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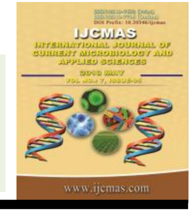

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Evolving Safe and Strategic Supplementation to Ruminants Fed with Rice Gruel

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A B S T R A C T

The present study was carried out to evolve a safe and strategic supplementation to rice gruel by synchronizing nutrient delivery in the rumen for maximum microbial production. Three *in vitro* trials were conducted to study the rumen fermentation characteristics of non-protein nitrogen (NPN) and sulphur sources supplementation to rice gruel with basal diet (Bajra x Napier grass, CO4). Urea and diammonium phosphate (DAP) were used as NPN sources, while copper sulphate and sodium sulphate were used as sulphur sources. The results indicated that sodium sulphate was the choice of sulphur source and pH and ammonia concentration were not alarming enough to produce toxicity. Both urea and DAP supplementation produced beneficial effect by significantly ($P<0.05$) increasing the microbial biomass production and apparent dry matter digestibility. The results were validated in semi-continuous culture (RUSITEC) system. The results of the study clearly demonstrated that the pH was in the range, that were neither low (5.5) to induce acidosis nor high enough (7.4) to convert ammonium ion (NH_4^+) to free ammonia (NH_3) to promote ammonia permeability. The IVDMD (*In vitro* dry matter digestibility) of the various treatments were not significantly ($P>0.05$) different from each other and the microbial biomass yield was numerically highest in "basal diet + rice gruel +DAP + sodium sulphate" (28.31 ± 2.06 per cent).

Introduction

Many attempts at genetic level interferences *viz.*, cross breeding, genetic up gradation etc., were aimed to improve the production potential, but similar attempts in terms of feeding practices were difficult due to the adoption of region oriented traditional feeding practices and lack of awareness by farmers on scientific feeding management. This has led to stagnation of production in animals in spite of their high genetic potential. Hence, the traditional feeding practices are to be improved scientifically through strategic supplementation of nutrients in order to synchronize nutrient delivery in rumen. One such traditional feeding practice is the feeding of rice gruel/rice waste/rice water to ruminants especially cattle. This practice has more relevance in regions of sub-continent where rice forms the staple food for the human population. Rice gruel feeding is common in East coast of India mainly Andhra Pradesh, Tamilnadu and West Bengal (Rao *et al.,* 1995). In Bangladesh rice gruel has been used as a traditional supplement for milking dairy cattle and fattening beef cattle (FAO, 1999).

Rice gruel is a rich source of starch but poor source of other nutrients especially nitrogen. Starch on fermentation in rumen can be effectively used in the presence of nitrogen (Henning *et al.,* 1991). At the same time feeding of nitrogenous compounds without energy rich diets can lead to increase in the rumen ammonia concentration resulting in ammonia toxicity. Therefore feeding of gruel with nitrogenous compounds is a better practice. Nitrogen sources that are readily available and economical to farmers are urea and diammonium phosphate (DAP). Further, supplementation of sulfur and maintaining nitrogen: sulfur ratio is essential for the microbial protein synthesis from NPN compounds (Moir, *et al.,* 1968). Many veterinarians also perceive that feeding of rice gruel to cows produce acidosis.

Starch/energy content of the rice gruel is highly variable due to various factors such as dilution with water, addition of rice washings, addition of vegetable scraps, addition of excess rice and various cooking methods. This warrants the standardization of the energy level of the rice gruel is also essential. Hence, there is a need to standardize the ratio of energy to nitrogen (NPN) compound and ratio of nitrogen to sulfur to be added to the rice gruel for the effective utilization of energy and nitrogen sources by the rumen microbes to augment the production. With considering these factors, the present study was performed to evolve a strategic supplementation for rice gruel in order to synchronize nutrient delivery in rumen.

Materials and Methods

Survey on rice gruel feeding

A survey was carried out in Villupuram District, Tamil Nadu to assess the existing feeding practice with reference to rice gruel feeding and its impact on the animal health / production. The survey was focused with separate sets of questionnaires on two issues *viz.,* (a) rice gruel feeding practice followed and (b) impact of feeding rice gruel on animal, to elicit respective information from the concerned target groups *viz.,* the farmers and veterinary doctors.

The rice gruel samples were collected from same farmers and dispatched in iced containers to the Department of Animal Nutrition, Madras Veterinary College. The gruel contained only decanted liquid. The samples were stored at 4°C until further analysis of dry matter (AOAC, 1995) and total carbohydrate contents of rice gruel (Hedge and Hofreiter, 1962).

