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### CAN RUMINANT METABOLIZABLE ENERGY OF BARLEY, CHICKPEA AND LENTIL STRAW BE PREDICTED USING CHEMICAL COMPOSITION?

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#### KEYWORDS

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Regression  
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#### ABSTRACT

This study attempted to generate simple and robust models to predict metabolizable energy (ME) content of barley, chickpea and lentil straw using chemical composition. Crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and ME of 1933, 487 and 489 straw samples of barley, chickpea and lentil respectively were determined using near infrared reflectance spectroscopy. The samples belonged to 1933 genotypes of barley, 79 genotypes of chickpea and 66 genotypes of lentil. Barley samples were collected from experimental locations of International Center for Agricultural Research in the Dry Areas, Morocco. Chickpea and lentil samples were collected from Ethiopian Institute of agricultural Research experimental locations. Data of each crop was randomly divided into two sets, a training set (75% of the data) and a deployment set (25% of the data). Crude protein, NDF, ADF and ADL were regressed on ME and Box-cox transformed ME of the training sets to generate prediction models. Coefficients of these models were used to calculate residuals and prediction error (PE) in both training and deployment sets. Criteria used in the screening algorithm were low PE (95<sup>th</sup> percentile of  $PE \leq 4$ ) and homogenous residuals in both training and deployment sets.

Barley and chickpea models were unable to predict ME of deployment samples with a 95<sup>th</sup> percentile of PE less than 4. Heterogeneity of residuals of the deployment set was found in lentil model (positive residuals= 64% of overall residuals). Accordingly, chemical composition from NIR is a poor predictor for ME of straws of barley, chickpea and lentil to formulate rations for farm management and a direct measurement of ME of these straws is still required.

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## 1 Introduction

In the predominantly mixed crop-livestock systems of developing countries, straw is key feed for livestock in terms of quantity and quality especially during the dry season. Grain yield of one ton is associated with approximately 1.33 t of straw in barley (Sundstøl, 1988), 1.75-8.74 t of straw in chickpea (Wamatu et al., 2017a) and 1.68 - 9.33 t of straw in lentil (Wamatu et al., 2017b). In dry areas of the West Asia and North Africa region, straw substitutes a considerable proportion of diets of sheep in summer and winter (ICARDA, 1986).

Energy content of feeds is important to determine the optimal level of incorporation of the feeds into diets of ruminants. Energy content is also valuable information for pricing straw for marketing purposes. Farmers in India price sorghum stover according to actual or estimated fodder quality (Blümmel & Rao, 2006). Blümmel & Rao (2006) reported that digestibility of stover, which is closely related to energy content, explained 75% of variation in stover prices. Usable energy content of forages available for ruminants is expressed as metabolizable energy (ME) (CSIRO, 2007). Metabolizable energy of a given feed is traditionally determined by subtracting energy of feces, urine and methane from gross energy (Kearl, 1982) but is now commonly assessed *in vitro* via the Hohenheim Gas Production method (Menke & Steingass, 1988). Some feeding standards use ME to express energy content of feeds like Kearl, (1982) and CSIRO, (2007) while other standards use it to estimate net energy (NRC, 2007).

Gas production technique is an accurate method to determine ME of feeds which based on recording gas emission from an incubation of 0.2g of sample in 100 ml of rumen fluids for 24 h (Menke & Steingass, 1988). Measurement of gas production method requires specialized apparatus, access to rumen fluid, technical skill and is time consuming, requiring a minimum of 24 hours.

Wide varietal and environmental variation in chemical composition and energy content of straw was reported in barley (Capper, 1988), chickpea (Wamatu et al., 2017a) and lentil (Wamatu et al., 2017b). Published tables which describe feeding value of feedstuffs do not address varietal and environmental variation. Therefore, farmers and researchers cannot rely on tabulated values of ME of straw to formulate rations for purposes of research and farm management.

Organic matter digestibility of forages is affected by its chemical composition (Givens et al., 2000). It has been reported that ME of forages correlates strongly and positively with CP and negatively with ADF (Yang et al., 2018). However, the correlation between ME and NDF of forages was weak (Yang et al., 2018). Accordingly, it is expected that there might be a relationship between ME and

chemical composition of barley, chickpea and lentil straw which gives a chance to predict ME using a simple model.

