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Morphological, Chemical and *In Vitro* Traits for Prediction of Stover Quality in Pearl Millet for Use in Multidimensional Crop Improvement

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ABSTRACT

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The paper reports on the variations in organic matter digestibility (OMD), organic matter intake (OMI), digestible organic matter intake (DOMI) and nitrogen (N) balances of 40 pearl millet stover fed to sheep *ad libitum* and investigated relationships between these *in vivo* variables and morphological, chemical and *in vitro* stover quality traits. Highly significant differences ($P<0.0001$) were observed for all *in vivo* variables. Plant height and stem diameter were consistently significantly (at least $P=0.0002$) inversely related to OMD, OMI, DOMI and N balance. Except for OMD ($P=0.16$) stover nitrogen content was significantly (at least $P=0.0005$) positively related to OMI, DOMI and N balance. The fiber constituents neutral (NDF) and acid (ADF) detergent fiber and acid detergent lignin (ADL) were consistently inversely related to OMD, OMI, DOMI and N balance. (at least $P=0.0005$). Stover *in vitro* digestibility, metabolizable energy content and extent and rate of *in vitro* gas production were highly positively (at least $P<0.0009$) related to the *in vivo* variables while lag and half time of *in vitro* gas production were negatively associated (at least $P=0.0004$) with the *in vivo* variables. Combined morphological, chemical and *in vitro* variables in stepwise multiple regressions accounted for 70 to 84%% of the variations observed in the *in vivo* variables. Application of stringent cross validation procedures reduced the variation in OMD, OMI, DOMI and N balance accounted for by the combined morphological, chemical and *in vitro* variables mostly moderately to 71, 49, 79 and 76%, respectively. Relatively simple traits can predict stover quality in breeding programs for dual-purpose pearl millet cultivars.

Key words: Stover quality, Pearl millet, Multidimensional crop improvement.

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INTRODUCTION

Pearl millet provide a crucial fodder resource for ruminant animals in smallholder crop-livestock systems in most of the arid and semi-arid zones of the Indian subcontinent (Kelley and Rao, 1995). This is especially the case where (1) the dry season is too long (6 months) for native pasture resources to maintain animals until the next rainy season, and/or (2) an increased population density has drastically reduced the area of fallow/ common property land which traditionally provided dry season grazing. However, key criteria for fodder quality such as protein content, digestibility and metabolisable energy content of pearl millet stover are often poor. Attempts to improve stover quality by chemical treatments such as ensiling with urea found little acceptance among farmers and stover upgrading is now largely targeted through genetic improvement (Reddy *et al.*, 1995; Hash *et al.*, 2000). In fact, estimates based on *ex-ante* impact assessments predicted that improvement of stover quality through genetic enhancement can result in benefit: cost ratios of 15: 1 and higher livestock productivity (Kristjanson and Zerbini, 1999). Multidimensional crop improvement requires the close collaboration of livestock nutritionist and plant breeder. Livestock nutritionists have proposed many laboratory traits for fodder quality assessments, but validation of these indicators through actual livestock productivity trials are limited, and often applied only to the higher quality feedstuffs used in industrialized livestock feeding. Basing dual-purpose millet genetic improvement work on untested laboratory traits could clearly present a major set back to the breeding objective. The present work therefore, compares and tests a wide range of morphological measurements (botanical fraction, stem diameter, residual green leaf area, plant height), chemical constituents (nitrogen, the fiber constituents NDF, ADF and ADL), *in vitro* (rate and extent of *in vitro* gas production), apparent digestibility and metabolisable energy in their ability to predict the organic matter digestibility, organic matter intake, nitrogen balance and digestible organic matter intake of 40 millet stover fed to sheep.

MATERIALS AND METHODS

Pearl millet stover used

A total of 40 pearl millet stovers comprising 21 cultivars (including experimental varieties) were tested. Two cultivars were grown at different management (2 soil types, 2 dates of harvest) and 7 cultivars were replicated in the fields and stover from both field reps were fed. The stover was harvested after grain maturity, chopped with a mechanical chopper (Hardcase) and artificially dried in diesel-heated drying bins to a residual moisture content of $<12\%$, filled in gunny bags and barn-stored till feeding trials

Stover analysis and feeding trials

At harvest 15 plants from 4 replications were fractionated into leaf blade, leaf sheath and stem. Stem diameter (SD) was taken with a caliper near the ground and residual green leaf area was measured using a leaf area meter. Stover and stover fractions were analyzed for nitrogen (N) by auto-analyzer, neutral detergent fiber (NDF) and acid detergent fiber (ADF), acid detergent lignin (ADL) by Goering and Van Soest (1970), *in vitro* apparent digestibility by Menke and Steingass (1988) and for rate and extent of *in vitro* gas production by Blümmel and Ørskov (1993) methods.

