Full Length Article

EVALUATION OF NUTRITIONAL CHARACTERISTICS OF DIFFERENT PULSE CHUNNIES USING NEAR INFRARED REFLECTANCE SPECTROSCOPY AND ITS VALIDATION WITH WET CHEMISTRY ANALYSIS

Jagadamba Kunja[1], Ch. Harikrishna[2]*, A. Saratchandra[3], M. Venkateswarlu[4]and M. Gnana Prakash[5]

Department of Livestock Production Management
College of Veterinary Science, P. V. Narsimha Rao Telangana Veterinary University
Rajendranagar, Hyderabad-500 030, Telangana State

ABSTRACT

Nutritional evaluation of different pulse chunnies was carried using Near Infrared Reflectance Spectroscopy (NIRS) and validated with wet analysis for inclusion in livestock feeds. The portable NIRS spectrophotometer FOSS XDS RCA and FOSS 6500 chemometrics software were used for the study. A total of 324 samples of four pulse chunnies (red gram (RGC), green gram (GGC), black gram (BLGC) and bengal gram (BGC)) were assessed using NIRS. It revealed that RGC had significantly (P<0.01) highest DM content than other three samples. The mean CP content was highest (P<0.01) in GGC and lowest in BGC and significantly (P<0.01) higher ether extract (EE) was recorded in BLGC and RGC and lowest in BGC samples. The ash content was significantly (P<0.01) highest in BLGC and lowest in RGC samples. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents were higher (P<0.01) in BGC and lower in GGC samples. Wet analysis shown, the DM content did not differ (P>0.05) among pulse chunni samples, while significantly (P<0.01) higher organic matter was recorded in RGC and lowest in BLGC samples. The CP was higher (P<0.01) in BLGC and lower in BGC and significantly (P<0.01) highest crude fibre recorded for BGC, RGC and was lowest in BLGC and GGC samples. Highest (P<0.05) EE was observed in BLGC while lowest in BGC. There was no significant (P>0.05) difference in nitrogen free extract among pulse chunnies, while ash content was significantly (P<0.01) higher in BLGC and lower in RGC samples. NDF, ADF and ADL were significantly (P<0.01) higher in BGC and lower in GGC samples. The study concludes that the NIRS technique can be used to screen large number of samples in lesser time for using them in livestock feed formulations as there is a high correlation between NIRS and conventional feed analysis.

Key words: NIRS, nutrients screening, pulse chunni, wet chemistry analysis

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[1]Assistant Professor and Ph.D. Scholar

[2]Professor, Dept. of Livestock Farm Complex, corresponding author Email Id: drhkvet@gmail.com

[3]Associate Dean, College of Dairy Technology, Kamareddy.

[4] Professor and Univ. Head, Dept. of Animal Nutrition, CVSc., Mamnoor.

[5] Professor and Univ. Head, Dept. of Animal Genetics and Breeding & and; Comptroller, PVNRTVU.

INTRODUCTION

Analysis of animal feed stuffs for major nutrients is one of the important pre requisite in livestock management as knowledge of nutrients of feeds and nutrients required by animals is essential to meet animal feeding standards (Tedeschi *et al.,* 2010). Conventional feed analytical methods have been used to evaluate nutritional composition of feed materials, however cost, time and labour requirements are the short comings in this process while fast and reliable non-destructive methods are more attractive and acceptable than laborious techniques (Carlier *et al*., 2009). Likewise, requirements for new analytical methods emphasize performance, sensitivity, reliability, speed, simplified use and low cost for high volume (Cheli *et al*., 2012). Increased power and widespread use of personal computers and the concomitant development of multivariate statistical procedures in the field of chemometrics have resulted in the use of NIRS as an alternative to traditional methods for determining nutritive value of feeds and forages, as it is rapid and can perform multiple analysis with one operation and very limited information is available on the use of NIRS to study the chemical composition of pulse chunnies. Bruno-Soares *et al*. (1998) and Decruyenaere *et al.* (2009) suggested that the most common chemical traits predicted from forages using NIRS include moisture, DM, OM, CP, NDF, ADF, total ash, crude fat or ether extract and ADL.

