



## Correlation and determination of the Metabolizable Energy (ME) of tropical forage with nutrient content for ruminants

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### ABSTRACT

The metabolizable energy (ME) of tropical forages measured by in vivo method in ruminants had a high degree of accuracy but requires a long time and is expensive. One method that can be done is the ME estimation model. The objectives of the present study were carried out to investigate the relationship between tropical forage nutrient content and ME for ruminants as well as determine and validate a model for estimating ME of tropical forage based on nutrient content. A total of 26 forage samples consisting of 14 types of grass and 12 legumes were obtained after data pre-processing or data cleaning and data normalization. Forage samples will be grouped into 3, Grass + Legume (G+L=26), grass (R=14), and legume (L=12). The database used is Crude Protein (CP), Extract Ether (EE), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and hemicellulose as well as ME with in vivo experiments. The initial stage is preprocessing data. Nutrient content and ME were analyzed using Pearson Correlation and followed by multiple linear regression to determine the ME estimation model. However, validated used the mean absolute deviation (MAD), root means square error (RMSE), and mean absolute percentage error (MAPE). The results showed that there was a significant and highly significantly correlated between nutrient composition and ME in the Grass + Legume, Grass, and Legume groups so it could be used to determine ME. There are 9 regression equations with significance and have high R<sup>2</sup> and after being validated with the lowest MAD, RMSE, and MAPE values, three regression equations are obtained with one each for each group Grass + Legume (G+L), Grass (R), and Legumes (L). It is concluded that the regression equation of ME of tropical forage is  $ME_{R+L} = 12.429 - 0.122 \text{ ADF}$  for Grass + Legume,  $EM_R = 15.609 - 0.115 \text{ NDF}$  for Grass, and  $EM_L = 3.726 - 0.186 \text{ CP}$  for Legume.

### Introduction

Forage is the main component in meeting the nutritional needs of ruminants more than 70% of consists of forage (Farizaldi, 2011). The productivity of ruminant's livestock is largely determined by the quantity and quality of forage (Jermias *et al.*, 2021) and also continuity. In general, the forage given to ruminants is in the form of grasses and legumes. However, forages in Indonesia are classified as tropical forages which have higher fiber content and lower protein when compared to tropical and subtropical forages (Archimede *et al.*, 2018). Most of the forage grown in the tropics has a low energy

metabolic content (Sadarman *et al.*, 2022) while the relatively higher quality of subtropical forage makes ruminants have lower energy requirements compared to the tropics (Haryanto, 2012).

Estimation of feed energy partition for ruminants has not been widely carried out. TDN is an energy unit that is still used in Indonesia and has been estimated (Sutardi, 1980; Jayanegara *et al.*, 2019; Indah *et al.*, 2020) that specifically calculate TDN forage based on its digestibility. However, not all of the energy in the feed can be completely absorbed by ruminants but will also be wasted through feces, urine, methane gas, and body heat (Santika *et al.*,

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2022) in more detail determined ME which can be determined by reducing gross energy consumption fecal energy, urine energy, and energy in the form of methane gas (CH<sub>4</sub>). Nevertheless, measuring the ME using the *in vivo* method had high degree of accuracy but requires a long time and is expensive (Wahyudi et al., 2022).

ME can be predicted quite accurately based on nutrient content to estimate energy concentrations in feedstuffs and rations (Sung and Kim, 2021). The method of determining forage quality by proximate and Van Soest analysis is used to prepare balanced and economical rations (Dumadi et al., 2021). Proximate analysis is intended to determine the percentage of nutrients in feed based on chemical properties including water content, protein, fat, fiber, nitrogen-free extract, and ash which are used to determine feed quality because the procedure is easy and relatively inexpensive (Wahyudi et al., 2022). Van Soest analysis is a method used to determine fiber in animal feed (Ferreira and Thiex, 2022) which can be measured based on its solubility in a detergent solution (Sahid et al., 2022).

The objectives of the present study were carried out to investigate the relationship between forage nutrient content and metabolizable energy (ME) for ruminants as well as determine and validate the model for estimating the ME forage based on nutrient content.

## Materials and Methods

### Experimental design

This research was conducted using the desk study method and the data observed was obtained from previously published scientific articles in the last 10 years starting from student final assignments (essay, thesis, and dissertation) as well as national and international scale scientific journals.