In vitro studies on inclusion of rice gruel to basal diet supplemented with NPN and sulphur source

The quantity of nitrogen required for effective supplementation for rice gruel was determined as suggested by McMeniman *et al.,* (1976). The quantity of urea / DAP needed to supplement the determined quantity of nitrogen was based on the concentration of the nitrogen in them. The effect of sulphur supplementation to NPN from two sources (copper sulphate/sodium sulphate) was also studied to identify the appropriate source. The quantity of sulphur to be supplemented with any of the two NPN compounds was based on the sulphur to nitrogen ratio of 1:10 and the quantity of sulphur source to be supplemented was based on the concentration of the sulphur present in the source that was used.

The experiment was split into two *in vitro* studies conducted simultaneously using the strained rumen liquor sourced from the same animal. While one *in vitro* study addressed the IVDMD of basal diet at 48 hours, following Tilley and Terry method (1963) and assessed the microbial biomass following Blummel *et al.,* (1997) with or without gruel, NPN source and sulphur source, the other addressed the liberation of ammonia (Conway, 1962) and pH variations at periodical intervals of 30, 60, 90 and 120 minutes with or without gruel, NPN source and sulphur source. There were eight treatment groups with four replicates in each of the six runs carried out.

The quantity of gruel required for IVDMD studies was estimated based on the rumen capacity of 73 liters of an adult animal weighing 500 kg is 3.0 ml. Since the objective of this study was to examine the scope of toxicity on supplementation with NPN compounds, double the dose of gruel (6 ml) was tested.

Thus corresponding level of nitrogen (urea- 2.106 mg; DAP-10.992 mg) and sulfur (copper sulphate- 0.7614 mg; sodium sulphate- 0.45 mg) sources were added to 6 ml of gruel along with 80 ml of strained rumen liquor and one gram of basal diet to test the liberation of ammonia and pH at periodic interval.

Batch culture results reexamined in semi continuous culture using RUSITEC

The promising results that emerged in the *in vitro* trial were reexamined in semi continuous culture using Rumen Simulation Technique (RUSITEC) to validate the batch culture results. The quantity of rice gruel to be fed in RUSITEC was extrapolated from the rumen volume for a 500 kg cow fed on forage alone. The corresponding quantity of urea/DAP/ Sodium sulphate was included to form the four treatments (Table 1). After six days of adaptation period 10 g basal diet (on DMB) was incubated for 24 hours; the bags were removed from the reaction vessel and washed twice with 40 ml of artificial saliva. The bags were washed in running tap water till the wash water was clean and were dried at 60^{o}C for 48 hours and weighed.

The substrate apparently degraded was calculated as follows:
Substrate apparently degraded (%) =

Weight of nylon bag with sample before incubation – Weight of the nylon bag with sample after incubation
-- × 100
Weight of the sample

The undegraded dried sample residues were subjected to NDF analysis for estimating the *in vitro* true degradability. The microbial biomass was calculated from the equation quoted by Blummel *et al.,* (1997). The rumen liquor was collected during the initial period of incubation, (30, 60, 90 and 120 min interval) and analysed for the ruminal ammonia concentration (Conway, 1962) and pH.

Statistical analysis

Completely randomized block design (CRD) was used as experimental design in this study. Data were analyzed using analysis of variance (ANOVA) as per procedure of statistical analysis system (SAS/SPSS, 1999, version 10.0 for windows). When significant differences (P<0.05) were detected the multiple range test was used to separate the mean value.

Results and Discussion

Survey results revealed that 55 per cent of farmers feed rice gruel along with bran. Among them, 73 per cent of farmers feed 2.5 litres of rice gruel / animal / day. Concurring with the results of this survey Das and Tripathi (2008) had also reported that in stall fed cattle / buffaloes in sundarbans delta, it is common to feed paddy straw along with rice gruel, rice washed water, rice bran and kitchen waste. Survey on impact of feeding rice gruel on health status of animals was carried out among 30 field veterinarians of Villupuram District.

The survey revealed that veterinary doctors perceived that rice gruel feeding adversely affect animal health by exhibiting three major symptoms like indigestion, acidosis and bloat or their combination. Only 17 per cent of veterinarians reported that they believe that feeding rice gruel to cows do not affect animal health.