Early attempt to predict ME of feed for poultry nutrition is traced to 1956 (Carpenter & Clegg, 1956). Anderson et al. (2012) reported that ME of corn coproducts for pigs could be predicted using chemical composition. Similarly, Armstrong et al. (1964) tried to predict ME of dried grasses for sheep fattening using apparent digestibility and chemical compositions. Metabolizable energy content for ruminant of sugar cane, sugar cane silage, soybean silage, mombaça silage (*Panicum maximum* cv. Mombaça), corn silage, Tifton-85 hay (*Cynodon* spp.) and chopped elephant grass (*Penisetum purpureum* cv. Cameroun) was predicted using chemical composition (Magalhães et al., 2010). However, these models cannot be used to predict ME for other feeds and individual prediction equations of ME for ruminants must be identified for feed other stuffs (Robinson et al., 2003).

According to our knowledge, there are no studies identified the potential of chemical composition of barley, chickpea and lentil straw to predict ME. Therefore, this study aims to determine robust and accurate models to predict ME of barley, chickpea and lentil straw using chemical composition.

## 2 Materials & Methods

### 2.1 Sampling and chemical analysis of straw

Samples of barley straw representing 1933 genotypes (one sample per genotype) were collected from field experiments in Marchouch (33°33'38.2"N 6°41'0 24.7"W), and Jemma-Shaim (32°21'9.3"N 8°50'32W) research stations in Morocco during the 2016-2017 season genotypes included 1017 two-row genotypes, 912 six-row and 4 three-row genotypes. A total of 487 (79 genotypes) chickpea and 489 lentil (66 genotypes) samples were collected from 7 and 8 multi-locational trials respectively in Akaki (08°53'N 38°49'E; 2200 m.a.s.l), Debre Zeit (08°44'N 38°58'E; 1900 m.a.s.l), Chêfe Donsa (08°57'N 39°06'E; 2450 m.a.s.l) and Minjar (08°44'N 38°58'E; 1810 m.a.s.l), Ethiopia. Samples were ground to pass through a 1mm screen and scanned using near infrared reflectance spectroscopy (FOSS 5000 with WINISI II software) to measure crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and ME using equations calibrated and validated for a wide range of barley, chickpea and lentil straws. The performance of the near infrared reflectance spectroscopy prediction equations is presented in Table 1. For equations' calibration, CP was analyzed according to AOAC (2005) (method 954.01 using Kjeldahl (protein/nitrogen) Model 1026, Foss Technology Corp), NDF was assayed without a heat stable amylase and expressed inclusive of residual ash (Van Soest et al., 1991), ADF was analyzed according to Van Soest et al. (1991)

and expressed exclusive of residual ash, ADL was determined by solubilization of cellulose with sulphuric acid according to Van Soest et al. (1991) and ME were measured in rumen microbial inoculum using the *in vitro* gas production technique as described by Menke & Steingass (1988). All samples were analyzed at the International Livestock Research Institute laboratory in Addis Ababa, Ethiopia. Details on the near infrared reflectance spectroscopy equations used in this study are presented in Table 1.

Table 1 Performance of near infrared reflectance spectroscopy prediction models

	Standard error of calibration (%)	Standard error of prediction (%)
<b>Chickpea straw (n=190)</b>		
CP	0.21	0.425
NDF	0.85	1.3
ADF	0.64	1.09
ADL	0.22	0.36
ME	0.06	0.036
<b>Lentil straw (n= 111)</b>		
CP	0.6	0.62
NDF	2.13	2.2
ADF	1.88	1.83
ADL	0.59	0.63
ME	0.996	0.05
<b>Barley straw (n= 105)</b>		
CP	0.37	0.508
NDF	2.26	2.38
ADF	1.83	2.26
ADL	0.47	0.68
ME	1	1.2

ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; ME: metabolizable energy; NDF: neutral detergent fiber.

## 2.2 Statistical analyses

Data of every crop was divided into two different sets, a calibration set (~85% of the data) and a validation set (~15% of the data) using Puchwein (1988) algorithm. The calibration set was used to develop prediction models and the validation set was used to determine the accuracy of the models in predicting ME of new set of samples.

Interquartile range method (Zwillinger & Kokoska, 2003) was used to identify the existence of outliers using the following equation:

$$\text{Lower bound} = Q1 - (IR \times 1.5)$$

$$\text{Upper bound} = Q3 + (IR \times 1.5)$$

Where Q1 and Q3 are the first and the third quartiles respectively and IR is the interquartile range. Observations of LW which fall out these boundaries were considered outliers.