The database observed is the nutrient content of tropical forages consisting of Ash, Crude Protein (CP), Extract Ether (EE), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and hemicellulose as well as ME experiments which are measured directly on ruminants (*in vivo* experiments). Nutrient content in percentage of dry matter (% DM) and ME in MJ/kg DM.

**Table 1.** Summary of nutrient content (%DM) and metabolizable energy (MJ/kg DM) of tropical forage

Nutrients Content	Grass + Legume			Grass			Legume		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Ash	12.20 ± 3.31	26.00	6.66	12.62 ± 4.59	26.00	6.66	11.81 ± 1.49	15.10	9.17
CP	16.98 ± 6.76	34.60	3.60	12.22 ± 5.58	22.60	3.60	21.27 ± 4.49	34.60	14.90
EE	2.17 ± 0.87	5.27	9.00	1.91 ± 1.01	05.27	1.00	2.40 ± 6.66	3.36	0.90
NDF	48.55 ± 12.69	84.60	26.78	57.86 ± 12.43	84.60	84.60	40.17 ± 4.42	48.88	29.90
ADF	33.75 ± 7.98	71.10	19.66	35.53 ± 10.45	71.10	71.10	32.14 ± 4.54	40.55	19.66
Hemicellulose	14.81 ± 9.28	33.19	2.40	22.34 ± 7.16	33.19	33.19	8.03 ± 4.43	17.72	2.40
ME	8.22 ± 1.61	11.07	3.78	8.42 ± 1.95	11.07	11.07	8.05 ± 1.26	10.63	6.60

CP = Crude Protein, EE = Extract Ether, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, ME = Metabolizable Energy.

A total of 26 forage samples used for analysis, consisting of 14 types of grasses and 12 legumes were obtained after data pre-processing or data cleaning and data normalization. Forage samples will be separated into three groups: Grass + Legume (G+L=26), Grass (R=14), and Legume (L=12). The nutritional composition of forages is described in Table 1.

### Data analysis

The data were analyzed using the two-tailed Pearson Correlation (Rice, 2007). If it is significantly correlated, it will be followed by Multiple Linear Regression (Devore and Berk, 2012) with the following mathematical model:

$$Y = \alpha + \beta_1x_1 + \beta_2x_2 + \dots + \beta_nx_n + \epsilon$$

where  $Y$  is EM,  $\alpha$  is a constant,  $\beta$  is estimation coefficient regression from an independent variable,  $x$  is an independent variable, namely the nutrient

content of forage,  $n$  is nutrient content (1, 2, ...), and  $\epsilon$  is an error.

Multiple Linear Regression is used to determine the ME estimation model that has the highest probability and determinant coefficient. Furthermore, to determine if the estimation model provides good actual information, validation is carried out by comparing the values of the mean absolute deviation (MAD) (Lam et al., 2021), root means square error (RMSE) (Devore and Berk, 2012), and mean absolute percentage error (MAPE) (Mynsbrugge, 2010) between the ME estimation model and other estimation models. Data is processed using Microsoft Excel 2010 and IBM SPSS version 22.

## Results

### Correlation between nutrient content and ME

The correlation value between forage in nutrient content and ME can be seen in Table 2. The coefficient correlations were negative and very significant ( $p < 0.01$ ) between ash and ADF with ME being very powerful ( $p < 0.01$ ) from group Grass + Legume. Ash and ADF have a negative correlation with ME so if ash and ADF increase, ME will decrease. Likewise, phenotypic correlations were significant ( $p < 0.05$ ) for the correlation between nutrient content and ME only at ADF for the Grass group with a negative correlation thus indicating that the lower the NDF, the ME of forage in ruminants will increase. For the Legume group, the correlation between ME with ash and NDF was negative and significant ( $p < 0.05$ ), and also CP and NDF were

highly significant ( $p < 0.01$ ) but just CP was a positive correlation. Therefore, only CP can increase the masses along with the increased concentration of its content in legumes.

Ash and ADF are negatively correlated to ME so if ash and ADF increase it will decrease ME. However, in hemicellulose content, there is a positive correlation so if hemicellulose increases, ME will also increase. Hemicellulose has a positive correlation with ME so if it increases, ME will also increase. CP has a positive correlation with ME, it could be due to legumes having a relatively higher CP content compared to grasses. ADF has a negative correlation with ME so it will cause a decrease in ME if ADF increases, which can be caused ADF containing lignin which is a limiting factor in forages.

