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MODELLING ANIMAL SYSTEMS PAPER

Predicting the metabolizable energy intake of ruminants using digestibility, ruminal methane production and fermentation data

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SUMMARY

Obtaining accurate estimates of the metabolizable energy (ME) intake (MEI; MJ/day) of individual grazing ruminants is an important requirement for effective nutritional management and genetic selection of energy efficient ruminants. Diet digestibility and the daily methane production rate (MPR; MJ/day) of ruminants can be closely linked with their MEI, so published data were examined to determine whether MEI could be accurately estimated from digestibility, MPR and other parameters able to be measured on grazing animals. Four modelling approaches were assessed or developed to estimate MEI: (i) a published fixed proportional relationship between the non-metabolizable losses of MPR and urinary energy (UE; MJ/day); (ii) the proportion of energy digestibility (EngDig); (iii) MPR and the ruminal factors that influence the stoichiometric relationships between MPR and MEI; and (iv) the calculated ME arising from rumen fermentation (ME_f; MJ/day). Data to develop the models ($n=61$) were collected across three publications (Paper) where the Paper effect was treated as a random-effect variable. Each of the models (1–4) was challenged with an independent data set ($n=19$). The inclusion of ME_f ($P=0{\cdot}01$) to predict MEI [MEI = 0·18 (2·03) + 3·42 (0·36) × sqrt(ME_f) (D.F. = 57; residual log likelihood = 173·6)] had the lowest mean square error of prediction (MSEP) when challenged with the independent data set; mean bias of −0·42 MJ/day ($P<0{\cdot}05$), MSEP = 0·68 MJ/day and the bias, slope and random components of the MSEP were, as a proportion, 0·26, 0·13 and 0·61, respectively. None of the models estimated MEI with sufficient accuracy to be useful for identifying individual animals with above average energetic efficiency. A critical limit to any model seeking to estimate MEI from MPR and fermentation traits appears to be the variation between animals and between diets, in the proportion of digested energy which is fermented relative to that which is made available by mammalian digestion, and this is evaluated.

INTRODUCTION

Metabolizable energy (ME) intake (MEI; MJ/day) is frequently the principal constraint to ruminant growth (Beauchemin *et al.* 1995; Hegarty *et al.* 1999) and, as such, is an important input in feeding standards and models which seek to describe or predict animal growth (SCA 1990; AFRC 1993). MEI of grazing animals has traditionally been estimated indirectly from measures of dry matter (DM) intake (DMI) derived from markers or pasture cuts and from laboratory- or marker-based estimates of DM digestibility (Dove & Mayes 1991). In recent years, new field methods have been developed for estimating diet digestibility (Coates 2000), methane production and perhaps CO_2 production in grazing ruminants (Johnson *et al.* 1994; Pinares-Patiño *et al.* 2007). It was hypothesized that combining measurements such as digestibility, methane production rate (MPR; MJ/day), characteristics of fermentation stoichiometry and rumen volatile fatty acids (VFA) proportions would allow accurate prediction of the MEI of grazing ruminants. Estimating MEI from animal measurements only would dispense with the need to sample pasture, or pursue the complexities of diet selectivity and

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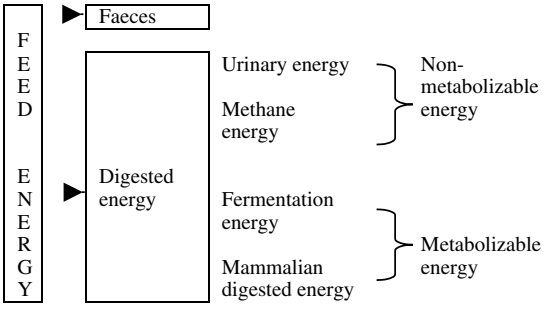

Fig. 1. Partitioning of feed energy into FE and DE that are either non-metabolizable [UE and methane energy] or is rendered metabolizable as either products of fermentation (VFA and microbial cells) or products arising from mammalian digestion of the diet.

digestibility in order to determine MEI. Such a simple technique is required to provide a more cost-effective means of identifying animals of high feed-use efficiency without lengthy testing (Archer *et al*. 1997). The current paper develops several models using digestibility, MPR and fermentation data obtained from controlled feeding and respiration studies. For the development and challenge of the models, we used published data.

