


# Comparison of equations to predict the metabolisable energy content as applied to the vertical strata and plant parts of forage sorghum (*Sorghum bicolor*)

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## ABSTRACT

**Context.** Nutritive values, particularly energy content of tropical forages, need to be accurately assessed so that rations can be more precisely formulated. **Aims.** The research aimed to collate and compare equations used to predict metabolisable energy content in forage sorghum (*Sorghum bicolor* (L.) Moench) to ascertain the effect of vertical strata on metabolisable energy content to assist in producing silage of defined quality. **Methods.** Twenty-four predictive metabolisable energy equations derived from international feeding standards were compared using forage sorghum samples grown under fertiliser and growth stage treatments. Samples were separated into leaf, stem and seed heads (where present) over four vertical strata. **Key results.** Equations based on digestibility with crude protein were robust in the prediction of metabolisable energy and had application to routine laboratory use. **Conclusions.** The current study suggests that predictions based on digestibility and crude protein content are best placed for metabolisable energy application. Such equations should be originally based on measured metabolisable energy content to establish a regression so as to be used for predictive purposes, and satisfy the biological requirement of *in vivo* and the laboratory measurement relationship with acceptable statistical error. Chemical composition relationships predicted different metabolisable energy contents. **Implications.** Improved accuracy of the prediction of metabolisable energy content in tropical forages will provide better application of production models and more accurate decisions in ration formulation.

**Keywords:** crude protein, digestibility, feed assessment, feeding systems, *in vitro*, ruminants, tropical forages, wet chemistry.

## Introduction

Estimation of metabolisable energy (ME) content underpins all international feeding standards, with different ones developed across regions and organisations such as in Australia (Nutrient Requirements of Domesticated Ruminants (NRDR); Freer *et al.* 2007), the UK (Agricultural and Food Research Council (AFRC); Alderman and Cottrill 1993), France (INRA; Martin-Rosset 1990), Scandinavia (NorFor; Volden 2011), East Germany (Rostock; Beyer *et al.* 2003), North America (National Research Council (NRC); NRC 2001) and South America (BR-Corte; Valadares Filho *et al.* 2016). Metabolisable energy content is estimated by various equations based on two approaches, that determined by chemical composition (e.g. NRC and BR-Corte standards) and that determined by some estimate of digestibility (e.g. AFRC and NRDR standards). Historically, the equations were developed on the basis of calorimetry, whereby ME content was measured and then related to various attributes of the diet such as chemical composition or *in vivo* digestibility using large data sets (e.g. Blaxter and Wainman 1964). Over time, the original data sets and their characteristics were overlooked, and the equations applied outside their original source of feed types. McLennan (2005) showed that ME content estimated by the Australian feeding

standards (SCA) and Cornell net Carbohydrate and Protein System (CNCPS) developed at Cornell University USA differed when applied to tropical forages. The gold standard in establishing these relationships must involve a measurement of the ME content through calorimetry and establishing a regression relationship to some chemical composition or *in vivo* or *in vitro* digestibility estimate. Calorimetry studies are rarely used now and so the equations in the various feeding standards are applied to situations often not in alignment with the original data sets.

Forage sorghum (*Sorghum bicolor* (L.) Moench) is widely grown for grazing or the production of silage to be fed to dairy and beef cattle. The whole crop may be grown and harvested or strata may be harvested (at least two cuts of varying height) to produce silage of varying quality and yield. The ME content of the harvested strata needs to be accurately estimated so that particular strata can be included in ration formulation in total mixed rations, partial mixed rations or grazed to a particular depth or level of utilisation (Benvenuti *et al.* 2016). When developing various forage-based systems for feeding dairy cows, it was noticed that estimates of ME content differed markedly as a result of the different approaches on the basis of either chemical composition or *in vitro* digestibility used by different, or the same, laboratories. In Australia, ME content has been estimated by chemical composition or some *in vivo* or *in vitro* methods, some of which utilise near-infrared spectroscopy (NIRS) relationships. The Queensland Department of Agriculture and Forestry dairy group have had over 7000 samples analysed by Dairy One Forage Laboratory (Ithaca, NY, USA) and Australian laboratories, with outputs being generated by NIRS and wet chemistry. There is not always agreement among methods and the choice of which ME value to use in various feed formulation models becomes problematic.

