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Impact of Dietary Input of Rice Based Concentrate Rations on *In Vitro* Rumen Fermentation Characteristics in Goats

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ABSTRACT

In Tamil Nadu, a southern state of India, it is a common practice to feed rice to goats reared especially in urban and semi urban areas. Hence, a study was conducted to document the *in vitro* rumen fermentation characteristics in goats, in rations with high levels of rice based concentrate. The study tested four roughage to concentrate ratios *viz.,* 45: 55, 40: 60, 35: 65 and 30: 70 through *in vitro* gas production technique in six runs, using goat rumen liquor at different incubation hours (9, 12, 18 and 24). The concentrate component of the ration comprised of a blend of broken rice (70%) and de-oiled rice bran (30%). Significantly ($P < 0.05$) lowest *in vitro* ruminal pH for all incubation hours was observed in roughage to concentrate ratio of 30:70 and significantly ($P<0.05$) highest *in vitro* ruminal pH for all incubation hours was observed in roughage to concentrate ratio of 45:55. Increasing the concentrate caused linear increase in the *in vitro* gas production, i*n vitro* apparent dry matter degradability and total volatile fatty acids for all incubation hours in a significant ($P < 0.05$) manner. The percentage of acetate and propionate production significantly ($P<0.05$) declined and increased respectively with increase in concentrate. Significantly ($P<0.05$) highest energetic efficiency was observed in 30:70 roughage to concentrate ratio at all incubation hours.

KEY WORDS: Rice based concentrate, Roughage to concentrate ratio, Rumen fermentation, volatile fatty acid

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INTRODUCTION

Goats play an important role in the food and nutritional security of millions of landless, marginal and small farmers in India. They produce a variety of products, mainly meat, milk, skin, fibre and manure which contribute to the economy of the farmers. Goats depend mainly on natural pasture and crop residue, to meet their nutritional needs. However, an increase in human population has resulted in a decrease in grazing land and availability of fodders. It is often necessary to feed concentrates to provide the nutrients that are deficient in a roughage only diet, especially in the case of lactating females and kids with the potential for rapid growth. In certain situations, concentrates are a more economical source of nutrients than roughages. Moreover, in the intensive management systems, it is a common strategy to feed large amounts of grains due to the lack of quality forage. In Tamil Nadu, a southern state of India, it is a common practice to feed rice to live stock because the area under paddy crop occupies highest share among food grains production and rice is a cheap and abundantly available feed resource for livestock. Murugeswari et al.(2018) also observed that 71.5% of farmers in unorganized sector of Tamil Nadu feed rice to their cattle in a cooked form. Such type of feeding is also prevalent in the goat population of the state. Goats reared in the urban and semi urban regions are fed mostly food / kitchen waste predominantly comprising of rice in some form or the other.

The breakdown of a large amount of readily fermentable materials can lead to a decline in ruminal pH and an increase in volatile fatty acid and lactic acid levels, which often induces metabolic acidosis (Sun et al., 2010).The decreases in rumen pH, acetate percentage, and acetate: propionate ratio with the increase in the percentage of concentrate have been observed frequently in the literature (Abijaoude et al.,2000; Serment et al.,2011).However, most of the earlier studies are focused in cattle, studies in goats are negligible. The earlier studies also focused on the rumen fermentation disorders associated with the incorporation of concentrate up to 50% of the ration but this study aimed to study the impact of a higher incorporation of rice-based concentrate (blend of rice and rice bran) up to 70% of the ration, on *in vitro* rumen fermentation characteristics. Documentation of *in vitro* rumen fermentation characteristics at high levels of rice-based concentrate in goats will help ascertain its advantages and disadvantages and therefore help evolve strategies to counteract the ill effects.

MATERIALS AND METHODS

The study was carried out during the year of 2020 at Institute of Animal Nutrition, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India. The study was designed to evaluate the effect of roughage to concentrate ratio on *in vitro* rumen fermentation using goat rumen fluid. The experiment had a 4 x 4 factorial design wherein the effect of roughage to concentrate ratio of 45: 55, 40: 60, 35: 65 and 30: 70 on ruminal fermentation at four different incubation (9, 12, 18 and 24 hours) periods each with six replicates was studied by using *in vitro* gas production technique as per the method described by Menke et al.(1979) using goat rumen fluid as inoculum. Dried Cumbu Napier hybrid grass ($CO(CN)_4$) was used as roughage and concentrate ration was prepared by mixing broken rice and de-oiled rice bran at the level of 70 and 30 % respectively. The ingredient composition and calculated nutritive value of the experimental rations used for *in vitro* gas production studies is presented in Table 1.