According to FAO (2009), pulses were used to an extent of 6.8 MT and 7.3 MT in developing and developed countries, respectively in concentrate livestock feeds. Chunni is actually a raw and dusty mixture of grain and husk of pulses and can be used as potential feed substitute for livestock (Saha and Ray, 1998). Chunni consists of broken pieces of endosperm including germ coat and husk obtained as by-product during the processing of pulses (NDDB, 2012) and constitute 15-20 % of total weight of pulses (Reddy *et al*., 2000). Various pulses namely red gram (*Cajanus cajan*), black gram (*Vigna mungo*), green gram (*Vigna radiata*) and bengal gram (*Cicer arietinum*) are grown as food crops by farmers in India. In spite of availability in large quantities, these pulse chunnies are not efficiently used in livestock feeding. Hence, the present study was planned to assess nutritional characteristics of four different pulse chunnies using NIRS tools and its validation with conventional feed analysis. NIRS is a physical technique with the potential of allowing cost effective, rapid and accurate determination of the chemical composition and nutritive value of different feeds and forages (Flinn, 1991), a high correlation between NIRS and chemical analysis shows the potential use of it for prediction of feed quality (Agelet and Hurburgh, 2010).

MATERIALS AND METHODS

Collection and preparation of samples and site of the study

A total of 324 samples {Red gram chunni (RGC) -100, Black gram chunni

(BLGC) -91, Green gram chunni (GGC) -59 and Bengal gram chunni (BGC) -74} were collected from 22 different places in the states of Telangana and Andhra Pradesh for this study. The samples were prepared for analysis by grinding to uniform size using Willey mill and allowed to pass through 1 mm screen, mixed thoroughly to reduce errors and finally stored in air tight containers for further analysis. Nutritional characteristics of chunnies were studied using a NIRS in Nutritional laboratory at International Livestock Research Institute located at ICRISAT, Patancheru, India and compared with conventional wet analysis tests carried out at Innovation Grant under National Agricultural Higher Education Project (IG-NAHEP) Feed Analytical Laboratory, College of Veterinary Science, Korutla, Jagtial.

Analysis of pulse chunni samples using NIRS technique

The feed samples were dried at 60 degrees for 48 hours, ground to pass through 1mm sieve for achieving uniform particle sizes. Chunni samples were filled in 50 mm diameter round NIRS cups and the filled cups were used for scanning. The feed samples were scanned in spinning sample cells at 2 nm intervals for their measurements, such as inverse of the reflectance (log 1/R) over the NIR spectral range from 400 to 2498 nm in reflectance mode by using a spectrophotometer FOSS XDS RCA and FOSS 6500 (FOSS, Denmark) and Win ISI II & IV (version 1.50 and 4.6.8) was used as the chemometrics software (Infrasoft International, LLC, PA, USA).

The calibration equations were obtained by using the modified partial least squares method (MPLS) which is usually recommended approach for natural product estimations (Shenk and Westerhaus, 1991). Different mathematical treatments were used on first and second derivatives to get the optimum number of regression coefficients. The NIRS global calibration equation for all general feed samples were then used to predict unknown samples (around 2698 to 5090 feed samples) of the related population. The feed constituents of the unknown feed samples predicted were namely dry matter, ash, nitrogen, neutral detergent and acid detergent fibre and acid detergent lignin.

Evaluation of pulse chunni samples using wet chemistry analysis

The collected chunni samples were analysed for their proximate constituents in duplicate or triplicate (AOAC, 2012) and for fibre fractions (Van Soest *et al.,* 1991).