In this paper, we undertake a comparison of various equations to predict the ME content of different components of two forage sorghum varieties grown under various N fertiliser conditions and sampled at different stages of growth. The equations were derived from feeding standards used in Australia, including the NRC and CNCPS systems, AFRC and Agricultural Development and Advisory Service (ADAS; Stratford-upon-Avon, UK) systems, SCA and NRDR (CSIRO) system and some specific publications that underpin equations used in these feeding standards (e.g. Minson 1984). A similar approach to compare equations was undertaken by Robinson *et al.* (2004). Equations from the NRC, University of California at Davis (UC Davis) and ADAS were compared and it is important to note that their approach did not determine the correct equation but did conclude how the equations differ and the biological basis of each equation. In an ideal world, the ME content would be determined by calorimetry, but the process is time inefficient and not practical when needing to make ration formulation decisions. Hence, there is a need to have some

chemical composition or *in vitro* procedure to predict a ME value. In some circumstances, ranking plant parts or sample types may be adequate to make appropriate decisions but in other cases an accurate value is required in the application of feeding and ration formulation models.

## Materials and methods

### Forage sorghum samples

Two forage sorghum varieties (Graze-N-Sile and Mega sweet) were grown at Gatton Research Facility (27°32'45"S, 152°19'44"E) during the summer of 2016. Treatments evaluated included high (30 kg N/ha week) and low (2 kg N/ha week) fertiliser application rates using Easy N (Incitec Pivot Fertilisers), and two stages of plant growth (grazing stage at approximately 1 m in height, and silage stage at soft dough stage) in replicated plots ( $n = 4$  replications/treatment). At sampling, plants were harvested 15 cm above the ground, plant height was measured, then samples were cut into four equal vertical strata (Benvenuti *et al.* 2016). Each stratum was then separated into leaf, stem and seed heads (where present). Samples were dried in an oven at 60°C and ground through a 2 mm screen (Retsch Mühle rotary grinder, Germany). A total of 100 samples were selected from our experimental forage sample set and used for further analysis. These samples were selected to represent a diverse range of nutritional parameters across all components across all strata.

### Laboratory analysis

Subsamples were sent to the Dairy One Forage Lab to determine crude protein (CP), ethanol-soluble carbohydrates, lignin, crude fat, acid detergent fiber (ADF), amylase, sodium sulfite-treated neutral detergent fiber and mineral content by using their wet-chemistry services (Dairy One 2007). The Dairy One Forage Lab uses a multiple component summative approach, using total digestible nutrients (TDN) for ME prediction employing a CNCPS approach (Eqn 1 in Table 1). These values are derived from the composition of those components in the sample and a theoretical digestibility of those components or a lignin-derived modification of NDF digestibility (Weiss *et al.* 1992).

Subsamples were analysed locally by using an *in vitro* procedure (Daisy ANKOM method, two-stage rumen fluid pepsin procedure corrected against known *in vivo* standards of tropical forages). Estimations were then made of dry-matter digestibility (DMD), organic matter digestibility (OMD) and digestible organic matter in the DM (DOMD; Holden 1999). Organic matter (OM) was determined by ashing dried samples at 600°C in a muffle furnace (Modutemp, Midvale, WA, Australia) for 3 h. Ash-free neutral detergent content was determined according to the method of Goering and

**Table 1.** Equations included in the current analysis that are commonly used to predict metabolisable energy (ME) content of forages.