Table 1. Ingredient composition (% DMB) and calculated nutritive value of the experimental rations used for *in vitro* gas production studies

S.No.	Ingredients	Roughage concentrate ratio			
		45:55	40:60	35:65	30:70
1.	Cumbu Napier hybrid grass ($CO(CN)_4$)	45	40	35	30
2.	Concentrate mixture	55	60	65	70
	Composition of concentrate mixture				
1.	Broken rice	70	70	70	70
2.	Deoiled rice bran	30	30	30	30
	Calculated nutritive value (% DMB)				
1.	Crude protein	10.6	10.6	10.6	10.6
2.	Crude fibre	17.0	15.8	14.6	13.4
3.	Calcium	0.26	0.25	0.23	0.20
4.	TDN	65.4	66.4	67.3	68.3

Different roughage to concentrate ratio containing substrates of 0.200 ± 0.01 g was accurately weighed and transferred to 100 ml glass syringes whose pistons were lubricated with vaseline prior to the study. Goat rumen fluid was collected from the slaughter house from six goats immediately after slaughter and transported to laboratory in a thermos flask maintaining a temperature of 39°C and flushed with CO_2 to maintain anaerobic condition. At the laboratory, the collected ruminal contents were strained through four layer cheese cloth with continuous flushing of CO_2. Goat rumen fluid was mixed with buffer solution (Menke et al.,1979) in the ratio of 1: 2 (v/v) and 30 ml of mixed solution was transferred into the syringes containing substrate which were kept in a water bath at 39°C, for incubation periods of 9, 12, 18 and 24 hours.

At the end of each incubation period pH, total gas production was recorded and gas production values were blank corrected. At the end of the respective incubation hours, the entire contents of the syringes were centrifuged at 20,000 g for 30 minutes at 4ºC and supernatant collected from the centrifugation process were used for volatile fatty acid estimation in gas chromatography (Model No. Thermo scientific 1110 GC) equipped with flame ionization detector as per the procedure of Li et al. (2014). For the VFA analysis in gas chromatography, 2.5 mL of clear supernatant was mixed with 0.5 mL of metaphosphoric acid (25%) to precipitate the proteins. For calibrating standard curve, volatile fatty acids *viz.*, acetate, propionate and butyrate from Sigma-aldrich were used as external standards.

Sediment of the centrifuged samples was transferred to pre-weighed filter crucible and *in vitro* apparent degradability of dry matter of the substrate of roughage to concentrate ratio were assessed as per the procedure described by Blummel et al. (1997).

The following formulae were used for arriving at stoichiometric derivations

Energetic efficiency of VFA = 0.622 A + 1.092 P + [1.56 B × (100 / A)] + P + 2 B (Orskov et al., 1968), Where, A - represents acetate (%),P - represents Propionate (%), B - represents Butyrate (%). Data were analysed statistically using one way analysis of variance (ANOVA) to compare the means using IBM® SPSS® Statistics version 20.0 for Windows® software as per the Snedecor and Cochran (1989). The critical difference between the groups was analysed by Duncan's multiple range test.

RESULTS AND DISCUSSION

Optimum roughage to concentrate ratio is crucial for proper rumen fermentation and availability of nutrients. However, in order to improve the productivity of ruminants, a high forage diet is commonly switched to a high concentrate diet, which alters the rumen ecosystem. In the present study it was observed that a significant ($P < 0.05$) decline in the pH was caused with increase in the ratio of concentrate, concurring with the results of Nagadi (2019) who had reported that the pH value decreased as the amount of concentrate mix in the ration was maximal (100 %). Increasing the concentrate caused linear decline in the *in vitro* ruminal pH in a significantly ($P < 0.05$) manner at all incubation hours (Table 2). The results of this study are concurrent with that reported by Kumar et al., (2013), who had observed that pH was greater ($P<0.05$) in diet with 80:20 roughage to concentrate ratio compared to diets with 50:50 and 20:80 roughage to concentrate ratio (6.38 vs 6.17 and 6.07) The decline in ruminal pH could also be attributed to increased ruminal total volatile fatty acids (Liu et al.,2013).

$$\text{A / P ratio} = \frac{\text{Acetate}}{\text{Propionate}}$$

$$\text{Non- glucogenic ratio (NGR)} = \frac{\text{Acetate} + (2 \times \text{Butyrate})}{\text{Propionate}}$$

Table 2. *In vitro* ruminal pH of rations having different roughage to concentrate# ratios at different incubation hours (Mean*± SE)

