



## Nutritive evaluation of pulse *chunies* available in India by rumen simulation technique (Rusitec)

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### ABSTRACT

*Chunies* of pulses viz. black gram (*Vigna mungo*, BGC), bengal gram (*Cicer arietinum*, BnGC), red gram (*Cajanus cajan*, RGC) and green gram (*Vigna radiata*, RGC) were analyzed for their proximate composition. Degradability of dry matter was studied by Rusitec method. The degradability after 48 h of incubation corroborated to the values obtained with standard method. BGC (206.57) and GGC (232.29) are good protein supplements (g/kg DM) and BnGC (406.87) and RGC (309.68) are high in fiber content (g/kg DM). After 48 h of incubation, IVDMD (%) varied significantly from 50.06 (RGC) to 77.81 (BGC). The gas production ranged from 630.00 (BGC) to 1685.00 (BnGC) ml. The effective degradability of DM was higher in BGC and GGC than other *chunies*. It was concluded that nutritive value of BGC and GGC were better compared to BnGC and RGC and may be a good protein source for ruminants.

**Key words:** Agro industrial by-products, IVDMD, NCFR, Pulse *Chunies*, Rusitec

Cereals and legumes are conventionally used as the animal feeds (Fagberno and Arowosoge 1991). Increased consumption of processed food by human leads to production of large quantities of agro industrial by-products (AIBP). Fortunately, indigenous ruminants have the potential to utilise these AIBP with greater efficiency. So, continuous efforts are being made to maximize the incorporation of AIBP in animal feeds to reduce the competition as well as to economize the ration. Pulse *chunies* are major byproduct obtained during the processing of pulse grains for human consumption (NDDB 2012), which consists of broken pieces of endosperm including germs and husks. It constitutes 15–20% of total weight of pulses (Reddy *et al.* 2000). Black gram (*Vigna mungo*), bengal gram (*Cicer arietinum*), red gram (*Cajanus cajan*) and green gram (*Vigna radiata*) are grown as cash crops by the farmers in India. As per FAO (2012), pulses were used to an extent of 6.8 MT and 7.3 MT in developing and developed countries, respectively, in concentrate livestock feeds for livestock. In spite of large availability of *chunies*,

these are not efficiently used in the livestock feeding.

Rumen simulation technique (Rusitec) is an advanced and more precise technique to study the rumen metabolism and digestibilities of the feeds, which simulate the condition of rumen in the laboratory conditions. Thus, the present study was undertaken to estimate the chemical compositions and nutritive values of various pulse *chunies*.

### MATERIALS AND METHODS

**Collection and sample preparation:** Six samples each of *chuni* from red gram (RGC), black gram (BGC), bengal gram (BnGC) and green gram (GGC) were collected from the local markets of Andhra Pradesh and Karnataka, India and pooled and ground to pass through 1 mm sieve size in Wiley mill and eventually mixed thoroughly to reduce the sampling errors and finally stored for further analysis. The samples were ground in a cyclotech to reduce the particle size before estimation of *in vitro* DM degradability (IVDMD).

**Collection of rumen liquor:** Rumen liquor (RL) was collected from 5 freshly slaughtered sheep and then was pooled and strained with 4 layered muslin cloths. Then RL was flushed with carbon dioxide to support the livability of anaerobic organisms. The livability was checked under microscope for confirmation.

**Chemical compositions of the *chunies*:** The *chuni* samples were analyzed for proximate constituents (AOAC 1997) and fibre fractions (Van Soest *et al.* 1991).