Variation in feed intake and daily MPR

If an accurate estimate of MEI has to be obtained from measurement of the non-ME losses, then an awareness of the sources of variation in methane and energy production and their errors of measurement or prediction is required. The quantity of ME obtained from an ingested feedstuff (Fig. 1) is a function of the digested energy (DE; MJ/day) minus the losses in methane (MPR; MJ/day) and urinary energy (UE; MJ/day) (Armstrong 1964), where the DE is a function of gross energy intake (GEI; MJ/day) minus faecal energy (FE; MJ/day), i.e. DE = GEI − FE.

In animals consuming feed *ad libitum*, the value of MEI obtained will depend on the duration of measurements. For housed sheep, the coefficient of variation (CV) for daily hay intake is 0·09–0·12 and repeatability of daily intake for animals on a constant diet is 0·59–0·61 (Sheehan *et al*. 1985). Lee *et al*. (1995) used alkane capsules to estimate that the CV of DMI for grazing sheep was 0·20–0·27. For beef cattle fully fed on a pellet diet, a period of at least 35 days is desirable to obtain a stable estimate of DMI when estimating residual feed intake (Archer *et al*. 1997) and short-term cycles in daily feed intake have been observed (Stroup *et al*. 1987). One implication of large day to day variation in DMI is that estimates of MEI will also require correspondingly long-term data on any predictive parameters. Short-term calorimetric and fermentation studies (e.g. Murray *et al*. 1978; Hegarty *et al*. 1994; Wright *et al*. 2004) will be inadequate as a basis for estimating the long-term MEI of grazing ruminants.

The influences of diet composition and level of intake on average daily methane production have been extensively reviewed (Blaxter & Clapperton 1965; Kirchgessner *et al*. 1995; Kurihara *et al*. 1997; Pelchen & Peters 1998). In long-term controlled feeding studies, the CV of methane production across days is approximately 0·072 (Blaxter & Clapperton 1965). In grazing sheep consuming pasture *ad libitum,* the CV of methane production over days is 0·13 of the mean (range 0·022–0·426: Ulyatt *et al*. 1999). In dairy cattle, both within-animal (0·069–0·101) and between-animal variation (0·062–0·278) in methane production changed with diet (Vlaming *et al*. 2008).

Individual animals have been recorded with extremely low MPRs on both grain (Johnson *et al*. 1991; Goopy & Hegarty 2004) and pasture diets (K. Joblin 2004, personal communication). While the low frequency of these atypical animals is unlikely to cause significant error in estimating the average methane production of a population, it is of concern in obtaining MEI estimates for individual animals. The current paper reports the development and evaluation of models predicting MEI from related traits and tests whether such predictions can be improved by addition of easily collected data on rumen or digestibility characteristics or derived descriptors of rumen fermentation energetics.

MATERIALS AND METHODS

Four models were assessed or developed for prediction of MEI: model 1 used only a published fixed proportional relationship between the non-metabolizable losses of DE (MPR and UE) and MEI; model 2 was developed with the inclusion of the proportion of energy digestibility (EngDig); model 3 was developed with the inclusion of MPR and ruminal factors that influence the stoichiometric relationships between MPR and MEI (VFA proportions and pH) as well as rumen ammonia concentration (NH_3; mg N/100 ml) as a possible indicator of UE; and model 4 was developed with the inclusion of the calculated ME arising from rumen fermentation (ME_f; MJ/day). Models 2, 3 and 4 were developed using the combined studies of Jentsch *et al*. (1972), Osakwe *et al*. (2004) and Mwenya *et al*. (2004), respectively. All models were then challenged using the combined data of Itabashi *et al*. (1984), Carulla *et al*. (2005) and Pinares-Patiño *et al*. (2003). Data used in both development (models 2–4) and challenge of models (Models 1–4) are summarized in Table 1.