S. No	Roughage to concentrate# ratio	*In vitro* ruminal pH			
		Incubation hours			
		9	12	18	24
1.	45:55	6.62^{c} ±0.01	6.52^{c} ± 0.02	6.42^{b} ± 0.02	6.38^{c} ± 0.03
2.	40:60	6.60^{bc} ± 0.01	6.46^{ab} ± 0.03	6.45^{b} ± 0.01	6.36^{c} ± 0.01
3.	35:65	6.59^{b} ± 0.01	6.47^{ab} ± 0.02	6.43^{b} ± 0.01	6.28^{ab} ± 0.04
4.	30:70	6.52^{a} ± 0.01	6.43^{a} ± 0.01	6.32^{a} ± 0.01	6.23^{a} ± 0.03

*Mean of six observations
Mean values bearing different alphabets as superscript within columns differ significantly ($P < 0.05$)
#Roughage - Cumbu Napier hybrid grass $CO(CN)_4$, Concentrate- broken rice (70%) and de-oiled rice bran (30%)

Increasing the concentrate caused linear increase in the *in vitro* gas production for all incubation hours in a significant ($P < 0.05$) manner (table 3). Significantly ($P < 0.05$) lowest *in vitro* gas production for all incubation hours was observed in roughage to concentrate ratio of 45:55 and significantly ($P < 0.01$) highest *in vitro* gas production for all incubation hours was observed in roughage to concentrate ratio of 30:70. It can be postulated that higher starch from higher levels of rice in the roughage to concentrate ratio of 30:70 resulted in higher total gas production. De Bover et al.(2005) and Sallam et al.(2007), also reported that total gas production values were positively correlated with soluble fraction / starch content of the feed. Gas production in the rumen is the result of fermentation of carbohydrates to volatile fatty acids i.e., acetate, propionate and butyrate and gas production from protein fermentation is relatively lesser compared to carbohydrates (Makkar, 2004). The higher gas production that was observed in this study, in the rations with increasing concentrates might be due to increased production of propionate, as carbon dioxide is produced when propionate is made by ruminal bacteria via the succinate: propionate pathway (Seshaiah et al., 2014).

Table 3. *In vitro* gas production of rations having different roughage to concentrate# ratios at different incubation hours (Mean*± SE)

S. No	Roughage concentrate ratio#	*In vitro* gas production (mL / 0.2 g substrate)			
		Incubation hours			
		9	12	18	24
1	45:55	12.3^{a} ± 1.45	17.2^{a} ± 1.16	33.5^{a}± 0.58	53.6^{a} ± 1.86
2	40:60	13.6^{a} ± 1.87	26.3^{b} ± 0.88	49.4^{b} ± 0.47	59.0^{b} ± 1.53
3	35:65	21.6^{b} ± 2.19	32.6^{c} ± 1.38	51.6^{b} ± 0.58	61.6^{bc} ± 0.67
4	30:70	26.3^{b} ± 1.65	38.7^{d} ± 1.16	56.2^{c} ± 1.16	65.3^{c} ± 1.45

*Mean of six observations
Mean values bearing different alphabets as superscript within columns differ significantly ($P < 0.05$)
Roughage - Cumbu Napier hybrid grass $CO(CN)_4$, Concentrate - broken rice (70%) and de-oiled rice bran (30%)

In vitro dry matter degradability (IVADMD) was significantly ($P < 0.05$) higher when the concentrate component of the ration increased at all incubation hours of study (Table 4). Similar results of increased IVDMD (47.6, 61.6 and 67.3 %) with increased concentrate ratios (80:20, 50:50 and 20:80) were also reported by Kumar et al.(2013). The report of Van Dung et al. (2014) was also comparable with the results of the present study. The authors reported that increase in concentrate diet (up to 80%) could improve dry matter degradability (65.4%). The presence of high levels of soluble substrate in high concentrate ration may be the reason for improved *in vitro* dry matter degradability.

Increasing the concentrate caused linear increase in the *in vitro* production of total volatile fatty acids for all incubation hours in a significant ($P < 0.05$) manner (Table 5). Acetate production was significantly ($P < 0.05$) declined and propionate production was significantly ($P < 0.05$) increased with increase in concentrate at all incubation hours except at 24 hours incubation (Table 6).