Equation number	Author	Equation
Equations based on chemical composition		
1	CNCPS; NRC (2001)	DE (Mcal/kg DM) = (TDN%/100) × 4.409 ME (Mcal/kg DM) = (DE (Mcal/kg DM) × 1.01) – 0.45 (dairy cattle) where TDN is: TDN IX (%) = tdNFC + tdCP + (tdFA × 2.25) + tdNDF – 7 (where: td, truly digestible; NFC, non-fibre carbohydrates, CP; FA, fatty acids (calculated as ether extract); NDF, neutral detergent fibre)
2	Minson (1984)	(a) ME (MJ/kg DM) = 0.260 CP (%) + 4.653 (b) ME (MJ/kg DM) = 21.574 – 0.207 NDF (%) (c) ME (MJ/kg DM) = 16.654 – 0.241 ADF (%) (d) ME (MJ/kg DM) = 13.764 – 0.165 CP (%) – 0.118 NDF (%) (e) ME (MJ/kg DM) = 10.738 + 0.161 CP (%) – 0.131 ADF (%) (f) ME (MJ/kg DM) = 7.735 + 0.17 CP (%) – 0.335 lignin (%)
3	Abate and Mayer (1996)	ME (MJ/kg DM) = 8.11 + 0.1341 CP (%) – 0.1065 ash (%)
Equations based on digestibility		
4	ADAS; Morgan (1972)	ME (MJ/kg DM) = 0.84 + 0.14 DOMD (%)
5	Givens et al. (1990)	ME (MJ/kg DM) = 0.37 + 0.0142 DOMD (g/kg DM) + 0.0077 CP (g/kg DM)
6	Minson (1984)	(a) ME (MJ/kg DM) = 0.153 DMD (%) – 1.057 (b) ME (MJ/kg DM) = 0.15 OMD (%) – 1.126 (c) ME (MJ/kg DM) = 0.184 DOMD (%) – 1.827 (d) ME (MJ/kg DM) = 0.157 DOMD (%) + 0.059 CP (%) – 1.073
7	AFRC; Alderman and Cottrill (1993)	ME (MJ/kg DM) = 0.0157 DOMD (g/kg DM)
8	NRDR/CSIRO; Freer et al. (2007)	(a) ME (MJ/kg DM) = 0.172 DMD (%) – 1.707 (b) ME (MJ/kg DM) = 0.169 OMD (%) – 1.986 (c) ME (MJ/kg DM) = 0.194 DOMD (%) – 2.577
9	AFIA (2011)	ME (MJ/kg DM) = 0.203 DOMD (%) – 3.001
10	SCA (1990)	(a) ME (MJ/kg DM) = 0.18 DOMD (%) – 1.8 (b) ME (MJ/kg DM) = 0.16 OMD (%) – 1.8 (c) ME (MJ/kg DM) = 0.17 DMD (%) – 2.0
11	Freer et al. (2004)	ME (MJ/kg DM) = 0.172 DMD (%) – 1.71

Van Soest (1970) modified by Mertens (2002), by using the ANKOM system (ANKOM 200 Fiber Analyzer, Macedon, NY, USA). All other values were derived from Dairy One laboratory analysis and applied to the equations that required a chemical component of the sample, for example, CP content.

## Equations

In total, 24 equations that estimated ME content from either chemical composition, digestibility or both, were obtained from a range of Australian, UK and USA feeding standards (Table 1). Equations specific to forages were chosen where possible. Some equations were based on a wide-ranging data base of feed types (e.g. NRC equations), some had temperate grass only, with varying sample numbers included (e.g. UK-based equations of ADAS, Givens et al. 1990; AFRC, Alderman and Cottrill 1993), tropical grasses of the *Digitaria* genus (e.g. Minson 1984) and some a selected group of forage samples (not defined) from the ADAS data base on temperate forages (e.g. Freer et al. 2004, 2007;

AFIA 2011). Only data bases and equations based on calorimetry or *in vivo* digestibility with appropriate digestible-energy conversions were chosen and access to analytical data used in the equations was also required.

## Statistical analyses

The full set of predicted ME values generated by the 24 equations across all strata and plant parts was subjected to principal component analysis (PCA) to identify major groupings within the data. This identified clear separation of samples on the basis of plant parts (i.e. leaves, stems and seeds). Analyses to compare the predictions focussed on each of these plant parts separately.

To compare the predictions generated by the different ME equations, predictions from each equation were compared with the mean predictions generated by averaging across all equations. Mean predictions across all equations were calculated and used to represent an ME equation index, from low ME prediction values to high ME prediction values. Predictions from each equation were then regressed against