Model 1

A published fixed proportional relationship between the non-metabolizable losses of DE (MPR and UE)

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Table 1. *Range and mean of input variables used in developing optimized regression predictions of MEI (development data) and data used to challenge models (challenge data). Development data were sourced from Jentsch* et al. *(1972), Osakwe* et al. *(2004) and Mwenya* et al. *(2004). Challenge data were sourced from Itabashi* et al. *(1984), Carulla* et al. *(2005) and Pinares-Patiño* et al. *(2003)*

	MEI (MJ/day)	Gross EngDig	Methane (MJ/day)	Urine (MJ/day)	Molar proportions			NH_3 (mg N/100 ml)	pH	ME_f* (MJ/day)
					Acetate	Propionate	Butyrate			
Development data										
N	61	61	61	61	61	61	61	51	61	61
Min	3·3	0·482	0·49	0·52	0·613	0·123	0·052	9·0	6·0	3·8
Max	14·0	0·808	2·0	1·5	0·759	0·277	0·177	51·1	7·3	13·7
Mean	11·0	0·687	1·4	0·93	0·684	0·192	0·103	27·4	6·6	9·2
S.D.	2·26	0·0695	0·37	0·20	0·0387	0·0321	0·0333	8·28	0·32	2·36
Challenge data										
N	19	19	19	19	19	19	19	–	–	19
Min	7·9	0·530	0·74	0·62	0·580	0·175	0·054	–	–	6·2
Max	12·1	0·700	1·3	1·4	0·701	0·293	0·130	–	–	10·8
Mean	9·4	0·614	0·97	0·97	0·654	0·219	0·084	–	–	8·0
S.D.	1·32	0·0549	0·16	0·22	0·0375	0·0370	0·0176	–	–	1·42

* ME_f stands for ME present in VFA and microbial cells as estimated from daily MPR and VFA molar proportions, with a constant microbial growth efficiency.

and MEI was used (Eqn 1; SCA 1990). The non-metabolizable losses (Eqn 2) were substituted into Eqn (1) to give Eqn (3) (MEI_{p1}; MJ/day), where the observed values of MPR and UE were used (Table 1) to predict MEI_{p1}.

$$MEI = 0{\cdot}81 \times DE \quad (1)$$

$$0{\cdot}19 \times DE = (MPR + UE) \quad (2)$$

$$MEI_{p1} = 4{\cdot}26 \times (MPR + UE) \quad (3)$$

Model 2

A nonlinear regression was conducted to predict MEI (MEI_{p2}) from the observed EngDig (Eqn 4).

$$MEI_{p2} = \alpha + \beta \times \ln(EngDig \times 100) + Paper \quad (4)$$

Model 3

A nonlinear regression was conducted to predict MEI (MEI_{p2}) from the observed MPR and the observed values of pH and NH_3, and the ruminal proportions of acetate, propionate and butyrate (Table 1; Eqn 5).

$$MEI_{p3} = \alpha + \beta \times \ln(MPR) + \sum_{i=1}^{5} X_i + \sum_{i=1}^{4} \sum_{j=i+1}^{5} \gamma_{ij} X_i X_j + Paper \quad (5)$$

Model 4

Potential ME_f (MJ/day) was calculated by combining the observed MPR and VFA proportions using stoichiometry to estimate the supply of potential ME (as VFA and microbial cell energy) arising from rumen fermentation. The stoichiometric relationship between the observed ruminal VFA and the observed MPR means that, when MPR and VFA proportions are known, the energy being yielded via VFA can be readily determined (Wolin *et al.* 1997). Combined with an assumed microbial growth efficiency, a measure of MPR can be readily used to estimate the yield of energy (MJ/day) arising from rumen fermentation in potentially metabolizable forms (VFA and microbial cells). The stoichiometry of Czerkawski (1986) as modelled by Nolan (1998) was used in association with measured MPR and VFA proportions to determine energy of VFA plus cells (MJ) produced/MJ of methane for each data point in the development and challenge data sets. This value was multiplied by the observed MPR to calculate ME_f for each data point. In model 4, a nonlinear regression was conducted to predict MEI (MEI_{p4}) from the calculated ME_f (Eqn 6).

$$MEI_{p4} = \alpha + \beta \times \mathrm{sqrt}(ME_f) + Paper \quad (6)$$

It was assumed that the energy costs of cell synthesis and of cell maintenance were both fixed at 40 mmol ATP/g cells/day, and that energy loss through re-fermentation of microbial cells in the rumen was

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assumed to be 30 mmol ATP/g cells in running the model of Nolan (1998).

Statistical analysis

Models 2–4 were developed using the PROC MIXED procedure of SAS Inc. An analysis of covariance (ANOVA) was performed on each of the models. The covariates for each of the models were EngDig, MPR and ME_f for models 2, 3 and 4, respectively; all two-way interactions and additional covariates for model 3 were evaluated. The intercept–slope, Paper (the subject in the mixed procedure of SAS), EngDig, MPR and ME_f were the random-effect terms. Paper was the experimental unit. The Paper effect in the current study was solely an intercept shift; the TYPE = VC statement in the RANDOM statement was removed to achieve the shift in intercept. Terms were included in the model at $P < 0{\cdot}05$.

