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Effects of processing method of agro-industrial by-product on rumen fermentation characteristics for sheep by *in vitro* technique

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Abstract
Rumen fermentation studies were carried out *in vitro* technique (RUSITEC) by using the sheep rumen content as an inoculums to assess the rumen fermentation characteristics were assessed by fermenting the black gram husk, The mean pH of the rumen liquor was 6.89±0.03 or 6.92±0.04 respectively, when raw or water soaked black gram husk was incubated. The values for non-glucogenic ratio (NGR) calculated in the present study were 2.67 ± 0.19 and 2.53 ± 0.05. The total volume of gas produced was 1.49 ± 0.02 or 1.53 ± 0.02 L/day respectively when the raw or water soaked BGH was incubated. It was concluded that the water soaking of the husk produced significantly (p<0.01) more ammonia nitrogen (10.73 Vs 10.54 mg/100ml), microbial protein (26.26 Vs 24.52 mg/100ml) and total short chain fatty acids (59.68 Vs 51.71 m mol/d) than the raw husk. Water soaking of the husk significantly (p<0.01) increased the level of total absorbable nitrogen, microbial protein and short chain fatty acids compared to the raw husk.

Keywords: Black gram husk - rumen fermentation characteristics - RUSITEC

Introduction
Pulses occupy 68.32 million hectare area and contribute 57.51 million tones to the world's food basket. India shares 35.2 percent area and 27.65 percent of the global pulse production (Chaturvedi and Ali, 2002) [5]. India is also one of the largest producers of legumes in the world (Jain *et al.*, 1980) [9]. Black gram/Urad (*Phaseolus mungo* or *Vinga mungo*) ranks third among the pulses produced in India and occupies 6.60 percent of the pulse crops (Bhaid *et al.*, 1981) [3]. Black gram is one of the important pulses grown in Tamil Nadu both in Kharif and Rabi seasons. The production of black gram in Tamil Nadu was estimated as 2.25 million tones and it constituted 47.60 percent of the total production of pulses (Annon, 2022) [1].
Among the several agro-industrial by-products available pulse husks or chunies form major resources in terms of availability. Large quantities of pulse husks are available as by-product while processing the pulses in the mills. Pulse husk/chunies are available to the extent of 3 million tones in India per annum (Srinivasa Rao *et al.*, 1998) [18]. Black gram (*Vigna mungo*) husk is one such agro-industrial by-products available in substantial quantity as this pulse is grown as cash crop in vast areas of Tamil Nadu.
However, very little information is available in the literature on its nutritive value and its rumen fermentation characteristic for ruminants. Hence a study was proposed to assess the effect of processing methods on Rumen fermentation characteristics

Materials and Methods
Experimental design
Apparatus
The Semi-continuous culture system developed at the Department of Animal Nutrition Laboratory, Madras Veterinary College was adopted from the "RUSITEC" (Plate 2) and run essentially as described by Czerkawski and Breckenridge (1977) [6]. It consisted of eight 1 litre capacity reaction vessels immersed in water bath maintained at 39 °C.

Preparation of the samples for incubation
The samples of black gram husk for studies in RUSITEC were ground through 3 mm sieve. The ground samples were then sieved and particles passing through 2 mm sieve but retained by 0.85 mm sieve were collected (Dong *et al.*, 1997) [7] and stored in air-tight containers for further analysis.

Nylon bag size and pore size
Nylon bags of 12.5x7.5 cm size made up of precious woven monofilament polyester cloth with a specified pore size of 100 µm were used in the present study (Carro *et al.*, 1995) [4].

Sample size
Ten gram of raw or water soaked black gram husks on dry matter basis were weighed separately into each nylon bag for incubation in the rusitec. The sample size to bag ratio was 15mg/cm^2 (Carro *et al.*, 1995) [4].