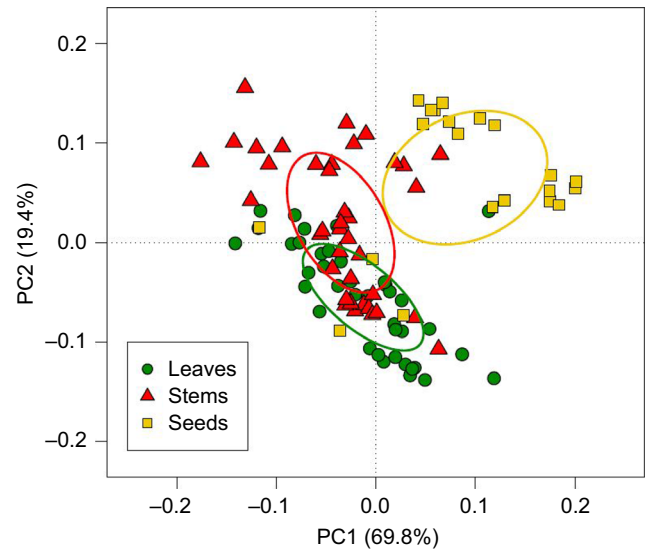
the ME equation index by fitting linear mixed-effects models. A comparison of the random intercept versus random slope and intercept model structures using the Akaike and Bayesian information criteria was made and showed that the random intercept model structure was most parsimonious for all three plant parts. The variability of the predictions generated by each ME equation along two dimensions was assessed by comparing the distance between the intercept and the overall mean intercept (delta intercept) of each ME equation, and the standard deviation (s.d.) of the predictions around the model fit. The s.d. was plotted against the delta intercept and selection of equations were based on a combination of criteria that were equations with the lowest s.d. and positioned within the 95% confidence interval of the mean delta intercept. These selected equations represent 'best bet' equations, in that they generate ME predictions that lie closest to the overall mean predictions across all ME equations combined and they have low variability across the range of ME values (i.e. have consistent predictive power across samples with a low and high ME). These 'best bet' equations were chosen for further comparison. The coefficient of determination ( $R^2$ ) was used to compare ME prediction of paired 'best bet' equations. The equations were also compared to investigate how consistently they ranked ME values across samples.

## Results

Estimates of ME content of sorghum samples were highly variable depending on the equation used. Estimations from the 24 equations were grouped on the basis of whether they were derived through digestibility or chemical composition. Furthermore, PCA showed that samples separated clearly on plant part (Fig. 1). Seeds separated clearly from leaves and stems along PC Axis 1, while leaves and stems separated more clearly along PC Axis 2.

The selected equations that had consistent predictive power across samples with a low and high ME were Eqn 6b for stems, Eqn 6d for leaves and Eqn 5 for seed heads (Table 2). Eqns 1 and 2b could also be classified as 'best bets' for stems and leaves respectively; however, in general the CNCPS equation overestimated ME compared with that estimated by other equations, while the NDF Minson (1984) equation (Eqn 2b) tended to underestimate ME on the same basis at low values of ME. Equation 2e was also considered adequate for seed heads but carried a lot of variation across low ME values. In terms of robustness (comparing across plant parts and agronomic conditions), the 'best bet' equations were Eqn 5 and 6d as both had tighter model fits and intercept close to the mean intercept. Both of these equations were based on DOMD and CP content.

There is considerable variation in prediction of ME content between the 'best bet' equations. Fig. 2 shows paired



**Fig. 1.** Principal component analysis of metabolisable energy (ME) content of forage sorghum samples estimated from 24 ME equations. Ellipses represent standard deviations of plant part centroids.

comparison of the 'best bet' ME equations. Fig. 2a, b compare the CNCPS Eqn (1) with the DOMD\_CP-based equations (Eqns 5, 6d). Model fits ( $R^2$ -values) for these comparisons were considered very poor and the regression coefficient slope values were  $<0.25$ , showing wide deviation from a 1:1 fit (Fig. 2a, b). The CNCPS equation (Eqn 1) showed a tendency for higher estimates of ME than did the Minson (1984) DOMD\_CP-based equation (Eqn 6d), except in stems in the top strata. There was close agreement in ME values between the Givens *et al.* (1990; Eqn 5) and Minson (1984; Eqn 6d) predictions (Fig. 2c). Both these equations are based on DOMD and CP, with the Givens *et al.* (1990) equation (Eqn 5) tending towards higher ME-value estimates. The 'best bet' Minson (1984) NDF (Eqn 2b) compared with Minson (1984) DOMD\_CP (Eqn 6d) showed considerable variation, particularly when the ME values of the seed heads were taken into account (Fig. 2d).

The leaves and stems of forage sorghum plants were examined for ME changes along the vertical strata and to compare the equations for any differences in ranking. Predictions based on digestibility and CP (Eqns 6d, 5) ranked similarly throughout the strata, while wet chemistry-derived predictions ranked differently from each other and from digestibility-based equations for both stems and leaves.