All models were evaluated for the assumption of normality and constant variance. A log transformation on DE and MPR was performed and a Shapiro–Wilk test ($P < 0{\cdot}05$) was performed using the UNIVARIATE procedure of SAS. A Levene's test of the residuals ($P < 0{\cdot}05$) was used to test the assumption of constant variance. All random-effects models used Restricted Maximum Likelihood (REML) for estimating variance components. The residual (Res) log likelihood is reported for each model. An adjustment was made on the observations (residual added to its corresponding *Y* predicted value) to take into account the collapsed observations from the multi-dimensional space into a two-dimensional space (St-Pierre 2001). The mean bias and mean square error of prediction (MSEP) were derived for each model and the sources of error decomposed into bias, slope and random error, as a proportion of MSEP, as outlined in a review by Tedeschi (2006). The statistical significance of each mean bias was evaluated using a paired *t*-test of the mean of the difference between observed and model-predicted values.

RESULTS

Model 1

The challenge to model 1 (MEI_{p1}), where the observed values of MPR and UE were used (Table 1) to predict MEI_{p1} (Eqn 3), indicated a mean bias and MSEP of 1·16 and 4·08 MJ/day MEI, respectively (Table 2). This error was attributable to substantial bias (underestimation, especially at low MEI; Fig. 2), as well as error associated with the slope of the regression (0·28; Table 2).

Model 2

The nonlinear relationship (Fig. 3) of model 2 (MEI_{p2}) indicated that EngDig was a significant factor

Table 2. *Mean bias, MSEP and decomposition of errors to bias, slope and random components, as a proportion of MSEP, in models predicting MEI (MJ/day) from: urinary and methane energy (model 1); DE (model 2); methane (model 3); or from energy arising from fermentation as estimated from methane, VFA proportions and stoichiometry (model 4)*

	Model			
	1	2	3	4
N	19	19	19	19
Mean bias* (MJ/day)	1·16	0·44	0·42	−0·42
MSEP (MJ/day)	4·08	2·13	1·25	0·68
MSEP: bias	0·327	0·092	0·143	0·262
MSEP: slope	0·280	0·190	0·0	0·125
MSEP: random	0·393	0·718	0·857	0·613

* $P < 0{\cdot}01$, $P = 0{\cdot}19$, $P = 0{\cdot}10$, and $P < 0{\cdot}05$ for models 1, 2, 3 and 4, respectively.

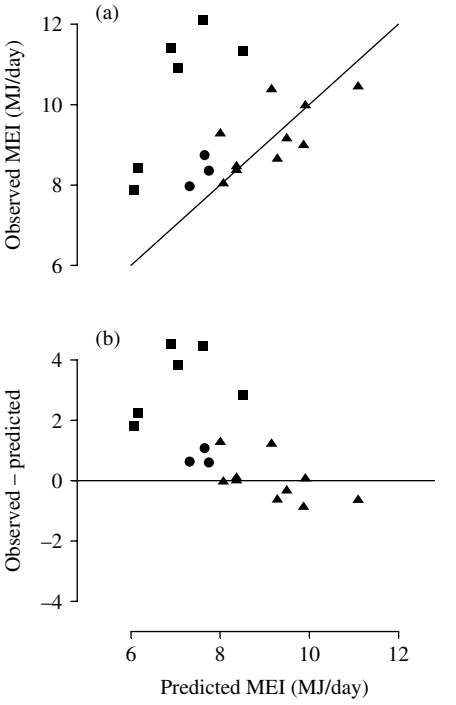

Fig. 2. Prediction (model 1; MEI_{p1} (MJ/day)) of MEI of sheep plotted against independent data (■, Itabashi *et al.* 1984; ●, Carulla *et al.* 2005; ▲, Pinares-Patiño *et al.* 2003): (a) observed *v.* predicted with the line $y = x$ and (b) the difference (observed − predicted) *v.* predicted with the line $y = 0$.