The probability distribution of ME in the training data set was depicted using the normal Q-Q plot. Box-cox procedure was used to confirm whether a power transformation of ME in the training set would increase predictability of constructed models (Box & Cox, 1964). The optimum power of transformation of ME was identified using a likelihood maximized Box-cox transformation with boundaries of -3 and +3 and a step of 0.25 (Box & Cox, 1964). Crude protein, NDF, ADF and ADL were used to construct linear models to predict ME in each crop. Coefficients of each constructed models were used to calculate residuals. The prediction error (PE) of each model was calculated using calibration set as follows:

$$PE = 100 \times \left( \frac{ME_p - ME_m}{ME_m} \right)$$

Where  $ME_p$  and  $ME_m$  are predicted and measured ME respectively.

Similarly, the validation error (VE) of the models was calculated using the validation set. The prediction models were screened in a stepwise approach which included residuals' magnitude (PE and  $VE \leq 4$ ) and homogeneity (independence of PE and VE from ME ( $r < 0.66$ ) and the symmetric distribution of residuals around zero). All statistical analyses were carried out using the Statistical Analysis System (SAS, 2003).

## 3 Results

All observations in the data had ME (MJ/kg) which lays within the outliers' boundaries which were 5.9-9.2 for barley, 5.8-8.9 for chickpea and 6.51-10.1 for lentil (Table 2a, 2b). Figure 1 shows the normal Q-Q plot of ME in barley, chickpea and lentil. Normal Q-Q plot of ME shows that distribution of ME was close to normal with some skewness in barley, chickpea and lentil. Results of Box-cox transformation procedure are presented in Table 3. Lambda which had the highest log-likelihood value was different form 1 in models of all crops. Relation between chemical composition and ME are presented in Table 4a,b and Figure 2. The 95<sup>th</sup> percentile of PE of models with non-transformed ME in all crops was higher than 4 (Table 5a). When ME was

Table 2a Chemical composition and metabolizable energy content of barley, chickpea and lentil straw samples of the training set

Crop	Mean	Minimum	Maximum	SD
<b>Barley</b>				
CP (g/kg DM)	69.2	50.3	98.1	6.24
NDF (g/kg DM)	738	684	781	12.7
ADF (g/kg DM)	485	429	532	14.1
ADL (g/kg DM)	71.4	49.7	90.3	5.74
ME (MJ/kg DM)	6.98	6.06	8.09	0.275
<b>Chickpea</b>				
CP (g/kg DM)	72.5	29.4	216	34.2
NDF (g/kg DM)	699	478	798	58.4
ADF (g/kg DM)	457	210	557	62.9
ADL (g/kg DM)	118	55.7	164	17.5
ME (MJ/kg DM)	7.37	6.16	9.56	0.511
<b>Lentil</b>				
CP (g/kg DM)	496	369	644	48.3
NDF (g/kg DM)	358	272	506	42.6
ADF (g/kg DM)	88.5	63.2	142	15.4
ADL (g/kg DM)	86.9	34.7	156	25.9
ME (MJ/kg DM)	8.35	7	9.5	0.45

SD: standard deviation; ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; ME: metabolizable energy; NDF: neutral detergent fiber.

Table 2b Chemical composition and metabolizable energy content of barley, chickpea and lentil straw samples of the deployment set

Crop	Mean	Minimum	Maximum	SD
<b>Barley</b>				
CP (g/kg DM)	26	14.3	46.6	4.7
NDF (g/kg DM)	795	721	843	19.7
ADF (g/kg DM)	553	470	601	21.8
ADL (g/kg DM)	87.2	54.1	102	6.57
ME (MJ/kg DM)	6.09	5.19	7.29	0.331
<b>Chickpea</b>				
CP (g/kg DM)	72.5	29.4	216	34.2
NDF (g/kg DM)	699	478	798	58.3
ADF (g/kg DM)	457	210	557	62.9
ADL (g/kg DM)	118	55.7	163	17.5
ME (MJ/kg DM)	7.36	6.15	9.56	0.511
<b>Lentil</b>				
CP (g/kg DM)	74.3	45.5	115	16.3
NDF (g/kg DM)	468	345	599	55.7
ADF (g/kg DM)	335	257	456	36.9
ADL (g/kg DM)	80.6	66	120	10
ME (MJ/kg DM)	8.5	6.98	9.86	0.548

SD: standard deviation; ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; ME: metabolizable energy; NDF: neutral detergent fiber.