## Discussion

This paper compares the prediction of ME content of forage sorghum fractions by a range of equations used in international feeding standards. The equations compared in this paper are not an exhaustive list but rather concentrate



**Table 2.** Equations with best predictive metabolisable energy (ME) content on the basis of standard deviation (s.d.) against delta intercept and selecting those equations that had the lowest s.d. and fell within the 95% confidence interval of the delta intercept.

Method	Equation	Feed system	Reference	Animal source	Original source	Original reference
TDN	Equation 1 DE (Mcal/kg) = (TDN %/100) × 4.409 ME (Mcal/kg) = (DE × 1.01) – 0.45 (dairy cattle)	CNCPS	Fox et al. (2004)	Cattle	<i>In vivo</i> digestibility trial with maintenance (calorimeter)	(NRC 2001)
Proximal analysis	Equation 2b ME (MJ/kg) = 21.574 – 0.207 NDF (%) Equation 2e ME (MJ/kg DM) = 10.738 + 0.161 CP (%) – 0.131 ADF (%)	Australia	Minson (1984)	Wether sheep	<i>In vivo</i> digestibility and chemical composition data	
<i>In vitro</i> (Tilley and Terry method)	Equation 5 MJ (MJ/kg DM) = 0.37 + 0.0142 DOMD (g/kgDM) + 0.0077 CP (g/kg DM)	UK	Givens et al. (1990)	Sheep	<i>In vivo</i> digestibility trial with maintenance (calorimeter)	(Terry et al. 1974)
<i>In vivo</i>	Equation 6b ME (MJ/kg) = 0.15 OMD (%) – 1.126 Equation 6d ME (MJ/kg) = 0.157 DOMD (%) + 0.059 CP (%) – 1.073	Australia	Minson (1984)	Wether sheep	<i>In vivo</i> digestibility and chemical composition data	

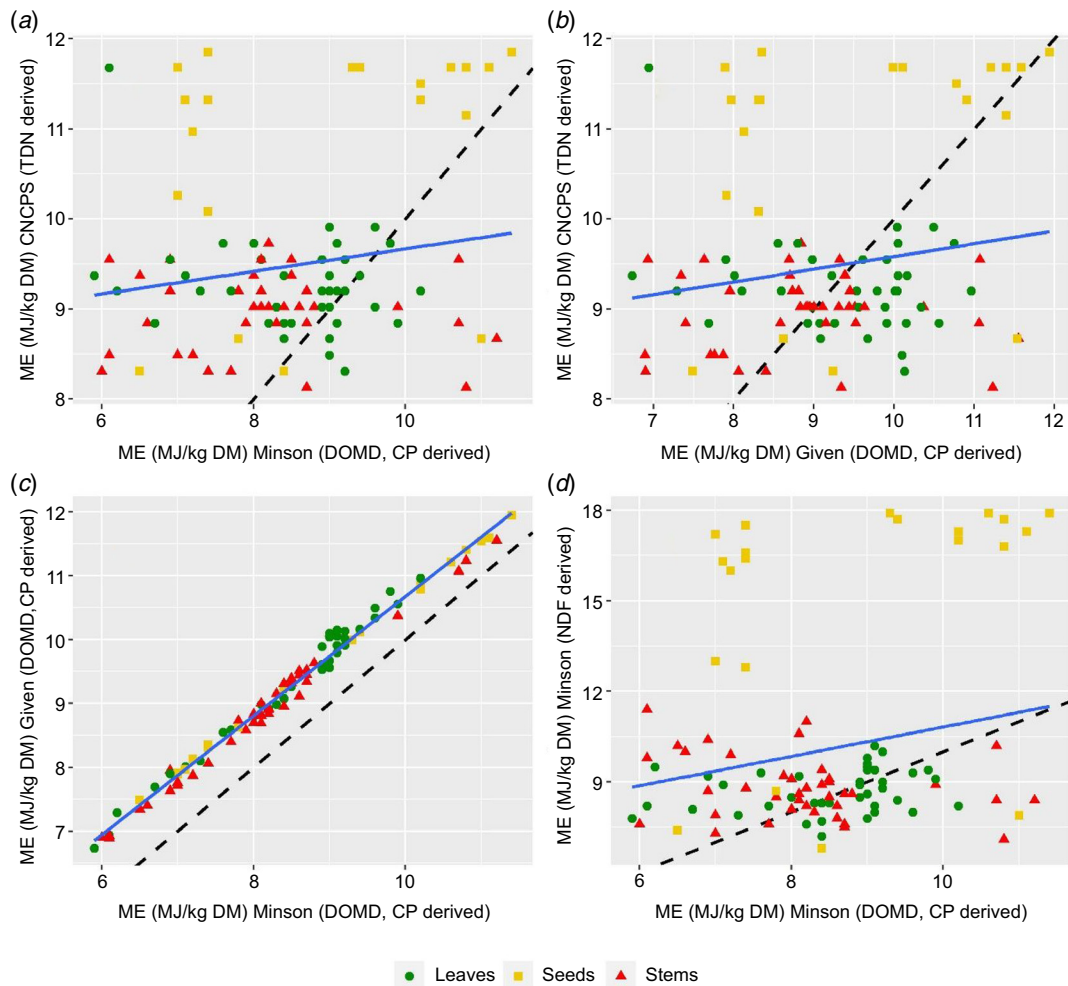
on those in use within Australia (USA NRC/CNCPS, UK ADAS and Australian CSIRO/NRDR systems). Only one equation was derived from a data base that exclusively used tropical forages (Minson 1984) but was limited to the *Digitaria* spp. The USA NRC/CNCPS system has a large number of undefined tropical forages in the data base but the extent to which these are related to *in vivo* estimates is unclear. The strength of any predictive set of equations must be based on their biological rationale and, hence, the original data base from which the equations were derived. This is not always clear in the various systems. Predicting ME from tropical forages is important for the following two reasons: first, to estimate ME content accurately so as to use models to formulate rations, interpret milk production or liveweight gain and predict intake through back calculation; and second, to rank (even if with less precise estimate of ME content) vertical strata for ME content so as to make decisions on cutting height or utilisation by grazing. The outputs by the various equations showed large variation (Fig. 2) and did not rank vertical strata similarly. Thus, a decision needs to be made as to which set of equations and system is best placed for application, provided they are based on tropical forages.