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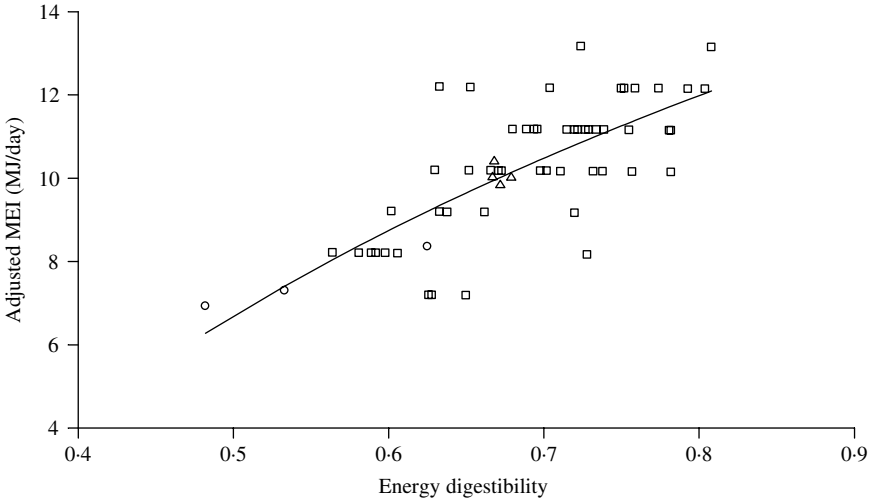

Fig. 3. Plot of adjusted observations of MEI (MJ/day) *v.* EngDig across studies (□, Jentsch *et al.* 1972; ○, Osakwe *et al.* 2004; △, Mwenya *et al.* 2004) with the nonlinear prediction (model 2; MEI_{p2} (MJ/day)).

in the model ($P=0{\cdot}02$; Eqn 7). The challenge to model 2 (Fig. 4) showed an improved MSEP relative to model 1 (2·13 *v.* 4·08 MJ/day MEI; Table 2) There was no substantial bias or error associated with the slope of the regression; the majority of the error was associated with the random component.

$$MEI_{p2} = -37{\cdot}36\ (6{\cdot}44) + 11{\cdot}26\ (1.63) \times \ln(EngDig \times 100) \quad (7)$$

(D.F. = 57; residual log likelihood = 187·2)

Model 3

The nonlinear relationship (Fig. 5) of model 3 (MEI_{p3}) indicated that MPR was a significant factor in the model ($P=0{\cdot}02$; Eqn 8). The observed values of pH and NH_3, and the ruminal proportions of acetate, propionate and butyrate and their interactions were not significant and were removed from the model. The challenge to model 3 (Fig. 6) showed an improved MSEP relative to model 1 (1·25 *v.* 4·08 MJ/day MEI; Table 2). There was no substantial bias or error associated with the slope of the regression; the majority of the errors were associated with the random component.

$$MEI_{p3} = 9{\cdot}21\ (1{\cdot}52) + 4{\cdot}72\ (0{\cdot}59) \times \ln(MPR) \quad (8)$$

(D.F. = 57; residual log likelihood = 185·5)

Model 4

The nonlinear relationship (Fig. 7) of model 4 (MEI_{p4}) indicated that ME_f contributed significantly to the model ($P=0{\cdot}01$; Eqn 9). The challenge to model 4 (Fig. 8) showed a substantial improvement in the MSEP relative to model 1 (0·68 *v.* 4·08 MJ/day MEI; Table 2). There was no substantial bias or error associated with the slope of the regression; the

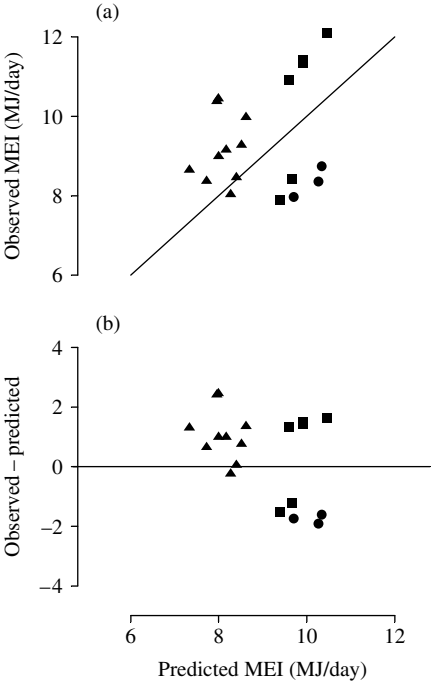

Fig. 4. Prediction (model 2; MEI_{p2} (MJ/day)) of MEI of sheep plotted against independent data (■, Itabashi *et al.* 1984; ●, Carulla *et al.* 2005; ▲, Pinares-Patiño *et al.* 2003): (a) observed *v.* predicted with the line $y=x$ and (b) the difference (observed − predicted) *v.* predicted with the line $y=0$.