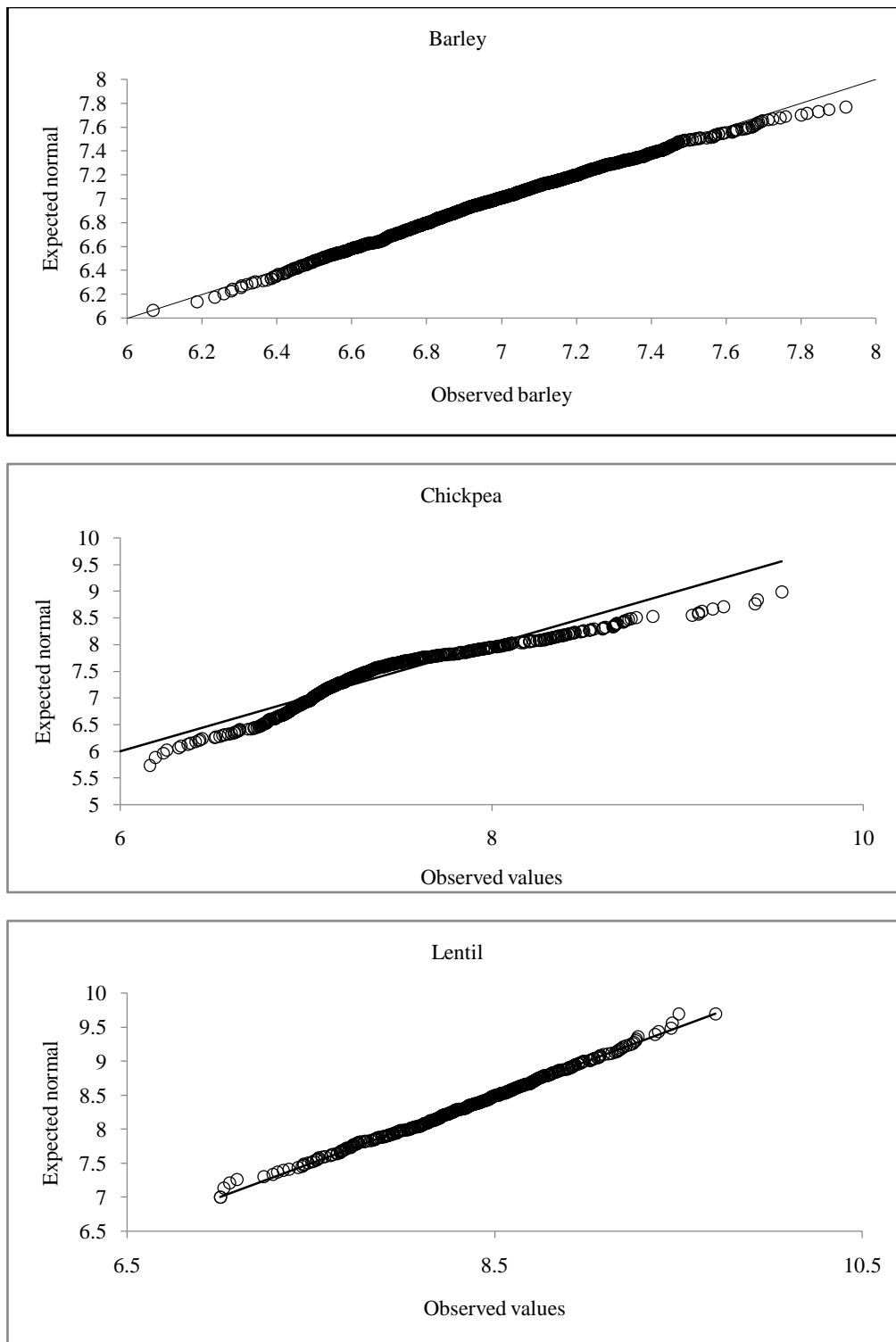


Figure 1 Q-Q normal plot of metabolizable energy of barley, chickpea and lentil straw of the training set

Table 3 Lambda values and their corresponding coefficient of determination and log likelihood values resulted from Box-cox transformation procedure

	Models	Lambda	Log-likelihood
<b>Barley</b>			
	NDF	-0.75	2667
	ADF	-0.5	3097
	ADL	-1	2746
	CP	-1	2311
<b>Chickpea</b>			
	NDF	0 <sup>c</sup>	1212
	ADF	0.5	1214
	ADL	-1.5	1296
	CP	-0.5	704
<b>Lentil</b>			
	NDF	1.25	629
	ADF	0.5	630
	ADL	0.75	48
	CP	2.25	364

ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; ME: metabolizable energy; NDF: neutral detergent fiber; R<sup>2</sup>: coefficient of determination; 0 denotes to Log<sub>10</sub> transformation.