The current study suggests that predictions based on DOMD and CP content are best placed for application and, providing they are based on measured ME content to establish a regression able to be used for predictive purposes, they satisfy the biological requirement of *in vivo* and laboratory measurement relationship with acceptable statistical error. Equations 5 (Givens et al. 1990) and 6d (Minson 1984) appear to be acceptable and are recommended to be used. These equations can also be easily applied through standard laboratory procedures, and NIRS can also be used for further application in the laboratory. The use of NIRS provides a rapid and accurate analysis, using the appropriate data set calculations for these laboratory parameters and,

additionally, the spectral analysis can be stored for further associative relationships (Poppi 1996). Those predictions based on chemical composition to predict TDN and ME, founded on measured *in vivo* and chemical composition relationships, were more variable but are equally able to be measured in a laboratory in a time frame that enables their widespread application. Total digestible nutrients from Dairy One is based on a summative equation using chemical composition and estimates of the digestibility of those components (Weiss et al. 1992). Equations 1 (NRC and CNCPS based) and 2 (Minson 1984) are appropriate but their predictions from those based on digestibility differed in some circumstances, which means that, practically, a research group should stick to one approach. Minson (1984), in his comparison of the same data set by a variety of regressions, suggested that the digestibility approach had less variation ( $R^2$  and relative standard deviation) and was preferred over chemical composition.

The present study could not ascertain which equation(s) are biologically correct. We compared two different approaches (chemical composition or digestibility estimates with or without some chemical parameter) and determined whether they agree numerically or rank similarly. For this data set of forage sorghum, they do not. This finding agrees with those of McLennan (2005) who demonstrated inconsistency between models, with CNCPS exhibiting considerable variability and higher estimates in ME contents than the SCA-estimated ME values. Robinson et al. (2004) highlighted this point and suggested various equations in a comparison of some USA-based systems and UK ADAS-based systems for a wider range of feed ingredients than was used here. Their conclusion that some variation needs to be accepted to have a simple analytical tool is valid and one we would endorse.