Table 4. *In vitro* volatile fatty acid (acetate, propionate and butyrate) production (Mean* ± SE) at different incubation hours of rations having different roughage to concentrate ratios

S. No	Roughage concentrate# ratio	Incubation hours											
		9			12			18			24		
		VFA (%)			VFA (%)			VFA (%)			VFA (%)		
		Acetate	Propionate	Butyrate	Acetate	Propionate	Butyrate	Acetate	Propionate	Butyrate	Acetate	Propionate	Butyrate
1	45:55	64.2^{c} ± 0.38	23.0^{a} ± 0.22	12.7^{a} ± 0.26	65.1^{b} ± 0.46	22.8^{a} ± 0.79	12.0 ± 1.26	66.9^{b} ± 1.52	22.9^{a} ± 1.25	10.0 ± 0.40	71.5^{c} ± 0.84	21.9^{a} ± 0.28	6.57^{a} ± 1.02
2	40:60	63.2^{c} ± 0.19	23.6^{a} ± 0.21	13.0^{ab} ± 0.02	64.5^{b} ± 1.50	22.4^{a} ± 0.66	12.9 ± 0.99	67.7^{b} ± 0.62	22.9^{a} ± 1.64	9.31 ± 1.05	69.4^{c} ± 0.94	23.7^{ab} ± 0.98	6.72^{a} ± 0.61
3	35:65	61.8^{b} ± 0.22	24.2^{a} ± 0.18	13.9^{b} ± 0.13	61.4^{b} ± 2.62	24.6^{a} ± 2.09	13.8 ± 0.65	66.9^{b} ± 0.51	22.8^{a} ± 0.13	10.2 ± 0.58	66.8^{b} ± 0.42	22.3^{a} ± 0.26	10.8^{b} ± 0.67
4	30:70	58.4^{a} ± 0.37	29.3^{b} ± 0.78	12.2^{a} ± 0.48	55.8^{a} ± 1.34	32.5^{b} ± 0.75	11.6 ± 1.29	63.2^{a} ± 0.81	27.0^{b} ± 0.53	9.70 ± 0.39	63.0^{a} ± 0.58	25.1^{b} ± 0.48	11.8^{b} ± 0.18

*Mean of six observations
Mean values bearing different alphabets as superscript within columns differ significantly ($P < 0.05$)
Roughage - Cumbu Napier hybrid grass $CO(CN)_4$, Concentrate - broken rice (70%) and de-oiled rice bran (30%)
NS – Non-Significant. Mean values do not differ significantly ($P < 0.05$)

The results of this study concurred with that of Granja-Salcedo et al.(2016) who had reported that in Nellore steers fed four different proportions of concentrate to roughage (corn silage): 30:70, 40:60, 60:40 and 80:20%, higher dietary concentrate levels resulted in a linear increase of propionic acid concentrations, a linear reduction of the ratio acetic acid to propionic acid ($P<0.05$).

Table 5. *In vitro* apparent dry matter degradability of rations having different roughage to concentrate[#] ratios at different incubation hours (Mean*± SE)

S. No	Roughage concentrate[#]ratio	*In vitro* apparent dry matter degradability (% DMB) Incubation hours			
		9	12	18	24
1.	45:55	$14.4^{a} \pm 0.30$	$23.8^{a} \pm 0.60$	$29.5^{a} \pm 0.58$	$38.6^{a} \pm 0.71$
2.	40:60	$16.7^{b} \pm 0.63$	$33.8^{b} \pm 0.53$	$41.9^{b} \pm 1.05$	$47.2^{b} \pm 1.73$
3.	35:65	$22.0^{c} \pm 0.39$	$36.9^{bc} \pm 1.58$	$45.5^{c} \pm 0.56$	$51.9^{b} \pm 1.87$
4.	30:70	$29.4^{d} \pm 0.85$	$38.1^{c} \pm 1.62$	$48.6^{d} \pm 1.29$	$65.7^{c} \pm 3.01$

*Mean of six observations
Mean values bearing different alphabets as superscript within columns differ significantly (P < 0.05)
[#] Roughage - Cumbu Napier hybrid grass $CO(CN)_4$, Concentrate - broken rice (70%) and de-oiled rice bran (30%)

With regard to butyrate production significant (P<0.05) variability was observed at 9 and 24 hours of incubation. Increasing the concentrate led to significant (P<0.05) increase in butyrate production.