Table 4a Regression of chemical composition on metabolizable energy of barley, chickpea and lentil straw

Dependent variable	Predictors	Coefficients (standard error)		R <sup>2</sup>	CV%
		Constant	b		
<b>Barley</b>					
	NDF	18.4(0.278)	-0.016*	0.513	2.68
	ADF	14.9(0.126)	-0.016*	0.714	2.11
	ADL	9.55(0.057)	-0.036*	0.556	2.63
	CP	5.48(0.067)	0.021*	0.24	3.44
<b>Chickpea</b>					
	NDF	12.7(0.099)	-0.00775*	0.781	3.24
	ADF	10.65(0.06)	-0.00721*	0.785	3.22
	ADL	10.46(0.052)	-0.0263*	0.811	3.02
	CP	6.83(0.036)	0.00726*	0.2352	6.07
<b>Lentil</b>					
	NDF	12.2(0.119)	-0.007*	0.708	2.91
	ADF	11.5(0.098)	-0.008*	0.709	2.91
	ADL	10.1(0.094)	-0.019*	0.434	4.06
	CP	8.07(0.074)	0.003*	0.035	5.3

ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; ME: metabolizable energy; NDF: neutral detergent fiber; R<sup>2</sup>: Coefficient of determination; CV: coefficient of variation; \*: standard error is less than 0.00001; P<0.001 for in all models.

Table 4b Regression of chemical composition on metabolizable energy of barley, chickpea and lentil straw

Dependent variable	Predictors	Coefficients (SE)			CV%
		Constant	b	R <sup>2</sup>	
Barley					
	NDF	-0.051(0.007)	0.00038*	0.513	2.05
	ADF	0.163(0.003)	0.0004*	0.713	1.05
	ADL	0.091(0.001)	0.0007*	0.558	2.61
	CP	0.17(0.001)	-0.0004*	0.242	3.42
Chickpea					
	NDF	1.17(0.006)	-0.00043*	0.769	1.61
	ADF	3.305(0.011)	-0.0013*	0.777	1.61
	ADL	0.381(0.001)	-0.000162*	0.2060	2.95
	CP	0.38(0.001)	-0.00016*	0.2060	2.94
Lentil					
	NDF	22.4(0.253)	-0.017*	0.71	3.64
	ADF	3.44(0.017)	-0.002*	0.712	1.45
	ADL	5.66(0.041)	-0.009*	0.436	3.05
	CP	110(2.33)	0.105(0.026)	0.035	11.8

ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; ME: metabolizable energy transformed according to results of Box-cox procedure; NDF: neutral detergent fiber; CV: coefficient of variation; \*: standard error is less than 0.00001; P<0.001 for in all models.