Six growing male *Deccani* sheep of mean body weight of about 20 kg were allocated to any one treatment and kept in metabolic cages facilitating measurements of feed intake, feed refusals and faeces voided and urine excretion by urinary funnels. The sheep were accustomed to a stover for two weeks followed by a 10-days faecal and urine collection period. Measured were organic matter digestibility (OMD) and intake (OMI) nitrogen balance, and digestible organic matter intake (DOMI).

Statistical analysis

The computer program SAS Version 9.1 (2006) was used. Differences in OMD, OMI, nitrogen balance and DOMI were analyzed by ANOVA using SAS Proc GLM. Simple linear relationships between morphological, chemical and *in vitro* measurement and *in vivo* data were analyzed by SAS Proc Corr. Multiple linear regression analysis including all morphological, chemical and *in vitro* measurement to predict OMD, OMI, nitrogen balance and DOMI were conducted by Stepwise methods using SAS Proc REG, setting a $P<0.05$ probability level for entry of a variable into a model. SAS cross validation procedures where observations from the variable to be predicted are not used in the development of the regression equation.

RESULTS AND DISCUSSION

Means and ranges in OMD, OMI, nitrogen balance and DOMI observed when feeding the 40 pearl millet stover to sheep are presented in Table 1. Significant ($P<0.05$) differences between different stover were observed for OMD, OMI, nitrogen balance and DOMI. The means and ranges observed *in vivo* across all 40 stover were very similar when restricted to the 21 cultivars grown under comparable conditions i.e. without variations imposed by variation in soil and date of harvest conditions except for the highest nitrogen balance (0.275 g/kgLW$^{0.75}$) which was observed with a cultivar harvested at physiological rate than full grain maturity. Highest cultivar-dependent nitrogen balance observed was (0.11 g/kg LW$^{0.75}$/d).Clearly considerable variations exist among cultivars in the potential to promote livestock productivity. A positive nitrogen balance indicates

Table 1. Mean, ranges and least significant differences for organic matter digestibility (OMD), organic matter intake (OMI), nitrogen balance (N-bal.) and digestible organic matter intake (DOMI) observed in forty pearl millet stover fed *ad libitum* to sheep.

Variable	Mean	Range	LSD
OMD (%)	55.2	47.7 to 62.5	2.5
OMI (g/kg LW$^{0.75}$/d)	48.9	36.9 to 59.6	5.9
N balance. (g/kg LW$^{0.75}$/d)	-0.011	-0.228 to 0.275	0.072
DOMI (g/kg LW$^{0.75}$/d)	27.1	18.7 to 35.1	3.0

body tissue accumulation (McDonald *et al*. 1988), in other words animals have gained weight despite been fed a sole pearl millet stover ration. Relative to live weight the mean intake of pearl millet was about (2.5%) a figure which is frequently used for crop residue based diets (NIANP, 2003). However, on higher quality pearl millet stover OMI could reach close to 3 percent of live weight which will present levels of energy intake above those required for maintenance requirements on pearl millet stover (Blümmel *et al*., 2007).

Correlations between morphological, chemical and *in vitro* stover traits and OMD, OMI, nitrogen balance and DOMI are reported in Table 2. Generally chemical and *in vitro* fermentation traits exhibited higher correlation with *in vivo* measurements compared to morphological traits. Interestingly variations in leaf blade: leaf sheath: stem ratio and in residual green leaf area had little effect on either OMD, OMI, nitrogen balance or DOMI. Plant height and stem diameter were consistently inversely related to any of the *in vivo* measurements with plant height showing slightly stronger correlations than stem diameter. Plant height and stem diameter are easily measured in the field and the two traits might be useful as very preliminary screening for stover quality. Kelley *et al*. (1996) investigated farmer perception of pearl millet stover quality and found the strongest preference to be for thin stems which agrees with the correlations observed in Table 2. In the work of Kelley *et al*. (1996), farmers did also prefer tall cultivars while tallness in the present work was inversely associated with *in vivo* measurements. It is conceivable that tallness preference relates more to stover yields than stover quality since quantity and quality traits are sometimes difficult to differentiate in rankings for farmer perception.