Statistical analysis

The data obtained from the study were analyzed statistically according to Snedecor and Cochran (1994). Analysis of variance (ANOVA) was used to test the significance of various treatments and the difference among the treatment means was tested for significance by Duncan's multiple-range and F Test (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical composition estimated by NIRS technique (% DM Basis)

The chemical composition of RGC, GGC, BLGC and BGC samples obtained through NIRS and results (Mean ± SE; %) presented in Table 1, showing a highly significant (P<0.01) difference in proximate and cell wall constituents among them. The mean DM content is ranged from 89.49 ± 0.06 (BGC) to 90.95 ± 0.05 (RGC). The DM was significantly (P<0.01) higher in RGC samples. Similar finding was reported by Williams (2001) for agricultural products. The CP content ranged from 7.74 ± 0.39 (BGC) to 22.08 ± 0.36 (GGC). The CP content was highest (P<0.01) in GGC and lowest in BGC. These results are in agreement with Bruno-Soares *et al.* (1998) who reported coefficient of determination (R^2) value of 0.98 for sorghum sample and Kalyan Chakravarthy (2015) reported the CP content of R^2 as 0.97 and co-efficient of determination in cross validation (1-VR) as 0.91, for dried sorghum. Similar to this study, Swart *et al*. (2012) also reported prediction values for CP as 0.97 % and 0.74 %, respectively in 94 samples used in independent validation. The EE content is ranged from 0.79 ± 0.07 (BGC) to 2.11 ± 0.05 (BLGC). Significantly (P<0.01) higher EE was seen in BLGC and RGC and was lowest in BGC samples. These findings are in agreement with Swart *et al*. (2012), who reported prediction values for EE was 0.89 % and 0.50 %, respectively. The total ash (TA) content ranged from 3.06 ± 0.08 (RGC) to 7.30 ± 0.29 (BLGC). The TA content was significantly (P<0.01) highest in BLGC and was lowest in RGC samples. The R^2 (0.77) and 1-VR (0.48) values reported by Kalyan Chakravarthy (2015) are in agreement with results of present study.

Ash content represents the inorganic portion comprising minerals and silica which are unavailable to the animal. The NIRS data recorded for the ash is rather precise in the present study. This might be because of the ability of minerals which are present in good quantity in chunni samples able to absorb in the NIR region. However, even better calibrations can be made for future prediction of ash by including more number of samples with accurate laboratory results. The NDF content ranged from 41.72 ± 0.92 (GGC) to 66.23 ± 0.99 (BGC) and was significantly (P<0.01) higher in BGC and lower in GGC. These results are in agreement with the findings of Swart *et al*. (2012), who reported prediction values for NDF were 0.95 % and 2.81 %, respectively and in partial agreement with Bruno-Soares *et al.* (1998) and Kalyan Chakravarthy (2015), who reported R^2 value of 0.93 in sorghum by using 110 calibration samples and 0.82 in dried sorghum samples.

The ADF content ranged from 25.31 ± 0.70 (GGC) to 56.45 ± 1.35 (BGC) and was highest (P<0.01) in BGC and lowest in GGC (Table 1). These results corroborate with the findings of Kalyan Chakravarthy (2015) and Bruno-Soares *et al.* (1998), who developed good NIRS calibration with R^2 (0.95) on dried sorghum. Swart *et al*. (2012) also reported the prediction values for ADF as 0.89 %

Table 1. Nutritional characteristics of pulse chunnies evaluated using Near Infrared Reflectance Spectroscopy (NIRS)