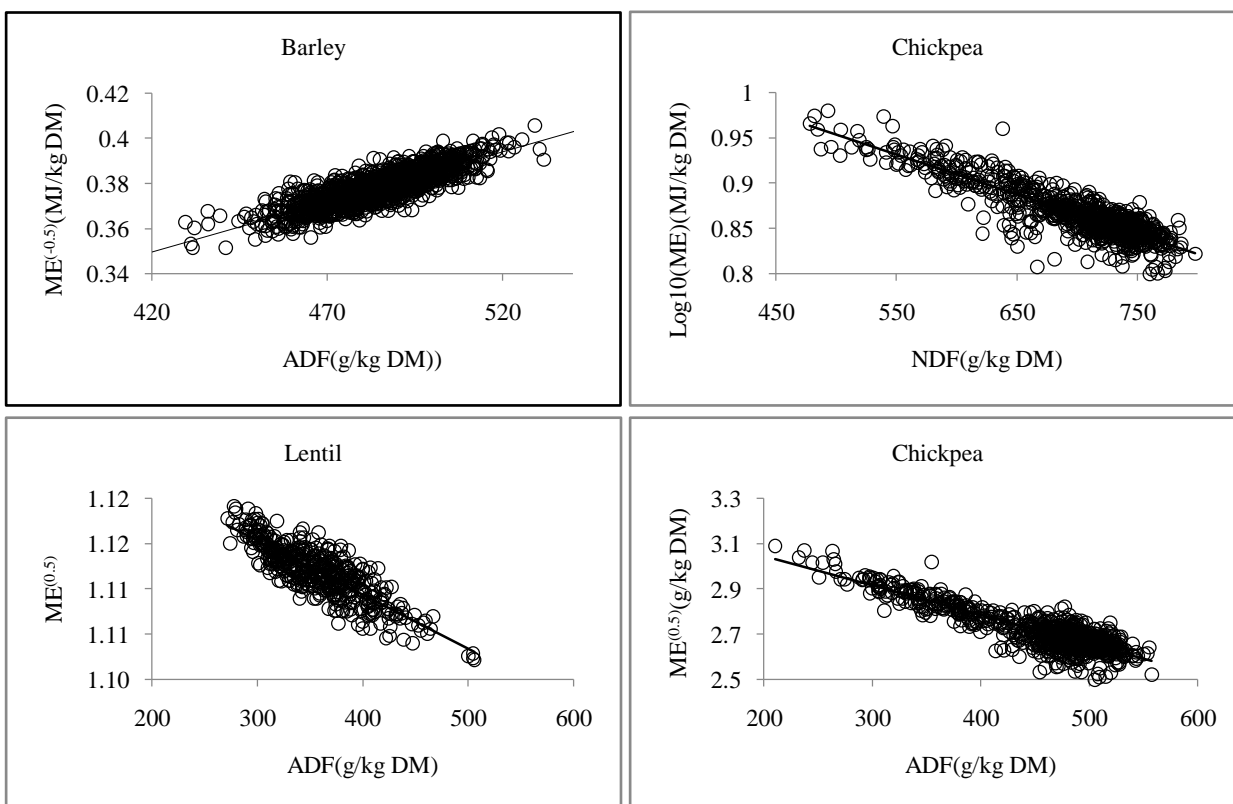


Figure 2 Metabolizable energy as a function of chemical composition of barley, chickpea and lentil straw.  
ADF: acid detergent fiber; DM: dry matter; ME: metabolizable energy; NDF: neutral detergent fiber

Table 5a Prediction error of models constructed to predict metabolizable energy of barley, chickpea and lentil straw using chemical composition

Crop	Predictor	75 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>
Barley				
	NDF	3.2	4.41	5.31
	ADF	3.73	4.96	6.02
	ADL	2.94	4.29	5.05
	CP	3.97	5.79	6.83
Chickpea				
	NDF	3.19	4.84	6.23
	ADF	3.21	5.31	6.67
	ADL	3.21	4.63	6.04
	CP	3.19	4.84	6.23
Lentil				
	NDF	3.25	4.89	5.81
	ADF	3.35	4.99	5.66
	ADL	5.08	6.56	7.45
	CP	6.04	8.7	10.6

ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; ME: metabolizable energy; NDF: neutral detergent fiber.

Table 5b Prediction and validation errors of models constructed to predict metabolizable energy of barley, chickpea and lentil straw using chemical composition

Crop	Predictor	PE			VE		
		75 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>	75 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>
Barley							
	NDF	2.95	4.04	4.69			
	ADF	1.39	1.92	2.39	4.04	5.13	5.8
	ADL	2.94	4.3	5.09			
	CP	3.91	5.76	6.79			
Chickpea							
	NDF	1.57	2.4	3.11	11.4	11.8	11.9
	ADF	1.62	2.61	3.39	36.2	37.10	37.6
	ADL	4.75	6.74	9.07			
	CP	3.09	4.89	6.21			
Lentil							
	NDF	4.07	6.19	7.25			
	ADF	0.16	0.25	0.28	1.99	2.89	3.42
	ADL	3.78	4.91	5.64			
	CP	13.6	20.2	23.9			

ADF: acid detergent fiber; ADL: acid detergent lignin; CP: crude protein; ME: metabolizable energy transformed according to results of Box-cox procedure; PE: prediction error; VE: validation error.



Table 6 Residuals' distribution and correlation between the dependent variable and prediction and validation error of the constructed models in training and deployment sets of barley, chickpea and lentil

Crop	Predictor	Correlation with the dependent variable		Negative residuals (%)	
		PE	VE	Calibration	Validation
Barley	ADF	-0.268*		71.8	
Chickpea	NDF	-0.1*		47.1	
Chickpea	ADF	-0.1*		44.5	
Lentil	ADF	-0.054	-0.107*	50.9	36

ADF: acid detergent fiber; NDF: neutral detergent fiber; \*:  $P \leq 0.05$ .