Among the chemical traits, NDF showed consistently the strongest (negative) relationship with OMD, OMI, nitrogen balance and DOMI. Next to NDF, ADF showed a similar trend and this was followed by lignin. Except for OMD, nitrogen content was significantly correlated to OMI, N balance and DOMI. Low nitrogen content is often considered the most severe limitation of cereal crop residues since rumen microbes require a minimum about 1 to 1.2% of nitrogen in the feed (Van Soest, 1994). However, in the present work fiber constituents and particularly measurements of *in vitro* digestibility and metabolisable energy content showed higher correlations with the *in vivo* measurements than stover nitrogen content, rejecting the assumption of nitrogen content been the most limiting factor in pearl millets stover quality.

In vitro digestibility and metabolisable energy content were closer related to the *in vivo* measurement than kinetic variables of *in vitro* gas production. The other variables-extent of gas production and rate of gas production were positively correlated to *in vivo* parameters although the extent of correlation was lesser than *in vitro* digestibility and metabolisable energy. Similarly the lag phase and half time of gas production were negatively correlated with *in vivo* measurements. Several of the chemical and *in vitro* traits accounted for more than 60 percent of the variations in the *in vivo* measurements and are therefore, suitable for use in accurate phenotyping for stover quality.

Table 2. Correlations between morphological, chemical and *in vitro* stover traits and organic matter digestibility (OMD), organic matter intake (OMI), nitrogen balance (N-bal.) and digestible organic matter intake (DOMI) observed in forty pearl millet stover fed *ad libitum* to Deccani sheep

Trait	OMD	OMI	N balance	DOMI
Morphological stover traits				
Plant height	-0.66 (<0.0001)	-0.66 (<0.0001)	-0.73 (<0.0001)	-0.71 (<0.0001)
Stem diameter	-0.55 (0.0002)	-0.63 (<0.0001)	-0.60 (<0.0001)	-0.67 (<0.0001)
Leaf blade proportion	0.39 (0.01)	0.23 (0.16)	0.32 (0.04)	0.48 (0.0019)
Leaf sheath proportion	-0.18 (0.26)	0.24 (0.14)	0.09 (0.56)	-0.0004 (0.99)
Stem proportion	-0.34 (0.029)	-0.28 (0.07)	-0.34 (0.03)	-0.47 (0.002)
Green leaf area	0.30 (0.056)	0.23 (0.15)	0.31 (0.048)	0.30 (0.058)
Chemical stover traits				
Nitrogen	0.22 (0.16)	0.61 (<0.0001)	0.60 (<0.0001)	0.52 (0.0005)
Neural detergent fiber	-0.79 (<0.0001)	-0.74 (<0.0001)	-0.82 (<0.0001)	-0.85 (<0.0001)
Acid detergent fiber	-0.59 (<0.0001)	-0.66 (<0.0001)	-0.80 (<0.0001)	-0.73 (<0.0001)
Acid detergent lignin	-0.77 (<0.0001)	-0.54 (0.0003)	-0.77 (<0.0001)	-0.70 (<0.0001)
In vitro fermentation and kinetic traits				
In vitro digestibility	0.85 (<0.0001)	0.66 (<0.0001)	0.86 (<0.0001)	0.82 (<0.0001)
Metabolisable energy	0.85 (<0.0001)	0.66 (<0.0001)	0.84 (<0.0001)	0.82 (<0.0001)
Extent of gas production	0.63 (<0.0001)	0.64 (<0.0001)	0.72 (<0.0001)	0.73 (<0.0001)
Rate of gas production	0.73 (<0.0001)	0.50 (0.0009)	0.66 (<0.0001)	0.70 (<0.0001)
Lag phase of gas production	-0.54 (0.0004)	-0.64 (<0.0001)	-0.68 (<0.0001)	-0.60 (<0.0001)
Half time of gas production	-0.73 (<0.0001)	-0.62 (<0.0001)	-0.74 (<0.0001)	-0.73 (<0.0001)