The total carbohydrate content of the gruel was found to be 5 g / litre (5.0137 ± 1.268) for thick and pasty rice gruel and 2 g / litre (1.855 ± 0.66) for thin and watery rice gruel. However, Perez *et al.,* (1987) reported much higher percentage (14.3 per cent) of starch in rice gruel obtained from rice cook for 20 minutes in ten weights of water. The dry matter content of the rice gruel was found to be 2 per cent (2.059 ± 0.161) for the thick and pasty rice gruel and 1 per cent (0.956 ± 0.097) for the thin and watery rice gruel. Addition of more water during the cooking process produces gruel of thin consistency having lower dry matter (Juliano, 1993).

The results of batch culture system were presented in table 2. Urea and DAP with sodium sulphate supplemented showed significantly highest ($P<0.05$) pH values, that were comparable to DAP or DAP with copper sulphate whereas rice gruel supplemented to basal diet had lowest pH values. This trend was maintained in all the incubation periods studied. Rice gruel was not able to significantly reduce the pH of basal diet within two hours of incubation. Within the treatment, between the various incubation intervals there existed no significant variation in pH values for all the treatments studied. Concurring with the findings of this study Chanjula *et al.,* (2004) had reported rumen pH of 6.8 in cows fed high level of cassava chips (75%) in their ration. Ali *et al.,* (2008) had also reported a pH of 7.08 on *in vitro* fermentation of urea and starch. In this study also urea and gruel feeding favored a pH of between 7.02 and 7.16.

Significantly lowest ammonia nitrogen ($P<0.05$) was observed in "Basal diet + rice gruel + Urea+ sodium sulphate" at incubation period of 30 minutes. The significantly highest ammonia nitrogen ($P<0.05$) was recorded in "Basal diet + rice gruel + Urea+ Copper sulphate" at 30 minute incubation, but were comparable to "Basal diet + rice gruel + Urea", "Basal diet + rice gruel + Diammonium phosphate" and "Basal diet + rice gruel + Diammonium phosphate + Copper sulphate" proved that sodium sulphate is a better supplemental sulphur source to urea / Diammonium phosphate as it could restrict ammonia nitrogen level within 7.35 mg/dl, whereas copper sulphate could not restrain liberation of ammonia.

Further sodium sulphate supplementation was effective in restraining the ammonia nitrogen levels below 9.48 mg/dl. Cameron *et al.,* (1991) had reported much higher (12.5 mg/dl) ammonia nitrogen when lactating cows were fed diets supplemented with starch + urea. Concurring with the findings of this study, Chanjula *et al.,* (2004) had reported 8.8 mg/dl as ammonia nitrogen concentration when urea was fed in rations with 75 per cent inclusion of cassava chips.

The microbial biomass production in 48 hours using basal diet in which was included rice gruel supplemented with NPN and sulphur sources revealed that the microbial biomass production on 48 hours incubation of basal diet alone was only 14 ± 2.5 per cent. Marginal improvement in microbial biomass production was evident on supplementation of rice gruel to the basal diet (17 ± 3.7 per cent). Urea supplementation /urea with copper sulphate supplementation / urea with sodium sulphate supplementation produced 18 ± 3.3, 18 ± 1.6 and 22 ± 8.4 per cent microbial biomass respectively. Similarly DAP / DAP with copper sulphate / DAP with sodium sulphate produced 17 ± 2.7, 19 ± 3.8 and 14 ± 2.4 per cent microbial biomass respectively.

It is thus evident that basal diet to which rice gruel is included supplemented with urea and sodium sulphate produces maximum microbial biomass. Considering all the results of this study, it was concluded that basal diet with rice gruel supplemented with urea / DAP along with sodium sulphate deserved to be studied further which was validate in semi continuous culture using RUSITEC. The results of pH, ammonia values recorded at periodical intervals, per cent dry matter degradability and per cent microbial bio mass production of basal diet at 24 hour interval for each feeding regimen in the semi continuous culture using RUSITEC are furnished in table 3. Significantly ($P<0.05$) lowest pH values was observed in “basal diet + rice gruel” irrespective of the incubation intervals. Significantly ($P<0.05$) highest pH values were observed with incubation of “basal diet + rice gruel and supplemented with NPN / sulphur sources. The trend was maintained in all the incubation intervals studied. Similar reports of lower pH values (6.03 to 6.70) in rumen liquor of Brahman cattle receiving diet containing urea from 0.2 to 1 per cent was also recorded by Chumpawadee *et al.,* (2006). Though effect of rice gruel on the pH of rumen liquor is not readily available in the literatures reviewed Cameron *et al.,* (1991) reported a lower pH (5.74) in rumen liquor of lactating cows fed diets that contain starch and urea. The results clearly indicate that inclusion of gruel significantly decreases pH but not low enough to produce acidosis. Compared to basal diet, adding urea / DAP moderately elevates pH but only below that of basal diet. The ammonia concentration in semi continuous culture system at periodical intervals for each feeding regimen experimented revealed that significantly ($P<0.05$) lowest ammonia concentration was recorded in “basal diet” at all incubation intervals and was comparable to “basal diet + rice gruel” though rice gruel inclusion to basal diet slightly increased ammonia concentration.