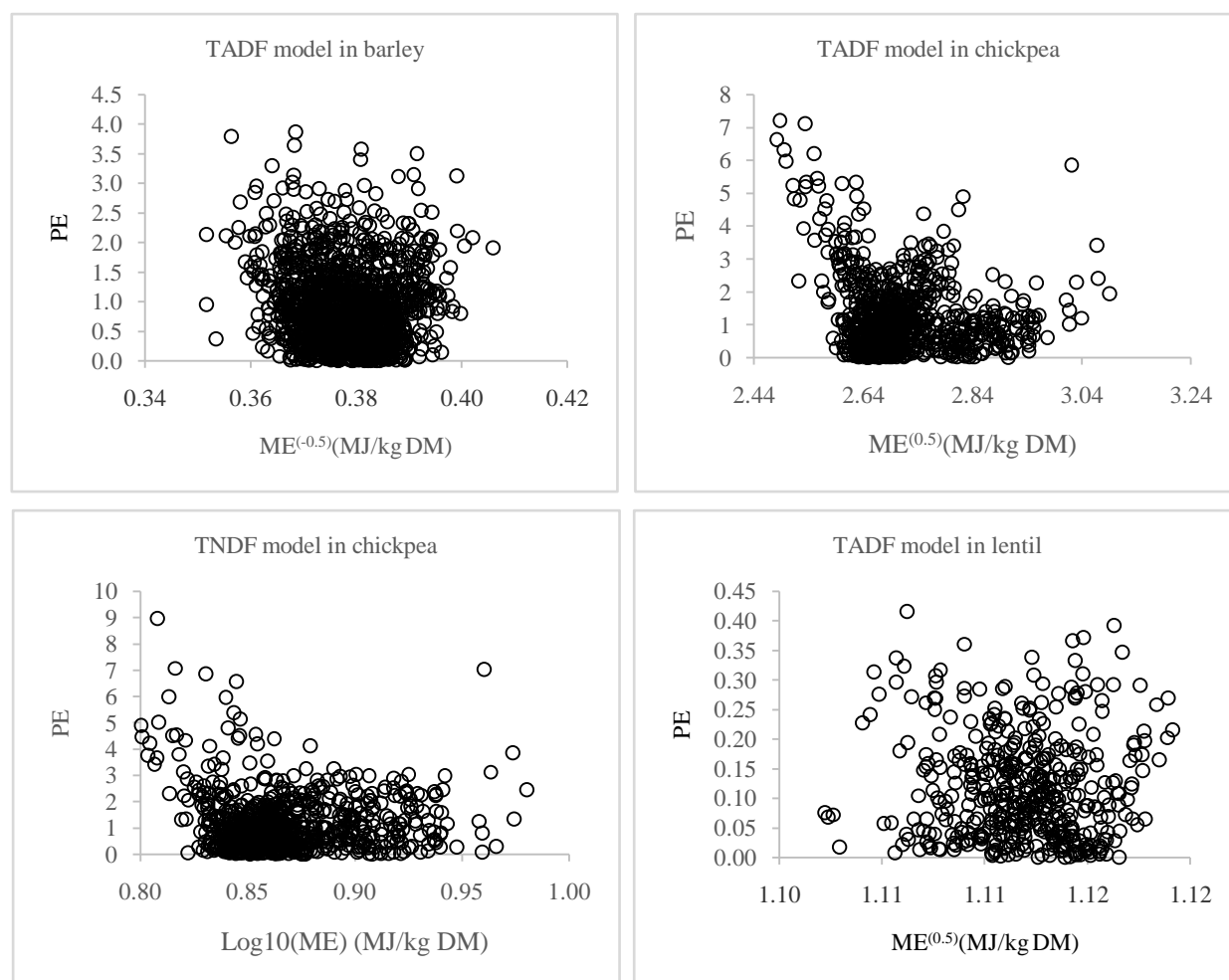


Figure 3 Relationship between the dependent variable and prediction error of constructed models of barley, chickpea and lentil training straw samples.

ADF: acid detergent fiber; DM: dry matter; ME: metabolizable energy; NDF: neutral detergent fiber; PE: prediction error; TADF: ADF model with transformed dependent variable; TNDF: neutral detergent fiber model with transformed dependent variable

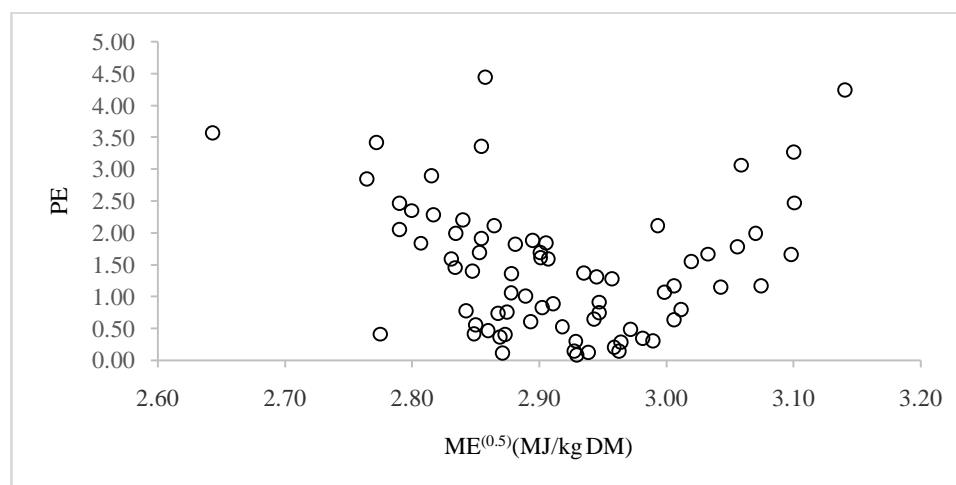


Figure 4 Relationship between the dependent variable and of prediction error of ADF model with box-cox transformed metabolizable energy in the deployment set of lentil