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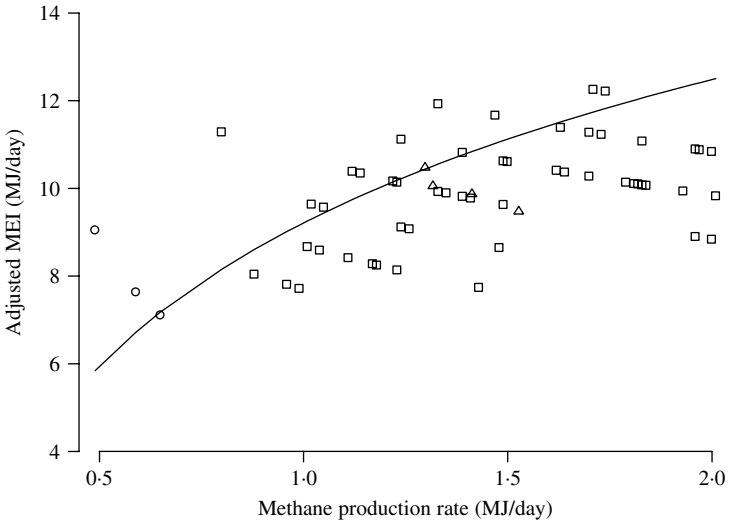

Fig. 5. Plot of adjusted observations of MEI (MJ/day) *v.* MPR (MJ/day) across studies (□, Jentsch *et al.* 1972; ○, Osakwe *et al.* 2004; △, Mwenya *et al.* 2004) with the nonlinear prediction (model 3; MEI_{p2} (MJ/day)).

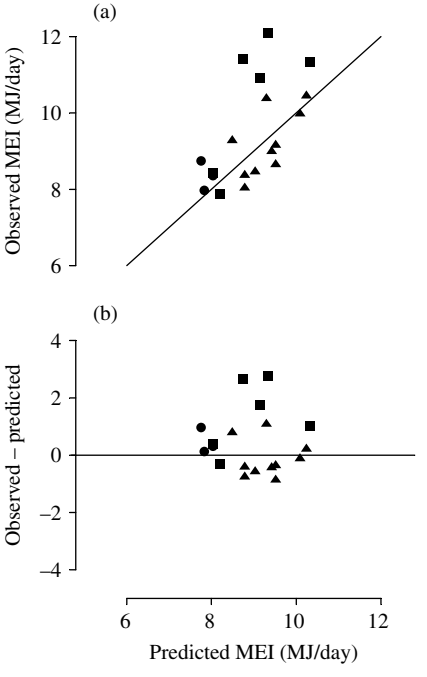

Fig. 6. Prediction (model 3; MEI_{p3} (MJ/day)) of MEI of sheep plotted against independent data (■, Itabashi *et al.* 1984; ●, Carulla *et al.* 2005; ▲, Pinares-Patiño *et al.* 2003): (a) observed *v.* predicted with the line $y=x$ and (b) the difference (observed − predicted) *v.* predicted with the line $y=0$.

majority of the error was associated with the random component.

$$MEI_{p4} = 0{\cdot}18\ (2{\cdot}03) + 3{\cdot}42\ (0{\cdot}36) \times \mathrm{sqrt}(ME_f) \quad (\text{D.F.} = 57;\ \text{residual log likelihood} = 173{\cdot}6) \tag{9}$$

Including ME_f (the potential ME in microbial cells and VFA arising from fermentation (MJ/day)) in model 4 (Eqn 9), rather than simply including VFA molar proportions, did improve the MSEP of prediction.