The data base on which the original equations were established is not often explicit. Only one data base was



**Fig. 2.** General relationship (-----) between equations estimating metabolisable energy (MJ/kg DM) in forage sorghum with plant parts separated and  $y = x$  (—). (a) Eqns 1 and 6a:  $y = 7.87 + 0.18 x$ ;  $R^2 = 0.045$ ;  $P = 0.03$ . (b) Eqns 1 and 5:  $y = 7.30 + 0.227 x$ ;  $R^2 = 0.0638$ ;  $P = 0.011$ . (c) Eqns 5 and 6a:  $y = 1.34 + 0.932 x$ ;  $R^2 = 0.9884$ ;  $P < 0.0001$ . (d) Eqns 6a and 2b: no relationship.

exclusive to tropical forage and was limited to *Digitaria* spp. (Minson 1984). However, this equation performed well and Eqn 6d is recommended to be used for tropical grass species, including forage sorghum. No relationships were exclusively established for tropical or temperate legume species. A similar conceptual relationship (DOMD and CP content) was established by ADAS on various temperate grass species, expanding and modifying the equations from 47 to 64 to 173 grass hays (Eqn 4, ADAS; Eqn 5, Givens *et al.* 1990). This also performed well and similarly to the Minson (1984) Eqn 6d (Fig. 2b) for comparison of both outputs. This may be expected, given the common parameters of the relationship; nevertheless, one was based on a tropical grass species, and one was based on a number of temperate grasses. Their close agreement suggests that the principle of conversion of digestible OM and protein to ME is universal as assumed and underpins all concepts of energy metabolism. Lipid content of forages is low and,

hence, unlikely to influence the result; however, with some supplements and compound feeds, lipid content can be increased and this will have an influence. Other equations are based on similar principles (Eqns 7, 8a–c, 9, 10a–c, 11). In particular in Australia, Eqn 10a–c (SCA 1990) and more recent Eqn 8a–c (NRDR; Freer *et al.* 2007) and Eqn 9 (AFIA 2011) and Eqn 11 (Freer *et al.* 2004) are derived using a subset of the ADAS data base (criteria not defined), which does not include tropical forages. As outlined above, this is not a major problem and there is close agreement between the output of the NRDR (Freer *et al.* 2007) and Minson (1984) equations (Eqns 8 and 6d respectively).

Laboratory procedures need to be carefully defined in ME prediction feeding tables and in feed analyses reports. Most of the early reports on *in vitro* procedures used a 1 mm grinding size and more recent *in vitro* procedures on tropical pastures used a 2 mm grinding size. Standards for *in vitro* digestibility need to be consistent with the *in vivo* standards. The original

*in vivo* standards are no longer available within Australia. It is recommended that new *in vivo* standards be established with a range of forages and that there be consistency in the approach. The original system devised by Blaxter and Wainman (1964) to determine digestibility and ME content with sheep fed at maintenance is recommended for consistency even for application to cattle. As with Minson (1984), there is no need for calorimetry (although that would be preferable), and a DOMD relationship based on well-established conversions to ME would appear adequate.

The ME equations rank ME content differently down the strata and ranking differences are complicated by separation of equations on plant part. There is disparity of ME values in equations for seed heads and stems, which may relate to the changeable carbohydrate fractions with maturity. Equations derived from chemical composition all rank similarly, as do those derived from digestibility data, which can also be inferred from the regression equations that simply relate the two estimates of ME content against each other without reference to strata (Fig. 2). Thus, as with a simple sample comparison, the strata comparison also suggests considerable variation among models in the estimation of ME content, which could contribute to errors in prediction of production. The pattern of change down the strata was different between equations. CNCPS overestimated ME values compared with Minson (1984) OMD-CP-derived equations, except for the top strata stems. It was of interest that the ME content in forage sorghum, as measured by most equations did not change markedly down the strata. Thus, whatever method was selected, cutting height of forage sorghum was not so important for delivering a silage product of defined ME content. The actual quantitative values for ME content are important as predictors of feed intake and milk production. As such, any differences in prediction of ME content will lead to differences and potential error in the prediction of intake and milk production.

## Conclusions

The prediction of ME content in forage sorghum is best applied using equations based on a combined regression using the parameters DOMD and CP. Such an approach would lend itself to the application of NIRS for rapid analysis. The *ad hoc* application of chemical composition to predict TDN and ME content on the basis of various chemical parameters in a multiple regression also has value but there is unacceptable variability between this approach and that based on some estimate of digestibility. This comparative ME equation approach needs to be validated across multiple forages. Australia also needs to develop feeding standards based on *in vivo* digestibility and current laboratory procedures for wider application by industry.

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**Data availability.** The data that support this study will be shared upon reasonable request to the corresponding author.

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