**Table 2.** Pearson correlation of coefficients between nutrient content and ME (N=26).

Nutrient Content (%BK)	Pearson correlation coefficient (r) of ME		
	Grass + Legume	Grass	Legume
Ash	-0.500**	-0.335	-0.617*
CP	1.107	-0.048	0.919**
EE	-0.224	0.059	-0.115
NDF	-0.156	-0.629*	-0.627*
ADF	-0.626**	-0.495	-0.735**
Hemicellulose	0.323	-0.500	-0.121

\*Significant at  $p < 0.05$ , \*\*Significant at  $p < 0.01$ , CP = Crude Protein, EE = Extract Ether, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, ME = Metabolizable Energy.

**Table 3.** Regression equation of ME on nutrient content in forage.

Group	Regression equation	MAD	RMSE	MAPE	Sig	R	R <sup>2</sup>
Grass + Legume (N=26)	$EM_{R+L} = 10.696 - 0.212 \text{ Ash}$	1.26	8.12	0.16	0.009	0.500	0.250
	$EM_{R+L} = 12.429 - 0.122 \text{ ADF}$	1.08	8.13	0.13	0.001	0.626	0.392
Grass (N=14)	$EM_R = 15.609 - 0.115 \text{ NDF}$	0.89	8.86	0.10	0.016	0.629	0.395
Legume (N=12)	$EM_L = 9.945 - 0.209 \text{ Ash}$	0.68	7.25	0.09	0.003	0.617	0.380
	$EM_L = 3.726 - 0.186 \text{ CP}$	0.43	7.31	0.07	0.000	0.919	0.844
	$EM_L = 10.153 - 0.066 \text{ NDF}$	0.81	7.24	0.11	0.029	0.627	0.393
	$EM_L = 10.833 - 0.096 \text{ ADF}$	0.62	7.28	0.08	0.006	0.735	0.541

\*Significant at  $p < 0.05$ , \*\*Significant at  $p < 0.01$ , MAD = Mean Absolute Deviation, RMSE = Root Means Square Error, MAPE = Mean Absolute Percentage Error, R = Correlation coefficient, R<sup>2</sup> = The coefficient of determination.

### Regression equation of ME

The prediction equations of ME of tropical forage for ruminants were determined with significance ( $p < 0.05$ ) and highly significant ( $p < 0.01$ ) (Table 3). Several variables can be used to determine ME but the best-fitted regression equation is an equation with a higher coefficient of determination in each group.

### Validation Regression Equation of ME

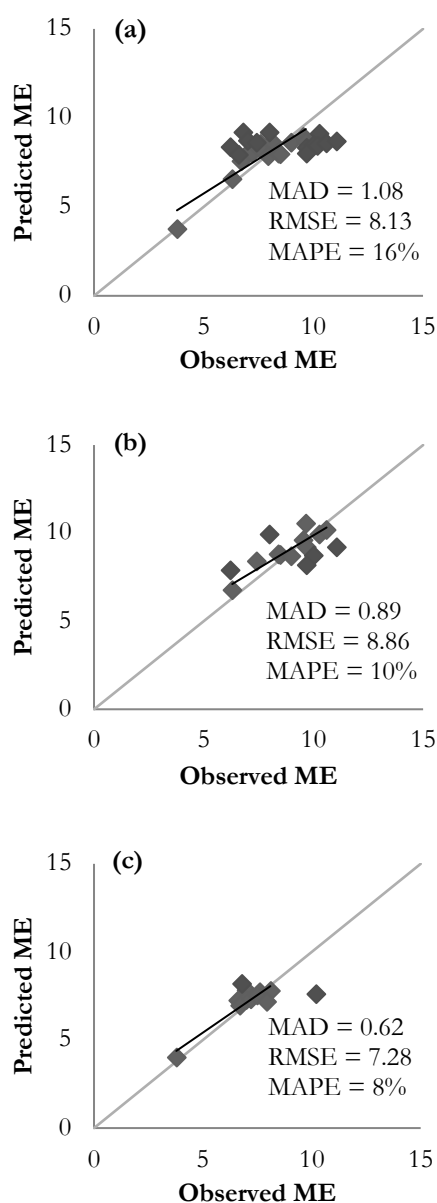
The validation using to indicates a good relationship between predicted and observed ME using MAD, MAPE, and RMSE. Each group with the best-fit equation has the lowest MAD, MAPE, and RMSE values. The regression equation expressed

with  $EM_{R+L} = 12.429 - 0.122 \text{ ADF}$ ,  $EM_R = 15.609 - 0.115 \text{ NDF}$ , and  $EM_L = 3.726 - 0.186 \text{ CP}$ . The ME estimation model is represented in Figure 1 shows the correlation between its model equation and observed ME.