DISCUSSION

The increased use of the SF_6 tracer-based 'emissions from ruminants using a calibrated tracer' (ERUCT) technique (Johnson *et al.* 1994) for measuring the methane production of grazing livestock and increasing use of faecal 'near infrared reflectance spectroscopy' (NIRS) to assess diet quality (Coates 2000) were the motivation for us to test whether variables measured by such techniques could be used to predict MEI of ruminants accurately. If successful, the approach could provide a way to estimate MEI of grazing ruminants without needing to consider diet selection or pasture sampling and analysis. The nonlinear regression for models 2–4 is in agreement with the study of Mills *et al.* (2003), which illustrated a nonlinear trend in the simulation of methane emissions *v.* MEI.

The average proportion of DE that is nonmetabolizable is accepted in the Australian feeding

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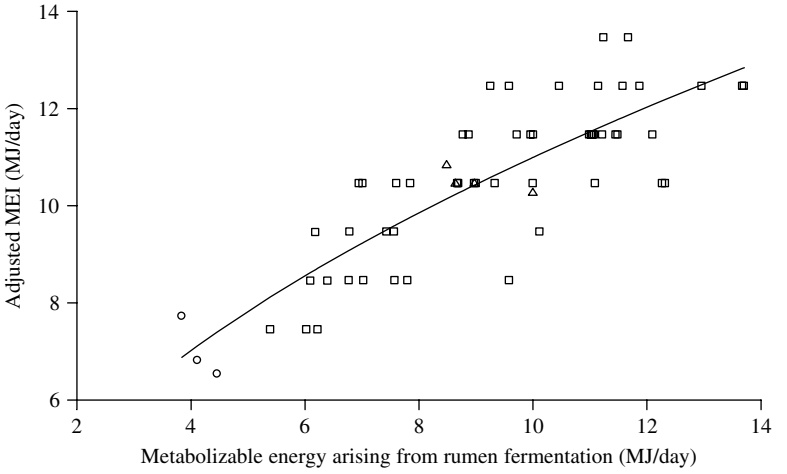

Fig. 7. Plot of adjusted observations of MEI (MJ/day) *v.* ME_f (MJ/day) across studies (□, Jentsch *et al.* 1972; ○, Osakwe *et al.* 2004; △, Mwenya *et al.* 2004) with the nonlinear prediction (model 4; MEI_{p4} (MJ/day)). ME_f was calculated by using the model of Nolan *et al.* (1998).

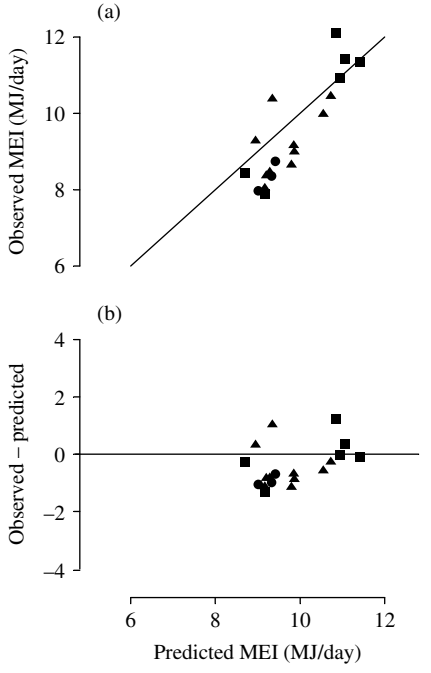

Fig. 8. Prediction (model 4; MEI_{p4} (MJ/d)) of MEI of sheep plotted against independent data (■, Itabashi *et al.* 1984; ●, Carulla *et al.* 2005; ▲, Pinares-Patiño *et al.* 2003): (a) observed *v.* predicted with the line $y = x$ and (b) the difference (observed − predicted) *v.* predicted with the line $y = 0$.

standards as being $0{\cdot}19 \times DE$ at maintenance energy intake (SCA 1990), and this partitioning of energy is relatively consistent within roughage-based diets. In the challenge data, non-metabolizable energy was $0{\cdot}16 \times DE$, in contrast with the $0{\cdot}19 \times DE$ in the model which would have contributed to the bias in model 1. Armstrong (1964) noted a significant positive effect of level of intake (relative to maintenance) on metabolizability of DE so not including an intake effect in model 1 would have contributed to its high MSEP and particularly the proportion of MSEP for slope (0·28; Table 2). The high MSEP of MEI_{p1} (4·08 MJ/day) associated with using the generic relationship in model 1 to predict MEI suggested that this model was unlikely to give precise predictions for individual animals or group averages. The need to measure UE loss in the field to predict MEI by model 1 further limits the usefulness of this model. UE is not readily measured in grazing animals, and energy in urine is principally present in the nitrogenous compounds (Bristow *et al.* 1992) and in urea in particular. The possible correlation between rumen ammonia concentration (NH_3) and UE was evaluated because up to 0·66 of the urea produced by ruminants is derived from ammonia drained from the digestive tract (Lobley *et al.* 1995). A significant association between UE and NH_3 existed in the development data set ($R^2 = 0{\cdot}61$), suggesting that NH_3 may provide an easily measured proxy for UE in estimating MEI. However, NH_3 did not subsequently explain a significant proportion of variance in model 3 so it was not retained in the model.