Nutrient	Chemical composition** (on % DMB; Mean ± SE)				SEM	P Value
	Red gram chunni (n=100)	Green gram chunni (n=59)	Black gram chunni (n=91)	Bengal gram chunni (n=74)		
Chemical composition:						
Dry matter	$90.95^{d} \pm 0.05$	$90.15^{c} \pm 0.05$	$89.88^{b} \pm 0.08$	$89.49^{a} \pm 0.06^{a}$	0.045	0.000
Crude protein	$14.29^{b} \pm 0.21$	$22.08^{c} \pm 0.36$	$20.25^{c} \pm 0.37$	$7.74^{a} \pm 0.39^{a}$	0.144	0.000
Ether extract	$2.10^{c} \pm 0.07$	$2.06^{b} \pm 0.04$	$2.11^{c} \pm 0.05$	$0.79^{a} \pm 0.07^{a}$	0.338	0.000
Total ash	$3.06^{a} \pm 0.08$	$6.36^{b} \pm 0.32$	$7.30^{c} \pm 0.29$	$6.59^{b} \pm 0.11^{b}$	0.043	0.000
Cell wall constituents:						
Neutral detergent fibre	$62.23^{c} \pm 0.76$	$41.72^{a} \pm 0.92$	$47.01^{b} \pm 0.98$	$66.23^{d} \pm 0.99$	0.708	0.000
Acid detergent fibre	$38.24^{c} \pm 0.42$	$25.31^{a} \pm 0.70$	$31.12^{b} \pm 0.67$	$56.45^{d} \pm 1.35$	0.731	0.000
Acid detergent lignin	$6.38^{c} \pm 0.08$	$4.75^{a} \pm 0.11$	$5.61^{b} \pm 0.13$	$8.29^{d} \pm 0.14$	0.089	0.000

n = Number of samples; **P** = Probability Value; **SEM**: Standard Error Mean

^{abcd}Means with different superscripts in a row differ significantly $^{**}(P<0.01)$

and 2.67 %, respectively. The ADL content ranged from 4.75 ± 0.11 (GGC) to 8.29 ± 0.14 (BGC), and was significantly (P<0.01) higher in BGC and lowest value in GGC. The reports of Kalyan Chakravarthy (2015) who stated R^2 coefficients for ADL of 0.49 and 1-VR values of 0.04 in sorghum samples, are in agreement with present findings. Bruno-Soares *et al.* (1998) developed satisfactory NIRS calibration with R^2 (0.73) for sorghum using 109 samples, when compared with the present findings. Stubbs *et al*. (2010) findings revealed the coefficient of determination (R^2; 0.72) and 1-VR (0.67) values for ADL in cereals, are comparatively better than the present study. The results obtained in the present study are in concurrence with Reeves (1988), who stated the precision in estimation of lignin by NIRS is generally lower than NDF or protein due to relatively low ADL content in samples. However, the accuracy of calibration can further be improved by including more number of samples collected at different stages of maturity since lignin content can vary with part of the plant. The present findings has shown that, the calibration model

Table 2. Chemical composition of pulse chunnies by conventional wet analysis

Nutrient	Chemical composition[#] (on % DMB; Mean ± SE)				SEM	P Value
	Red gram chunni (n=6)	Green gram chunni (n=6)	Black gram chunni (n=6)	Bengal gram chunni (n=6)		
Proximate composition :						
DM	90.30 ± 0.46	89.99 ± 1.00	90.22 ± 0.50	91.79 ± 0.71	0.359	0.284
OM**	96.16 ± 0.04^{c}	93.54 ± 0.15^{b}	91.62 ± 0.49^{a}	96.08 ± 0.73^{c}	0.448	0.000
CP**	16.40 ± 0.26^{b}	22.08 ± 0.36^{c}	26.88 ± 0.21^{d}	12.93 ± 0.12^{a}	1.328	0.000
EE*	2.13 ± 0.10^{b}	2.11 ± 0.18^{b}	2.14 ± 0.09^{b}	0.89 ± 0.20^{a}	0.089	0.028
CF**	22.93 ± 1.14^{b}	10.20 ± 0.82^{a}	9.29 ± 0.66^{a}	29.57 ± 7.56^{c}	2.539	0.002
NFE	54.71 ± 1.14	59.16 ± 0.60	53.32 ± 0.67	52.71 ± 8.35	1.989	0.963
Ash**	3.83 ± 0.04^{a}	6.45 ± 0.15^{b}	8.37 ± 0.49^{c}	3.90 ± 0.73^{a}	0.448	0.000
Cell wall constituents:						
NDF**	72.25 ±0.95^{b}	70.79 ± 1.21^{b}	60.16 ± 1.57^{a}	71.11 ± 2.92^{b}	1.330	0.000
ADF*	40.48 ± 0.75^{ab}	21.55 ± 1.95^{a}	26.50 ± 4.18^{a}	36.97 ± 9.10^{a}	2.871	0.055
ADL**	6.75 ± 0.08^{c}	4.98 ± 0.13^{a}	5.78 ± 0.20^{b}	8.47 ± 0.04^{d}	0.278	0.000
Cellulose	2.12 ± 1.04	3.09 ± 4.16	1.56 ± 0.78	6.51 ± 5.88	1.788	0.599
Hemi-cellulose	31.77 ± 1.62	49.24 ± 2.97	33.65 ± 4.64	34.14 ± 1.56	3.346	0.227