Table.1 Description of the various treatment groups used in RUSITEC

Treatment	Description of treatment
Basal diet	10 g Basal diet + 600 ml strained rumen liquor and volume made up to 850 ml with artificial saliva
Basal diet + rice gruel	10 g Basal diet + 600 ml strained rumen liquor + 29 ml gruel and volume made up to 850 ml with artificial saliva
Basal diet + rice gruel + Urea + Sodium sulphate	10 g Basal diet + 600 ml strained rumen liquor + 29 ml gruel with 10.1 mg urea supplemented with 2.175 mg sodium sulphate and volume made up to 850 ml with artificial saliva
Basal diet + rice gruel + DAP + Sodium sulphate	10 g Basal diet +600 ml strained rumen liquor + 29 ml gruel with 26.5 mg diammonium phosphate supplemented with 2.175 mg sodium sulphate and volume made up to 850 ml with artificial saliva

Table.2 Effect of urea / diammonium phosphate with or without sulphur source to rice gruel on the pH, ammonia nitrogen concentration (mg/dl) in rumen liquor at periodical incubation and per cent Microbial biomass production (Mean *± SE) as estimated in batch culture / *in vitro* study

Feeding regimen	pH values				Ammonia nitrogen concentration (mg/dl)				Microbial biomass production %
	Incubation period (minute)				Incubation period (minute)				
	30	60	90	120	30	60	90	120	
Basal diet	6.97^{bc} ± 0.03	6.96^{c} ± 0.02	7.00^{bc} ± 0.02	6.99^{cd} ± 0.03	8.12^{abcd} ± 0.90	8.85^{ab} ± 0.92	8.92^{ab} ± 0.94	8.97^{ab} ± 1.05	14.15^{NS} ± 2.566
Basal diet + rice gruel	6.89^{c} ± 0.04	6.95^{c} ± 0.03	6.95^{c} ± 0.02	6.96^{d} ± 0.02	7.85^{bcd} ± 0.92	8.64^{ab} ± 0.86	8.87^{ab} ± 0.97	8.92^{ab} ± 0.98	17.53^{NS} ± 3.678
Basal diet + rice gruel + Urea	6.98^{bc} ± 0.05	7.03^{abc} ± 0.03	7.01^{bc} ± 0.03	7.02^{bcd} ± 0.03	11.01^{ab} ± 1.03	10.33^{a} ± 0.87	9.85^{ab} ± 0.84	9.78^{ab} ± 1.05	18.57^{NS} ± 3.319
Basal diet + rice gruel + Urea + Copper sulphate	7.02^{bc} ± 0.05	7.02^{bc} ± 0.03	7.03^{bc} ± 0.03	7.01^{bcd} ± 0.02	11.68^{a} ± 1.16	11.01^{a} ± 1.32	9.29^{ab} ± 1.04	9.41^{ab} ± 1.09	18.27^{NS} ± 1.643
Basal diet + rice gruel + Urea + Sodium sulphate	7.16^{a} ± 0.07	7.12^{ab} ± 0.04	7.16^{a} ± 0.02	7.13^{a} ± 0.03	6.52^{d} ± 0.86	5.85^{b} ± 0.92	7.18^{b} ± 1.47	7.35^{b} ± 1.59	21.93^{NS} ± 8.384
Basal diet + rice gruel + Diammonium phosphate	7.04^{abc} ± 0.04	7.06^{ab} ± 0.04	7.04^{bc} ± 0.03	7.06^{abc} ± 0.03	10.35^{abc} ± 1.09	10.71^{a} ± 1.09	11.22^{a} ± 1.06	11.57^{a} ± 1.06	17.27^{NS} ± 2.728
Basal diet + rice gruel + Diammonium phosphate + Copper sulphate	7.05^{ab} ± 0.03	7.08^{ab} ± 0.03	7.09^{ab} ± 0.03	7.07^{abc} ± 0.03	9.35^{abcd} ± 0.84	11.70^{a}± 1.03	11.29^{a} ± 1.06	11.49^{a} ± 1.12	19.77^{NS} ± 3.809
Basal diet + rice gruel + Diammonium phosphate + Sodium sulphate	7.16^{a} ± 0.03	7.13^{a} ± 0.02	7.13^{a} ± 0.02	7.09^{ab} ± 0.03	6.97^{cd} ± 0.92	8.59^{ab} ± 0.79	8.96^{ab} ± 1.52	9.48^{ab} ± 1.48	13.75^{NS} ± 2.444