**IVDMD of pulse *chunies* by Rusitec:** The IVDMD was

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estimated by artificial rumen simulation technique, carried out in Rusitec (Czerkawski and Breckenridge 1977). To 500 ml of the pooled strained rumen liquor (5 freshly slaughtered sheep), 200 ml of artificial saliva (McDougall 1948) and 100 ml of distilled water were added in each reaction vessel. About 80 g cud was tied tightly in a nylon bag and placed in the sample box of reaction vessel. Then distilled water was added till over flow and cap was tightly screwed and placed in the groove in the water bath. Again these vessels were flushed with carbon dioxide to make the dead space conducive for anaerobic organisms. The flow rate of saliva (0.55 ml/min) and temperature (39°C) was adjusted properly. The microbial density was frequently observed under microscope. After switching on the machine for 24 h, a nylon bag containing 5 g of sample was incubated along with cud for next 24 h. Then the cud was replaced with another nylon bag containing the feed sample. So each feed sample was incubated for 48 h in their respective reaction vessels. After 48 h of incubation, the gas bags were analyzed quantitatively for the amount of gas produced during that period by water displacement method and the effluent collected in the effluent vessels was noted. Initially, gas and effluent were found to be high and then became constant after 5 days as the digestibility of DM remained constant after 4 to 6 days of incubation leading to similar amount of effluent production (standardization) (Czerkawski and Breckenridge 1977). After standardization, bags were taken out of reaction vessel after each 48 h of incubation and were first washed with artificial saliva and the washed out liquor from the bag was placed in the respective reaction vessels as they contained a large population of the microorganisms. In this way, 2–3 washings were done for each nylon bag. Thereafter, the nylon bags were washed under running tap water and squeezed strongly to remove as much liquid as possible till clear water came from the nylon bags with residues. Then these bags were dried in the spin drier (washing machine). The weight loss of the samples represented the DM degraded or digested and expressed as IVDMD in g/kg DM. Then the samples were dried in a hot air oven maintained at 65°C for a period of 3 days and the final weight was taken. The chuni samples were taken in triplicate.

DM disappearance (g/kg DM) =  $[(W_3 - W_4) / W_3] \times 1000$  where, W, empty bag weight; W<sub>1</sub>, weight of bag with feed sample before incubation; W<sub>2</sub>, weight of bag with residue after incubation; W<sub>3</sub>, % DM in the feed sample  $[(W_1 - W) \times DM\%]$ ; W<sub>4</sub>, residue DM weight  $[W_2 - W]$ .

**Cumulative DM degradation by Rusitec:** After standardization, 5 g of these *chunies* were weighed and tied in nylon bag and kept in each reaction vessel for a specified time. So all the reaction vessel were containing 4 bags (one bag of each *chuni*) for 0, 6, 12, 24, 36, 48, 72, 96 h on rotation and the degradation pattern was measured for respective incubations. The DM degraded was calculated for each incubation and the results were analyzed with NAAWAY software for effective degradation of the DM. Modified 2-stage digestion technique of Tilley and Terry

(Goering and Van Soest 1970) using sheep rumen liquor was applied for determining IVDMD for comparison.

**Statistical analysis:** The data obtained from the study were analyzed statistically as per Snedecor and Cochran (1989) and Duncan (1955). Single-way ANOVA was conducted to compare the mean values of the *chunies*. The t-test was conducted to compare the mean results obtained from the above 2 methods.

## RESULTS AND DISCUSSION

**Chemical composition of pulse chunies:** The crude protein (CP) (P<0.01) was highest in GGC followed by BGC, RGC and BnGC (Table 1). Crude fibre (CF) content was highest (P<0.01) in BnGC followed by RGC, GGC and lowest in BGC. Highest (P<0.01) total ash content was in BGC and lowest in RGC. Ramachandra and Nagabhusana (2006) reported similar trend for CP and CF for the pulse tested *chunies*. But few studies reported some variations from present observation which might be due to difference in physical conditions of growing pulses and/or variation in processing technique (Engtipi *et al.* 2006, Chandran 2005, Suryanarayana *et al.* 2006). The CP content in BGC was higher than the values of Reddy *et al.* (2000) and lower than those of Das *et al.* (2007). Ravi *et al.* (2005) and Radhakrishna *et al.* (2002) reported lower CP, higher CF as compared to the present findings. Feedstuffs should contain at least 10% CP for optimum microbial activity in the rumen (Norton 1998), so incorporation of GGC and BGC may be a potential protein source for ruminants which will support microbial activity in the rumen. Our results are comparable to the reports of NDDB (2012) (15–20% CP) for *chunies* which varies with its type.

**IVDMD of pulse chunies by Rusitec:** Digestibility of DM in g/kg DM (P<0.01) was higher in GGC and BGC than BnGC and RGC (Table 2). The DM degradation in BGC at 48 h of incubation was higher to the reported value of 54.23% by Jain (1986). Gas production (ml/24 h) in Rusitec

Table 1. Chemical composition of pulse chunnies (g/kg DM)