Incubation procedure
Rumen digesta was collected from three sheep maintained on grazing. It was thoroughly mixed and transported to the laboratory (within 30 minutes) in a pre-heated vacum flask. The rumen fluid was strained through a double-layered muslin cloth into a CO_2 filled beaker. The solid content in the muslin was squeezed to maximum to get the rumen liquor.
Each reaction vessel was charged with 500 ml of strained rumen liquor and 200 ml of artificial saliva (McDougall, 1948) [11]. One nylon bag (pore size 100 µm) containing 80g of rumen digesta solids (fibrous fraction from the rumen content straining) and another containing 10g dry matter of feed to be tested were placed into the perforated feed container and the assembly was put into the reaction vessel which was filled upto the brim with distilled water making the total volume of the container to one liter.
Artificial saliva was pumped at a constant ratio of infusion (650 ml/day) into the reaction vessel by a peristaltic pump. The effluent and fermentation gases were collected in effluent collection vessels (containing few drops of saturated $HgCl_2$ solution) and gas collection bags respectively. After 24 hours the solid inoculum was removed and a new bag of feed was placed in the feed container. Thus each reaction vessel at a time contained 2 bags introduced each in 2 consecutive days and removed 48 hours later.
The bag to be removed was allowed to drain, squeezed and washed in artificial saliva in a polyethylene bag. The washings were returned to the respective reaction vessels. The removed bags were further washed and dried at 60 ^oC for 48 hours. Each experiment totally consisted of 7 days adoption period followed by collection period.

Replication
Raw or water soaked black gram husk treatments were allotted three reaction vessels to each at random. The data were generated from two runs, thus yielding six measurements for each incubation period to each treatment.

Collection period
The liquid effluent was collected to study the various rumen parameters from the 8th day at 2, 4, 6, 8, 12, 18, 24, 36, and 48 hours interval. The pH of effluent was measured using digital pH meter. About 4.5 ml of sample from each reaction vessel was taken and 0.5 ml of 50 percent Trichloracetic acid was added for ammonia – N (NH_3N) analysis. For estimating the Volatile fatty acids (VFA) concentration 2.5 ml of the effluent was taken and 0.5 ml of 25 percent metaphosphoric acid was added and further 10 ml of effluent was stored in the refrigerator for analysis of microbial protein.

Rumen fermentation studies
The pH of effluents collected at various time intervals was determined using digital pH meter. The ammonia nitrogen concentration of the effluents collected was estimated colorimetrically as per the method of Weatherburn (1967). The microbial protein content was estimated as per the method of Makker *et al.* (1981) [10]. Total and individual volatile fatty acids concentration of the effluents was measured by gas chromatographic method as per the procedure of Chase (1990). The Netel make Gas Chromatograph Model "Omega qc" was used for this estimation.

Stoichiometry of the rumen fermentation
The following stoichiometric equations were used to predict various patterns (Orskov *et al.*, 1968) [13].

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

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

Gas analysis
At the end of specific time intervals the infusion of artificial saliva was stopped and the gas collected into the gasbag was measured for the total gas production and fractioned to CO_2 and CH_4 using potassium hydroxide.

Statistical analysis
The data obtained in different parameters were subjected to statistical analysis as per the procedure of Snedecor and Cochran (1968) [17].

Results and Discussion
Rumen fermentation characteristics of raw or water soaked black gram husk
Rumen fermentation characteristics of raw or water soaked black gram husk after 24 hours of incubation in RUSITEC is presented in Table 1.

pH
The mean pH of the rumen liquor was 6.89±0.03 or 6.92±0.04 respectively, when raw or water soaked black gram husk was incubated. The pH slightly increased though non-significantly when water soaked black gram husk was incubated.

Ammonia nitrogen
The ammonia nitrogen concentration was 10.54±0.27 or 10.73±0.16 mg/100 ml when raw or water soaked black gram husk was incubated. The ammonia nitrogen concentration was significantly (p<0.01) high when water soaked black gram husk was incubated. This could be due to the significantly reduced total tannins content and increased level of soluble fraction of degradable nitrogen in the water soaked black gram husk. The significantly high ammonia nitrogen concentration observed with water soaked black gram husk in the present study explains the reason for the increased pH value. Murugan (1991) [12] reported similar level of ammonia nitrogen in the rumen liquor sheep fed cottonseed hull or other cellulosic wastes. The values reported for pH and ammonia nitrogen in the present study coincided with the earlier reports of Sudakara Reddy *et al.* (2002) [19] and Radha Krishna *et al.* (2002) [15] for urad chuni and green gram chuni, respectively.