Table 3. Stepwise multiple correlations between morphological, chemical and *in vitro* stover traits and organic matter digestibility (OMD), organic matter intake (OMI), nitrogen balance (N-bal.) and digestible organic matter intake (DOMI) observed in forty pearl millet stover fed *ad libitum* to Deccani sheep

Trait and Model	Y-Variable	R^2	Probability
Model: Metabolisable energy	OMD	0.73	0.0001
Step 1: Metabolisable energy		0.73	0.0001
Model: NDF + Sheath Ratio + SD	OMI	0.70	0.0001
Step 1: NDF		0.54	0.0001
Step 2: Sheath Ratio		0.10	0.003
Step 3: SD		0.06	0.012
Model: NDF + SD + Extent	DOMI	0.84	0.0001
Step 1: NDF		0.73	0.0001
Step 2: SD		0.08	0.0005
Step 3: Extent		0.03	0.012
Model: IVOMD + Nitrogen + ADF	N-Bal	0.82	0.0001
Step 1: IVOMD		0.73	0.0001
Step 2: Nitrogen		0.05	0.005
Step 3: ADF		0.04	0.01

Table 3 presents relationships between combinations of morphological, chemical and *in vitro* traits and OMD, OMI, nitrogen balance and DOMI in stepwise multiple regressions. Except for OMD (where metabolisable energy content accounted for 73% of the variation therein) combinations of traits in stepwise multiple regression accounted for higher parts of the variation in *in vivo* measurements than any stover trait alone. The R^2 for OMI using a combination of NDF, sheath ratio and stem diameter was 0.70 while for DOMI a value of 0.84 was obtained using the NDF, stem diameter and extent of gas production. Similarly for nitrogen balance the R^2 was 0.82 using IVOMD, nitrogen and ADF. Clearly combinations of traits could predict important *in vivo* measurements such as DOMI and nitrogen balance with high accuracy. These *in vivo* measurements are very closely associated with actual livestock productivity such as meat and milk production. It will therefore, be feasible to predict economic benefit from meat and milk production upon feeding stover from certain cultivars of pearl millet. This information will support breeding and selections in case of trade-off effects between grain and stover traits.

Table 4 presents relationships between only chemical laboratory traits and OMD, OMI, nitrogen balance and DOMI. Except for OMI (where NDF and nitrogen content accounted for 61% of the variation therein), higher parts of the variation in *in vivo* measurements were accounted for by combination of chemical traits than by any trait alone.

Table 4. Stepwise multiple correlations between chemical stover traits and organic matter digestibility (OMD), organic matter intake (OMI), nitrogen balance (N-bal.) and digestible organic matter intake (DOMI) observed in forty pearl millet stover fed *ad libitum* to Deccani sheep

Laboratory trait and Model	Y-Variable	R^2	Probability
Model: NDF + ADL	OMD	0.71	0.0001
Step 1: NDF		0.62	0.0001
Step 2:ADL		0.09	0.001
Model: NDF + Nitrogen	OMI	0.61	0.0001
Step 1: NDF		0.54	0.0001
Step 2: Nitrogen		0.07	0.01
Model: NDF + ADF	DOMI	0.76	0.0001
Step 1:NDF		0.73	0.0001
Step 2:ADF		0.03	0.03
Model: ADF+ NDF+ Nitrogen	N-Bal	0.82	0.0001
Step 1: ADF		0.69	0.0001
Step 2: NDF		0.09	0.0005
Step 3:Nitrogen		0.04	0.008

The correlations between observed and OMD, OMI, N balance and DOMI predicted by morphological, chemical and *in vitro* traits (as in Table 2) used in a cross-validation mode where the predicted observation is not used in the establishment of the regression

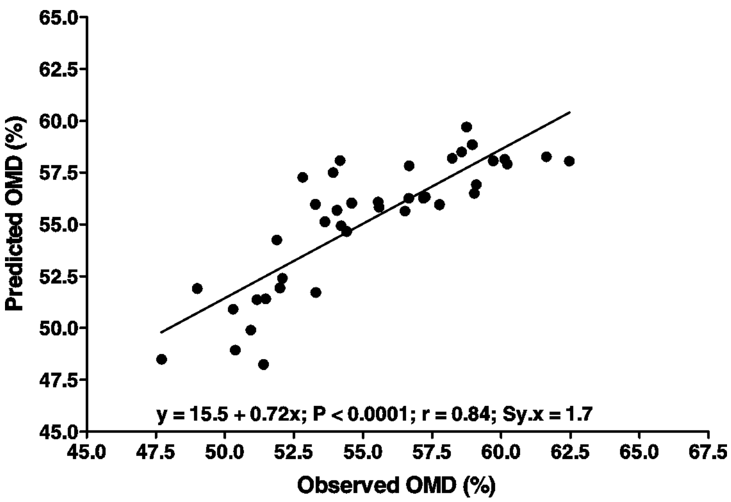

Fig. 1a. Relationship between observed and predicted organic matter digestibility (OMD) in 40 pearl millet stover