Attribute (g/kg DM)	Green gram	Black gram	Bengal gram	Red gram	SEM	P value
Dry matter	949.82 <sup>b</sup>	931.65 <sup>c</sup>	955.13 <sup>a</sup>	951.10 <sup>d</sup>	0.668	0.001
Crude protein	223.29 <sup>a</sup>	206.57 <sup>a</sup>	95.78 <sup>c</sup>	162.24 <sup>b</sup>	8.652	0.001
Ether extract	24.34	21.64	18.02	22.01	2.673	0.540
Crude fibre	159.20 <sup>c</sup>	131.40 <sup>d</sup>	406.87 <sup>a</sup>	309.68 <sup>b</sup>	2.471	0.001
Total ash	85.03 <sup>b</sup>	116.88 <sup>a</sup>	50.32 <sup>c</sup>	40.27 <sup>d</sup>	1.290	0.001
Nitrogen free extract	470.21	488.90	425.67	466.10	17.650	0.250
Lignin	42.59 <sup>a</sup>	30.51 <sup>b</sup>	16.04 <sup>c</sup>	24.96 <sup>bc</sup>	3.506	0.010
Silica	11.04 <sup>a</sup>	9.64 <sup>a</sup>	1.66 <sup>b</sup>	0.77 <sup>b</sup>	0.633	0.001

<sup>a,b,c,d</sup>Means in the row with different superscript differ significantly.

Table 2. *In vitro* DM degradability of common pulse *chunies* estimated by Rusitec

Attribute	Green gram	Black gram	Bengal gram	Red gram	SEM	P value
DM degradation (48 h, g/kg DM)	752.87 <sup>a</sup>	778.13 <sup>a</sup>	516.09 <sup>b</sup>	500.60 <sup>b</sup>	13.512	0.001
Gas production (ml/24 h)	835.00 <sup>b</sup>	630.00 <sup>c</sup>	1685.00 <sup>a</sup>	1630.00 <sup>a</sup>	70.387	0.001
Effluent production (ml/24 h)	630.00	617.50	672.50	665.00	47.935	0.830
Effective degradability (NAAWAY, %)	56.80 <sup>a</sup>	58.80 <sup>a</sup>	35.50 <sup>b</sup>	39.40 <sup>b</sup>	—	0.001

<sup>a,b,c</sup>Means in the row with different superscript differ significantly.

varied significantly among these *chunies* (Table 2). The effective degradability (%) of DM was higher in GGC and BGC as compared to BnGC and RGC.

The gas production ranged from 630 (BGC) to 1685 (BnGC) ml. The variation in gas production could be correlated to the fibre and the protein content of the sample as feedstuffs with high CP results in low gas production (Chenost *et al.* 2001). The quantum of gas produced from GGC and BGC was much lower ( $P < 0.01$ ) than that of BnGC which could be due to variation in their CP content (Table 2). Again, fermentable carbohydrates increase gas production, whereas addition of degradable nitrogen compounds to fibre rich feeds decrease gas production which is attributed to enhanced capture of nutrients and higher production of microbial protein as the carbon source is diverted from gas to microbial protein (Menke and Steingass 1988). Nitrogen released from protein degradation, combined with CO<sub>2</sub> resulted in less methane production leading to lesser gas production (Gatechaw *et al.* 1998) which may be the cause behind the lower gas production from GGC and BGC. The samples containing more crude fibre produced more gas (BnGC and RGC) than the samples with lower crude fibre (GGC and BGC). Methane producers are slow growing and last users of organic matter in the food chain and thus acts upon the indigested materials left after preliminary fermentation (Srinivas and Singh 1998) and thus produces more gas, which may be correlated to the higher gas production from BnGC and RGC (higher CF) than GGC and BGC (low in CF) as fibre fractions are fermented late. A positive correlation exists between non-fibre carbohydrate (NFC) content of feeds and gas production, while CP content is negatively correlated with gas production (Getachew *et al.* 2004, Maheri-Sis *et al.* 2008) which explains the higher gas production in BnGC and RGC. The effective degradability of BGC is superior to other *chunies*.

*Cumulative DM degradation of pulse chunies by Rusitec:* Cumulative DM degradation indicates the total gas

production from a *chuni* at a particular incubation, starting from the beginning. Cumulative DM degradation (g/kg DM) of *chunies* estimated by Rusitec (Table 3) indicated that degradability is a function of time. No significant variation ( $P > 0.05$ ) at 0 and 6 h but significant variations were observed at 12 ( $P < 0.01$ ), 24 ( $P < 0.01$ ), 48 ( $P < 0.01$ ), 72 ( $P < 0.01$ ) and 96 h ( $P < 0.05$ ) of incubations. GGC and BGC showed higher cumulative degradation in Rusitec at 24 to 96 h incubations than BnGC and RGC and no significant difference was observed between the later two. More than half of the digestible DM was digested within 6 h of incubation during which almost all the readily fermentable nutrients were used up, followed by the fermentation of fiber fraction of the sample. After 48 h of incubation very less degradation was observed in all the *chunies* probably due to non-availability of fermentable nutrients as they were used up by this time. The kinetics of DM disappearance (%) of paddy straw in Rusitec was 10.33, 15.62, 22.40, 24.35, 27.50, 30.51 and 33.24 at 0, 2, 6, 12, 24, 48 h intervals (Senthilkumar *et al.* 2012). Similar trend in kinetics of DM degradation was observed with *chunies*. With the above results, BGC and GGC were better available source of nutrients and were also easily degradable in the rumen than