* Mean of twenty four samples

Mean values in column bearing different superscripts differs significantly (P<0.05)

^{NS} Mean values does not differ significantly (P>0.05)

Table.3 Effect of urea / diammonium phosphate with or without sulphur source to rice gruel on the pH, ammonia nitrogen concentration (mg/dl) in rumen liquor at periodical incubation, per cent dry matter degradability and per cent microbial biomass production of basal diet (Bajra x Napier *var* CO4) at 24 hour interval and (Mean *± SE) as estimated in semi continuous culture / *in vitro* study

Feeding regimen	pH values				Ammonia nitrogen concentration (mg/dl)				Dry matter degradability %	Microbial biomass production %
	Incubation period (minute)				Incubation period (minute)					
	30	60	90	120	30	60	90	120		
Basal diet	7.36^{a} ± 0.03	7.30^{a} ± 0.02	7.34^{a} ± 0.02	7.33^{a} ± 0.02	4.81^{b} ± 0.35	4.64^{b} ± 0.68	6.02^{b} ± 1.02	5.62^{b} ± 0.65	32.30^{NS} ± 0.629	44.71^{NS} ± 1.147
Basal diet + rice gruel	7.17^{c} ± 0.04	7.18^{b} ± 0.02	7.07^{c} ± 0.02	7.10^{b} ± 0.03	5.78^{b} ± 0.72	6.65^{b} ± 0.56	6.56^{b} ± 1.44	7.95^{ab} ± 0.89	33.15^{NS} ± 1.755	46.66^{NS} ± 1.932
Basal diet + rice gruel + Urea + Sodium sulphate	7.23^{bc} ± 0.02	7.17^{b} ± 0.06	7.19^{b} ± 0.05	7.12^{b} ± 0.01	7.93^{a} ± 0.89	8.75^{a} ± 0.82	10.57^{a} ± 1.16	9.01^{a} ± 0.96	34.82^{NS} ± 1.616	47.19^{NS} ± 2.190
Basal diet + rice gruel + Diammonium phosphate + Sodium sulphate	7.29^{ab} ± 0.03	7.17^{b} ± 0.04	7.15^{bc} ± 0.02	7.13^{b} ± 0.02	8.73^{a} ± 0.31	9.80^{a} ± 0.67	10.22^{a} ± 0.52	9.98^{a} ± 0.61	34.22^{NS} ± 1.433	48.31^{NS} ± 2.062

* Mean of six samples
Mean values with in column bearing different superscripts differs significantly (P<0.05)
^{NS} Mean values does not differ significantly (P>0.05).

Ammonia concentration on addition of urea or DAP with sodium sulphate was significantly (P<0.05) increased. Decline in ammonia concentration was noticed after 90 min of incubation. Cameron *et al.,* (1991) reported higher ammonia nitrogen level (12.6 mg / dl) in rumen liquor on supplementation of corn starch with urea (0.67 per cent) to lactating dairy cows.

The IVDMD as indicated in the table 3 between the various treatments ranged between 32.30 and 34.82 per cent and were not significantly (P>0.05) different from each other treatment.

The results of the dry matter degradability showed that the degradability was more or less similar in "basal diet + rice gruel + urea + sodium sulphate" (34.22 ± 1.616 per cent) and "basal diet+ rice gruel + DAP + sodium sulphate" (34.82 ± 1.433 per cent). However, significant increase in dry matter degradability was recorded by Ali *et al.,* (2008) on addition of starch and biuret to low quality roughages like wheat straw.

The per cent microbial biomass production in 24 hours incubation for various feeding regimen was non-significant (P>0.05) among the various treatments, with a numerically higher value was recorded in basal feeds supplemented with rice gruel with NPN and sulphur sources. Highest microbial biomass production was noticed in "basal diet + rice gruel +DAP + sodium sulphate" (48.31 ± 2.062 per cent).

Extrapolation of the findings is indicative of the fact that 0.8775 g of urea or 2.29 g of DAP with 0.185 g of sodium sulphate can be fed along with 2.5 litres of rice gruel to an adult cattle weighing about 500 kg without any deleterious effects to maximize microbial bio mass production and improved digestibility.

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