Table 5. Correlations between observed and by cross validation procedures predicted organic matter digestibility (OMD), organic matter intake (OMI), nitrogen balance (N-bal.) and digestible organic matter intake (DOMI) observed in forty pearl millet stover fed *ad libitum* to sheep

Trait	OMD	OMI	N balance	DOMI
Morphological stover traits				
Plant height	0.61 (<0.0001)	0.62 (<0.0001)	0.67 (<0.0001)	0.71 (<0.0001)
Stem diameter	0.48 (0.002)	0.58 (<0.0001)	0.55 (0.0003)	0.63 (<0.0001)
Leaf blade proportion	0.28 (0.07)	-0.008 (0.96)	0.40 (0.01)	0.18 (0.27)
Leaf sheath proportion	-0.12 (0.47)	-0.023 (0.86)	-0.77 (<0.0001)	-0.48 (0.001)
Stem proportion	0.23 (0.15)	0.11 (0.49)	0.40 (0.01)	0.21 (0.18)
Green leaf area	0.20 (0.22)	-0.17 (0.28)	-0.21 (0.19)	0.12 (0.45)
Chemical stover traits				
Nitrogen	0.007 (0.96)	0.55 (0.0002)	0.55 (0.0002)	0.46 (0.003)
Neural detergent fiber	0.77 (<0.0001)	0.70 (<0.0001)	0.80 (<0.0001)	0.83 (<0.0001)
Acid detergent fiber	0.67 (<0.0001)	0.61 (<0.0001)	0.81 (<0.0001)	0.74 (<0.0001)
Acid detergent lignin	0.76 (<0.0001)	0.43 (0.005)	0.73 (<0.0001)	0.66 (<0.0001)
In vitro fermentation and kinetic traits				
In vitro digestibility	0.83 (<0.0001)	0.61 (<0.0001)	0.84 (<0.0001)	0.80 (<0.0001)
Metabolisable energy	0.84 (<0.0001)	0.61 (<0.0001)	0.82 (<0.0001)	0.80 (<0.0001)
Extent of gas production	0.59 (<0.0001)	0.60 (<0.0001)	0.69 (<0.0001)	0.69 (<0.0001)
Rate of gas production	0.68 (<0.0001)	0.41 (0.008)	0.66 (<0.0001)	0.60 (<0.0001)
Lag phase of gas production	0.48 (0.001)	0.58 (<0.0001)	0.54 (0.0004)	0.64 (<0.0001)
Half time of gas production	0.70 (<0.0001)	0.56 (0.0002)	0.70 (<0.0001)	0.71 (<0.0001)

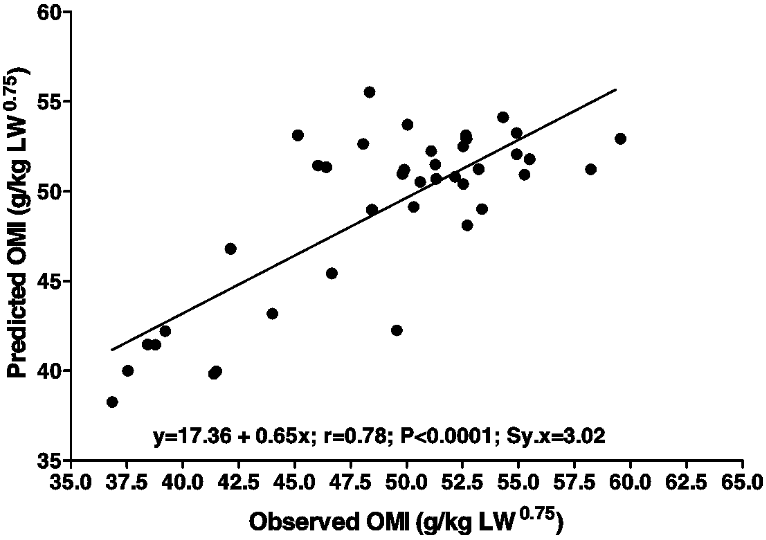

Fig. 1b. Relationship between observed and predicted organic matter intake (OMI) in 40 pearl millet stover