[#]Each value is an average of duplicate analysis; **n** = Number of samples; **P** = Probability Value; **SEM**: Standard Error Mean;

[abcd] Means with different superscripts row wise differ significantly [*](P<0.05); [**](P<0.01)

is 'satisfactory' and indicated the usefulness of NIRS for prediction of proximate and cell wall constituents effectively (Shenk and Westerhaus, 1991).

Chemical composition estimated by conventional wet analysis (% DM Basis)

The conventional feed analysis (Table 2) revealed that, the DM content of different chunni samples was in the range of 89.99

± 1.00 to 91.79 ± 0.71 and no significant difference (P>0.05) was observed among them. The organic matter was in the range of 91.62 ± 0.49 to 96.16 ± 0.04 and significantly (P<0.01) higher OM observed in RGC and lowest in BLGC.

There was a wide variation in CP content of samples with BLGC (P<0.01) having a maximum (26.88 ± 0.21) and a least value in BGC (12.93 ± 0.12) samples. Compared to present findings, Parthasarathi *et al.* (2016) reported highest (P<0.01) CP in GGC and BLGC samples. The crude fibre was in the range of 9.29 ± 0.66 to 29.57 ± 7.56, with significantly (P<0.01) higher CF in BGC and RGC and lowest in BLGC and GGC samples. Similar findings were reported by Parthasarathi *et al.* (2016), who reported highest CF (P<0.01) in BGC and lowest in BLGC. Ramachandra and Nagabhusana (2006) reported similar trend for CP and CF for the pulse chunni samples. 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 and Suryanarayana *et al.*, 2006). The CP content in BLGC obtained in this study was higher than the values of Reddy *et al*. (2000) and lower than those of Das *et al*. (2007). Ravi *et al*. (2005) also reported lower CP and higher CF as compared to the present findings, the feedstuffs should contain at least 10 % CP for optimum microbial activity in the rumen, hence incorporation of BLGC and GGC may be a superior protein source for ruminants to support microbial activity in the rumen. Our results are comparable to the reports of NDDB (2012) (15– 20 % CP), which merely depends on the type of chunni locally available for use as livestock feed. The variation in CP content may be due to differences in the soil type and fertility, variety, cultivation practices and proportion of broken pulse pieces and husk in chunni (Patel, 1961 and Ray, 1978). The EE content was in the range of 0.89 ± 0.20 (BGC) to 2.14 ± 0.18 (BLGC) revealing that the highest (P<0.05) EE was recorded in BLGC and lowest in BGC samples. The present findings corroborating the reports of Parthasarathi (2012), who reported higher EE in BLGC and lower in RGC and Ravi *et al*. (2005) also reported similar findings. On contrary, Engtipi *et al.* (2006) reported lower EE levels in RGC samples. The total ash (TA) content recorded was significantly (P<0.01) highest in BLGC and lowest in BGC and RGC samples and was quantified as 8.37 ± 0.49, 6.45 ± 0.15, 3.90 ± 0.73, 3.83 ± 0.04 %, respectively. Similar findings were reported by Ravi *et al*. (2005) and Parthasarathi *et al.* (2016), while Radhakrishna (1999) reported lower levels of TA in GGC.

