairy Research Institute, Karnal, Haryana, India.

Mandal, A.B. 2017. *Challenges of Feed Industries for Sustainable Poultry Production.* IPSACON, Lead paper and Souvenir. [*http://krishi.icar.gov.in/jspui/handle/123456789/18200*].

Menke, K.H. and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Resource Development*, **28**: 7-55.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D. and Scheinder, W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feed stuffs from the gas production when they are incubated with rumen liquor in vitro. *Journal of Agriculture Science Cambridge*, **92**: 217-222.

Morvay, Y., Bannink, A., France, J., Kebreab, E. and Dijkstra, J. 2011. Evaluation of models to predict the stoichiometry of volatile fatty acid profiles in rumen fluid of lactating Holstein cows. *Journal of Dairy Science*, **94**(6): 3063-3080.

NIANP. 2013. *Vision 2050*. ICAR-National Institute of Animal Physiology and Animal Nutrition. Bengaluru, Karnataka, India.

Orskov, E.R. 1975. Manipulation of rumen fermentation for maximum food utilization. *World Review of Nutrition and Dietetics*, **22**: 152-182.

Rao, S.B.N., Jash, S., Dineshkumar, D., Krishnamoorthy, P., Elangovan, A.V., Sivaram, M., Parthipan, S. and Selvaraju, S. 2016. Influence of detoxified neem seed cake on diet digestibility, body weight change, hormonal profiles, immune response and testicular gene expression in male sheep. *Animal Feed Science and Technology*, **211**: 41-49.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods (*11th edn.). The Iowa State University Press, Ames, USA.

SPSS. 2012. *Statistical Package for Windows*. Chicago, IL, USA.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science,* **74**: 3583-3597.