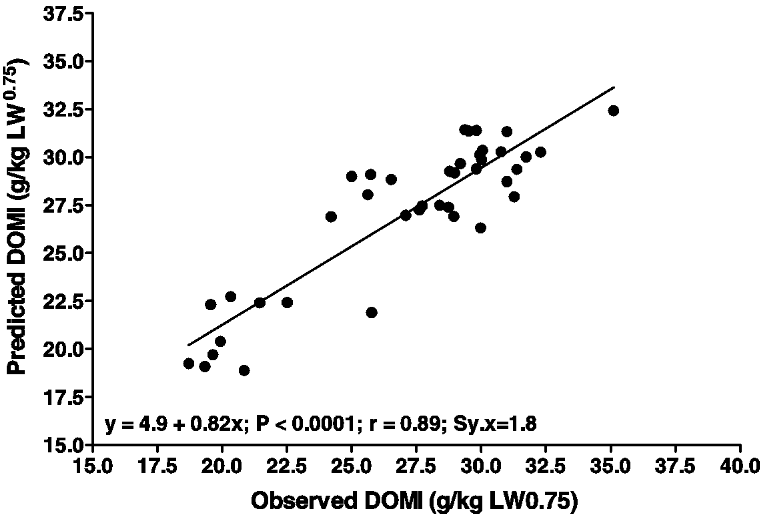

Fig. 1c. Relationship between observed and predicted digestible organic matter (DOMI) in 40 pearl millet stover

equations are reported in Table 5. Correlation coefficients (r) between observed and predicted OMD, OMI, N balance and DOMI were slightly less than observed for simple correlations analysis reported in Table 2.

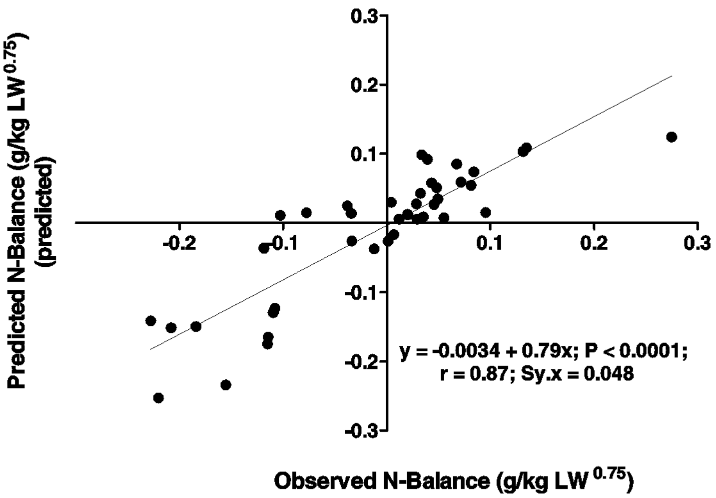

Fig. 1d. Relationship between observed and predicted nitrogen balance (N-Balance) in 40 pearl millet stover

The relationships between *in vivo* variables as predicted by the morphological, chemical and *in vitro* traits as entered in the stepwise multiple regressions in Table 3 but used in a cross-validation mode and actually observed *in vivo* variables are reported in Figures 1a-1d. The R-squares for predicted and observed *in vivo* variables were 0.71, 0.61, 0.79 and 0.76 for OMD, OMI, DOMI and nitrogen balance, respectively.

Cross validation procedures are quite stringent in that the predicted observation is not used in the development of the regression equation which makes it impossible for outliers and extreme values to influence the good-of-fitness of predicted versus observed values.

CONCLUSION

Pearl millet stover quality traits were identified that were highly correlated with important livestock productivity associated measurements such as OMD, OMI, DOMI and nitrogen balance. *In vitro* digestibility, metabolisable energy, cell wall (NDF) and cellulose (ADF) were found to be well suited for phenotyping for pearl millet stover quality. . In most cases combinations of various morphological, chemical and *in vitro* traits resulted in more accurate predictions of *in vivo* measurements than achieved by any single trait alone.

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