The NDF was in the range of 60.16 ± 1.57 (BLGC) to 72.25 ± 0.95 (RGC) and was significantly (P<0.01) higher in RGC and least value in BLGC samples. Similar findings were reported by Rao *et al.* (1998), Reddy *et al.* (2002) and Parthasarathi (2012). The NDF recorded was higher than the reports of Engtipi *et al.* (2006). The NDF for GGC (70.79 ± 1.29) was higher than the findings of Radhakrishna *et al.* (2002) and NDF of BLGC (60.16 ± 1.57) was higher than the reports of

Parthasarathi (2012). For BGC, the NDF value (71.11 ± 2.92) was higher than the reports of Awadhesh *et al.* (1998). The ADF is in the range of 21.55 ± 1.95 (GGC) to 40.48 ± 0.75 (RGC) and was highest (P<0.05) in RGC and lowest in GGC samples. Similar findings were reported by Parthasarathi (2012) though the trend is similar, the ADF for RGC (40.48 ± 0.75) was higher than the findings of Engtipi *et al.* (2006) and Parthasarathi (2012). For GGC, the ADF (21.55 ± 1.95) was lesser than the reports of Parthasarathi (2012). Acid detergent lignin (% DMB) was in the range of 4.98 ± 0.13 (GGC) to 8.47 ± 0.04 (BGC) and was significantly (P<0.01) higher in BGC and lower in GGC samples (Table 2). Lignin content recorded for RGC was lower than the findings of Engtipi *et al.* (2006) and similar to the findings of Chandran (2005) and higher than Parthasarathi (2012). The values reported for ADL in GGC by Radhakrishna *et al.* (2002) and Parthasarathi (2012) was in accordance with the present observations and Jain and Bhaid (1986) reported higher ADL content than this study. For BLGC, the reports of Rao *et al.* (1998) revealed higher lignin content, while Parthasarathi (2012) reported lesser lignin content than this study. ADL content in BGC was higher compared with findings of Das *et al.* (2007) and Parthasarathi (2012). The variations seen in the present study in proximate and cell wall constituents in common pulse chunnies might be due to genotype, climate, minerals level of soil and agronomic factors like application of various fertilizers which decides the mineral content of crop residues (Khanum *et al.*, 2007 and Parthasarathi, 2012).

Relationship between NIRS and wet chemistry methods

The correlation coefficients among chemical composition of various pulse chunnies between NIRS and conventional wet chemistry pertaining to ash and ether extract was positive, while significantly (P<0.01) positive correlation was observed in case of DM (0.937) for RGC samples. The correlation of ether extract, NDF, ADF and ADL was positive but significant negative (P<0.01) correlation was noted for ash content (-0.977) in GGC samples. The correlation of all proximate principles was positive for BLGC samples and positive correlation was also observed for DM, CP, EE, and ADL, while significantly (P<0.01) positive results were noted for ash and ADF (0.937 and 0.979, respectively) contents pertaining to BGC samples. These results are consistent with the findings of Kalyan Chakravarthy (2015) and Bruno-Soares *et al.* (1998) and similar to this study, Swart *et al*. (2012) also reported prediction values obtained by NIRS technique can be used for independent validation.

CONCLUSION

It is concluded that, the pulse screenings of the red gram, green gram, bengal gram and black gram revealed that they are rich in energy and protein which can be fed to the livestock economically in the place of conventionally used grains. The proximate composition and all the cell wall constituents recorded using wet chemistry analysis are similar to the results obtained through NIRS technique, revealing that the NIRS technique

is a potential tool, comparatively easy, relatively cheap and large number of samples can be screened in lesser time with minimum possible efforts compared to conventional method for inclusion in livestock feeds as there is a high correlation between NIRS and conventional wet chemistry analysis.

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