### Discussion

Pearson correlation is used to determine the degree of closeness of the relationship between content nutrient and ME through the correlation coefficient. Ash, NDF and ADF fractions were able to determine ME. This is because ash is considered a potential independent variable for predicting ME in

feed ingredients and rations (Sung and Kim, 2021). Tropical grasses have low quality due to the high content of fiber components, namely NDF and ADF content, while tropical legumes have better quality due to high CP and TDN content (Indah et al., 2020). Ash plays a role in the body of ruminants, but it is needed in small amounts, but if it was excessive, it will disrupt the metabolism of ruminants. However, CP has the biggest contribution in determining ME in legume forage. Legumes are a source of protein because it generally contains CP >18% (Sawen and Abdullah, 2020). Likewise, legumes have a cell wall component (NDF or ADF) that is negatively correlated with ME (Uslu et al., 2018).



**Figure 1.** Relation between predicted ME and observed ME of tropical forage in (a) Grass + Legume; (b) Grass; and (c) Legume groups

That regression equation (Table 3) could be established as a good predictor ME of tropical forage for ruminants. The ME prediction model in Table 3 shows that ME can be estimated from the nutrient content. Especially fiber fraction obtained from the Van Soest analysis because it has a higher R than nutrient from the proximate analysis. The fiber fraction that builds plant cell walls is the most important factor affecting forage consumption by ruminants. It can be effective cell wall properties that influence the degradation and digestibility of forage (Sriagtula et al., 2022).

In addition, it also has smaller MAD, RMSE, and MAPE values so that it can be used to determine ME in tropical forages. The selection of the best model needs to be seen from the resulting RMSE and MAPE values (Nikmatya et al., 2022). ME can be obtained by changes in the biochemical structure of forage nutrient content such as a decrease in the value of soluble carbohydrates, digestible protein, and dry matter digestibility which can reduce ME (Bedinan et al., 2022).

The results of the visualization (Figure 1) show that in general the estimated ME values can follow the pattern of observed ME values. Visualization of the results is given to describe the closeness of the relationship between the predicted nutritional and the actual values. Lee et al. (2000) suggested that the ME energy partitioning equation model with CF as a determinant of total fiber is lower when compared to ADF. It represents forage cellulose content, so CF needs to be replaced with ADF to increase the precision of the equation model. The fiber fraction, NDF and ADF can reduce the efficiency of energy utilization. This caused contains limited energy to sustain microbial growth; reduces the availability of nutrients in feeds that contain high fiber and tend to accelerate rumen filling and limit feed consumption (Phuong et al., 2013). Gierius et al. (2016) revealed that legumes have a stronger effect on higher digestibility and ME values due to differences in the ratio of leaves to stems and the level of maturity. The energy value in corn silage that was evaluated *in vivo* did not differ from the value evaluated by the regression method (Wei et al., 2018)

ME is the amount of energy that can be utilized by the body's cells from digestible energy. The nutrient content and quality of feed ingredients determine the digestibility of a feed ingredient (Holik et al., 2019). The digestibility of feed ingredients is affected by the fiber content, the balance of nutrients, and livestock factors which in turn will affect the metabolic energy value of a feed ingredient (Astutik et al., 2019). ME is energy that can be used by

ruminants to meet energy needs for both maintenance and production (Lassa et al., 2021).

## Conclusions

The results obtained from the present study indicated that nutrient content had a significant correlation with ME of tropical forage and could be used to estimate ME. Therefore, the ME estimation model of tropical forage is  $ME_{R+L} = 12.429 - 0.122$  ADF for Grass + Legume,  $EM_R = 15.609 - 0.115$  NDF for Grass, and  $EM_L = 3.726 - 0.186$  CP for Legume.

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