Table 6. *In vitro* production of total volatile fatty acids from rations having different roughage to concentrate[#] ratios at different incubation hours (Mean*± SE)

S. No	Roughage concentrate[#]ratio	Total volatile fatty acids (mmoL / dL) Incubation hours			
		9	12	18	24
1.	45:55	$9.74^{a} \pm 0.10$	$11.6^{a} \pm 0.63$	$14.5^{a} \pm 0.18$	$18.7^{a} \pm 0.15$
2.	40:60	$10.3^{a} \pm 0.12$	$12.6^{a} \pm 0.15$	$15.6^{b} \pm 0.32$	$20.0^{a} \pm 0.68$
3.	35:65	$11.7^{b} \pm 0.23$	$14.1^{b} \pm 0.18$	$19.6^{c} \pm 0.21$	$23.4^{b} \pm 0.44$
4.	30:70	$16.1^{c} \pm 0.49$	$20.1^{c} \pm 0.08$	$21.2^{d} \pm 0.26$	$26.5^{c} \pm 0.19$

*Mean of six observations
Mean values bearing different alphabets as superscript within columns differ significantly (P < 0.05)
Roughage - Cumbu Napier hybrid grass CO(CN)4, Concentrate - broken rice (70%) and de-oiled rice bran (30%)

Increasing the concentrate caused decrease in acetate propionate ratio for all incubation hours in a significant (P<0.05) manner (Table 7). Significantly (P<0.05) highest acetate propionate ratio was observed in 45:55 roughage to concentrate ratio at 9 and 24 hours of incubation. With regard to NGR, lowest value was observed in 30: 70 roughage concentrate ratio and no significant variation was observed in roughage toconcentrate ratio of 45: 55, 40: 60 and 35: 65 in all incubation hours.

Table 7. Influence of different roughage to concentrate# ratios on *in vitro* acetate to propionate ratio, non-glucogenic ratio and energetic efficiency at different incubation hours (Mean*± SE)

S. No	Roughage concentrate ratio#	Incubation hours: 9			12			18			24		
		Acetate Propionate Ratio	NGR	EE	Acetate Propionate Ratio	NGR	EE^{NS}	Acetate Propionate Ratio	NGR^{NS}	EE	Acetate Propionate Ratio	NGR	EE
1.	45:55	2.79^{c} ± 0.04	3.89^{b} ± 0.04	144^{a}± 1.30	2.86^{b}± 0.07	3.91^{b} ± 0.22	141± 3.78	2.94^{b}± 0.23	3.82 ± 0.26	133^{ab}± 3.50	3.27^{c}± 0.04	3.87^{ab} ± 0.10	117^{a} ± 3.52
2.	40:60	2.66^{bc} ± 0.03	3.77^{b}± 0.04	147^{a}± 0.33	2.88^{b}± 0.15	4.04^{b}± 0.12	144± 5.12	2.98^{b}± 0.23	3.81 ± 0.38	130^{a} ± 1.39	2.94^{b}± 0.15	3.50^{a}± 0.20	121^{a} ± 2.27
3.	35:65	2.55^{b}± 0.02	3.70^{b}± 0.03	152^{b} ± 0.71	2.55^{b}± 0.33	3.68^{b}± 0.39	153± 6.90	2.94^{b}± 0.02	3.83 ± 0.05	133^{ab}± 2.22	2.99^{bc} ± 0.02	3.96^{b}± 0.08	135^{b} ± 2.30
4.	30:70	1.99^{a}± 0.06	2.83^{a}± 0.11	154^{b} ± 0.92	1.72^{a}± 0.07	2.43^{a}± 0.09	158± 6.11	2.34^{a}± 0.08	3.06 ± 0.07	139^{b} ± 2.32	2.51^{a}± 0.07	3.44^{a}± 0.08	144^{c} ± 1.45

*Mean of six observations Mean values bearing different alphabets as superscript within columns differ significantly (P < 0.05) # Roughage - Cumbu Napier hybrid grass $CO(CN)_4$, Concentrate - broken rice (70%) and de-oiled rice bran (30%) NS–Non-Significant, Mean values do not differ significantly (P<0.05)

Significantly (P<0.05) highest energetic efficiency was observed in 30:70 roughage to concentrate ratio at all incubation hours except 12 hour of incubation. A decrease in acetate propionate ratio, non-glucogenic ratio and an increase in energetic efficiency were observed with increase in concentrate. The NGR is related to the efficiency with which VFA are used for productive purposes, as it provides an indication of the partitioning of energy between milk and body mass (Orskov et al., 1969; Van Knegsel et al., 2007). Orskov (1977), also reported that increasing concentrates caused reduction in NGR, the extent of which depended on type of roughage and processing of concentrate.

CONCLUSION

Increasing the dietary input of rice-based concentrate in ration caused decline of pH, increase in the *in vitro* gas production, *in vitro* apparent dry matter degradability (IVADMD) and total volatile fatty acids (TVFA) for all incubation hours in a significant manner. The percentage production of acetate declined and propionate increased with increase in rice-based concentrate. This study will help evolve strategies to overcome fermentation disorders.

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