ADF: acid detergent fiber; DM: dry matter; ME: metabolizable energy; PE: prediction error

transformed, the models with a 95<sup>th</sup> percentile of PE less than 4 were ADF model (TADF) in barley, chickpea and lentil. The NDF model with transformed ME (TNDF) predicted ME of chickpea straw with a 95<sup>th</sup> percentile of PE less than 4 (Table 5b).

Correlation between PE and the dependent variable in TADF model in barley, TNDF in chickpea, TADF in chickpea and TADF in lentil was weak ( $r < 0.27$ ;  $P < 0.05$ ) in all crops (Table 6). Distribution of residuals of selected models around zero is presented in Table 6. Frequencies of positive and negative calibration residuals were similar in TNDF model in chickpea, TADF model in chickpea and TADF model in lentil. In barley, negative calibration residuals were dominant in TADF model (71.8%).

An examination of figure 3 and figure 4 shows that PE of TADF model in barley, TNDF model in chickpea, TADF model in chickpea and TADF model in lentil did not agglomerate around specific values of the dependent variable.

The 95<sup>th</sup> percentile of VE was higher than 4 in TADF model in barley, TNDF and TADF models in chickpea but less than 4 in TADF model in lentil (Table 5b). The Correlation between the dependent variable and VE was weak in TADF model in lentil ( $r = -0.107$ ). Positive validation residuals dominated negative validation residuals in TADF model in lentil (64%) (Table 6). Figure 4 shows that there were no drifts in VE of TADF in lentil nor systematic relationship between VE and the dependent variable.

#### 4 Discussion and conclusions

Distribution of ME of all crops was deviated from normal as shown in Q-Q plots. This result is confirmed by results of Box-cox transformation procedure which showed that a power transformation of ME might increase the accuracy of prediction of ME using chemical composition. This agrees with McDonald (2009), Lesosky et al. (2013) and Goopy et al. (2017) who reported that transforming the response variable improved accuracy of simple linear regression model in predicting live weight of cattle using heart girth. Accordingly, non-transformed and transformed ME were regressed on chemical composition parameters to construct prediction models.

Metabolizable energy of commercially available forages ranges from 10 to 12.5 MJ/kg (Warren, 2018 personal communication – Unpublished data). Therefore, a difference of 0.5 MJ/kg ME would have a great impact on the resultant ration as 55 - 60% of the dry matter of the diet will be comprised of forages in dairy livestock (Warren, 2018 personal communication - Unpublished data). Accordingly, a maximum of 4% error on a dry matter basis for ration formulation for purposes of farm management, is accepted when ME is estimated (Warren, 2018 personal communication - Unpublished data).

All models with non-transformed response variable could not be used to predict ME to formulate rations for research and farm management as their 95<sup>th</sup> percentile of PE were higher than 4%. However TADF model in barley predicted ME of 95% of

prediction set samples with a PE less than 4 and PE was independent of ME, distribution of residuals around 0 was asymmetric with a dominance of negative residuals. That means ME of barely straw (~72%) tends to be underestimated by TADF model.

The prediction error of TNDF and TADF models in chickpea were less than 4 and residuals were homogenous. However, both TNDF and TADF models predict ME of 95% of chickpea validation samples with VE higher than 4. In lentil, the 95<sup>th</sup> percentile of the PE and VE in TADF model was less than 3 and the residuals were homogenous, however, positive residuals dominated validation samples (64%). That means TADF model overestimated almost two third of lentil straw samples in the validation set. Accordingly, NDF, ADF, ADL and CP are poor predictors for straw ME in barley, chickpea and lentil and direct estimation of ME of these straws is still required.

Relationship between chemical composition and digestibility of straw is expected to be affected by morphological structure. Precise prediction of ME of straw might be achieved using morphology-based equations. On that account, prediction equations of ME of morphological fractions of barley, chickpea and lentil straw using chemical composition has to be studied.

#### Conflict of Interest

Authors declare no conflict of interest regarding publication of this paper.

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