Table 1: Rumen Fermentation characteristics of raw or water soaked BGH at 24 hours of incubation in Rusitec

Parameter	Raw BGH	Water Soaked BGH
pH	6.89±0.03	6.92±0.04
Ammonia nitrogen (mg/100ml)	10.54^{a}±0.27	10.73^{b}±0.16
Microbial protein synthesis (mg/100ml)	24.52^{a}±0.61	26.26^{b}±1.57
Total short chain fatty acids (mmol/day)	51.71^{a}±0.38	59.68^{b}±0.67
Acetic acid (mmol/day)	33.49^{a}±0.45	36.98^{b}±0.60
Propionic acid (mmol/day)	15.05^{a}±0.62	18.19^{b}±0.39
Butyric acid (mmol/day)	3.18^{c}±0.37	4.51^{d}±0.24
A:P ratio	2.22±0.12	2.03±0.05
Non glucogenic ratio (NGR)	2.67±0.19	2.53±0.05
Total gas production (L/day)	1.49^{a}±0.02	1.53^{b}±0.02
CO_2:CH_4	1.90±0.04	1.88±0.05

$^{a, b}$ Mean value with different superscript in rows differ significantly (p<0.01)

$^{c, d}$ Mean value with different superscript in rows differ significantly (p<0.05)

Microbial protein synthesis

The microbial protein synthesis was 24.52 ± 0.61 mg/100 ml of rumen liquor when raw black gram husk was incubated. The corresponding value for water soaked BGH was 26.26 ± 1.57. Murugan (1991) [12] reported similar level of TCA – insoluble nitrogen in the rumen liquor of sheep and goat fed with groundnut hulls, cottonseed hulls or maize straw. Water soaking of black gram husk significantly (p<0.01) increased the microbial protein production. The significantly high level of soluble fraction of degradable nitrogen together with the rumen degradable protein and high level of NFE in the water soaked husk might have provided the optimum protein energy ratio that resulted in the significantly increased level of microbial protein production. Arora (1983) [2] suggested the rate of breakdown of dietary nitrogen, rate of absorption of ammonia, amino acids and energy availability to microbes as factors that influenced the microbial protein production in the rumen.

Volatile fatty acids

The total short chain fatty acids (TSCFA) produced on incubation of raw or water soaked BGH was 51.71 ± 0.38 or 59.68 ± 0.67 milli moles per day. The constituents of the short chain fatty acids like acetic, propionic and butyric acids were present at the level of 33.49 ± 0.45, 15.05 ± 0.62 and 3.18 ± 0.37 m mol/ day when raw BGH was fermented in the RUSITEC. The respective values were 36.98 ± 0.60, 18.19 ± 0.39 and 4.51 ± 0.24 for the water soaked BGH.

The concentrations of acetic, propionic and butyric acids were significantly lowest (p<0.01) when the raw husk was incubated in the RUSITEC compared to the water soaked husk. This trend was reflected in the short chain fatty acid concentration also. The ratio between the acetic, propionic and butyric acids produced were 65:28:6 for raw BGH and 62:30:8 for water soaked BGH.

Hungate (1966) [8] suggested 62:22:16 as the normal ratio between acetic, propionic and butyric acids in the rumen. It is obvious that while the level of acetate production was within the range suggested by Hungate (1966) [8], the propionate production was on the higher side and the butyrate on the lower side when the BGH was incubated.

The ratios between acetate to propionate were 2.22 ± 1.2 or 2.03 ± 0.05 respectively when the raw or water soaked BGH was incubated in the present study. However the variation was not significant. The values for non-glucogenic ratio (NGR) calculated in the present study were 2.67 ± 0.19 and 2.53 ± 0.05. Since the NGR reported in the present study was within the range suggested by Orskov (1975) [14].

Gas production

The total volume of gas produced was 1.49 ± 0.02 or 1.53 ± 0.02 L/day respectively when the raw or water soaked BGH was incubated. The level of total gas produced in the present study was in agreement with the report of Durand *et al.* (1988) for various concentrate by-products. The significantly high (p<0.01) level of gas produced when water soaked black gram husk was fermented may be attributed to the high level of digestibility of the husk. The significantly high level of gas production with incubation of water soaked husk could also be attributed to the significantly low level of total tannins as suggested by Singh (1978) [16]. The carbon dioxide to methane ratios were 1.90 ± 0.04 or 1.88 ± 0.05 respectively when raw or water soaked husk was incubated in the RUSITEC.

Conclusion

Black gram husk both as the raw or water soaked form qualifies as a feed ingredient of choice to produce greatest efficiency for growth and fattening in ruminants. And when the BGH was evaluated in the laboratory to assess its rumen fermentation pattern it was inferred that compared to the raw BGH the water soaked BGH produce significantly more NH_3-N, microbial protein and short chain fatty acids.

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