Table 3. Cumulative DM degradation (g/kg DM) of common pulse *chunies* in Rusitec

	Green gram <i>chuni</i>	Black gram <i>chuni</i>	Bengal gram <i>chuni</i>	Red gram <i>chuni</i>	SEM	P value
Time (h)						
0	170.84	138.92	115.98	92.90	43.117	0.64
6	490.70	460.34	315.87	362.82	53.240	0.18
12	557.34 <sup>a</sup>	652.63 <sup>a</sup>	319.90 <sup>b</sup>	374.35 <sup>b</sup>	53.240	0.01
24	707.30 <sup>a</sup>	699.39 <sup>a</sup>	389.56 <sup>b</sup>	423.59 <sup>b</sup>	53.240	0.001
48	746.40 <sup>a</sup>	743.69 <sup>a</sup>	524.67 <sup>b</sup>	447.23 <sup>b</sup>	53.240	0.01
72	807.26 <sup>a</sup>	818.39 <sup>a</sup>	621.05 <sup>b</sup>	496.12 <sup>b</sup>	53.239	0.01
96	819.19 <sup>a</sup>	833.15 <sup>a</sup>	637.85 <sup>b</sup>	578.93 <sup>b</sup>	53.240	0.03

<sup>a,b</sup>Means in the row with different superscript differ significantly.

BnGC and RGC. Chemical composition and nutritive value of these samples are dependent on the processing methods, proportion of grain and husk in the sample and stage of harvest and their geographical locations also.

A slight difference was observed at 48 h degradation while studying the DM degradation (Table 2) and cumulative DM degradation (Table 3) in Rusitec. The reason might be that, in degradation studies (kinetics) only 2 samples, whereas in cumulative degradation, 4 *chuni* samples (1 sample from each *chuni*) were kept in the reaction vessel which might have decreased the population of microorganism available for unit *chuni* sample for fermentation. But the variation observed was small and marginal.

*Comparison of degradability by Rusitec to traditional two stage digestion technique:* Similar DM degradability

Table 4. *In vitro* dry matter degradation (IVDMD) (48 h, g/kg DM) of pulse *chunies* estimated by two methods

Method	Green gram	Black gram	Bengal gram	Red gram	SEM	P value
Modified Tilley and Terry Method	776.20 <sup>b</sup>	803.86 <sup>a</sup>	528.04 <sup>c</sup>	551.07 <sup>c</sup>	9.46	0.001
DM degradation in Rusitec	752.87 <sup>a</sup>	778.13 <sup>a</sup>	516.09 <sup>b</sup>	500.60 <sup>b</sup>	13.51	0.001

a,b,c Means with different superscript differ significantly.

at 48 h of incubation was obtained for the respective *chunies* in modified Tilley and Terry method and Rusitec (Table 4). The higher degradability values observed in modified Tilley and Terry method could be due to the fine grinding of sample by Cyclotech allowing more surface area for microbial fermentation, but more importantly the same trend in DM degradability was observed for all the *chunies* in these methods. Both the techniques showed similar results when compared with earlier reported values in *in vivo* trials (Radhakrishna *et al.* 2002, Jain and Bhaid 1986 a,b,c,d). Previous reports in corroboration with present findings suggest that *in vitro* methods like modified Tilley and Terry and Rusitec are comparable to each other in regards to the DM degradability of the feed samples and can be used as alternatives to one another depending on the available facilities.

BBG, BGC, RGC and BnGC are available in large amounts in India but are rarely used scientifically in formulating TMRS for animals. BGC, BnGC, GGC and RGC were having more than 50% IVDMD with a maximum of 77% (BGC). These are having higher CP, CF and higher DM degradability thus may help in economizing and balancing the ration. GGC and BGC are potential substitute for the conventional feed stuffs in the animal feeding system and which can economize the livestock feeding.

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