Components of nonlinear models

Progression to nonlinear models to predict MEI reduced the mean bias and MSEP of MEI (Table 2). Faecal NIRS is increasingly being developed to

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describe diet characteristics, including digestibility (Coates 2000), especially for rangeland areas where diet selectivity can be high. If the faecal NIRS continues to be developed to predict digestible organic matter intake (Coates 2000) it may be able to predict MEI accurately. The lower MSEP when MPR was included in the model (MEI_{P3} *v.* MEI_{P2}) indicates the value of MPR as a predictor. In a meta-analysis of recent New Zealand data, Machmüller & Clark (2006) found that MPR was strongly correlated with estimated DMI across combined sheep and cattle data ($R^2 = 0{\cdot}86$), but less so within sheep data alone ($R^2 = 0{\cdot}26$–$0{\cdot}42$), indicating the need for other physiological predictors to be used in developing predictions of MEI based on MPR. It must also be remembered that these models have been developed and tested using MPR measured in respiration facilities. The precision of MPR measured by the ERUCT technique is lower, with a further between day CV of 0·034 attributable to the method itself (Grainger *et al*. 2007).

Table 3. *Variation in the whole tract digestibility of dietary energy and in the proportion of DE fermented in the reticulo-rumen across different diet types*

	Concentrate diets ($n = 16$)	Pasture and silage ($n = 12$)	Hays ($n = 9$)
EngDig			
Mean	0·82	0·74	0·59
S.D.	0·023	0·053	0·071
CV	0·028	0·072	0·120
Proportion of DE absorbed/eructated from reticulo-rumen			
Mean	0·66	0·57	0·74
S.D.	0·063	0·072	0·091
CV	0·095	0·127	0·123
Sources	a, b, c	d, e, f	a, b, c, d

Sources: a, Topps *et al*. (1968*a*); b, Topps *et al*. (1968*b*); c, Nicholson & Sutton (1969); d, Beever *et al*. (1972); e, Beever *et al*. (1978); f, Ulyatt & MacRae (1971).

Constraints to prediction of MEI from methane production

MPR in the rumen is the net result of the total energy fermented in the rumen and the partitioning of fermented matter into each of the VFA and microbial cell growth. Consequently, it was expected that estimating the energy captured in VFA and microbial cells synthesized in the rumen from VFA proportions and MPR (by the model of Nolan 1998) would have improved the ability to predict MEI, relative to that possible from MPR alone. This was the outcome achieved, with model 4 showing a lower MSEP than model 3, although with greater bias (Table 2). Nevertheless, the challenge data of Itabashi *et al*. (1984) illustrate that the prediction of MEI was improved in each of the models: model 2 (Eqn 7; Fig. 4); model 3 (Eqn 8; Fig. 6) and lastly model 4 (Eqn 9; Fig. 8). The independent variables EngDig, MPR and ME_f for models 2, 3 and 4, respectively, influence the prediction of MEI and progressively improved the prediction of MEI; that is, fermentation in the rumen had the greatest effect followed by methane and then digestibility.

However, MEI is the sum of both energy released through fermentation (as calculated in MEI_f) and energy that is digested in the small intestine, but not by fermentation (Fig. 1). Recognition of these two distinct components of ME obviates two additional sources of error in establishing a highly accurate MPR-based approach to estimating MEI, which are outlined and evaluated below.

1. How accurately can stoichiometric principles predict ME present in fermentation products, working only from knowledge of MPR and rumen VFA proportions?
2. How constant is the proportion of ME coming from fermentation relative to ME arising from mammalian digestion? If these proportions do vary, can they be readily predicted?

Adequacy of stoichiometry

The empirical chemical reactions of anaerobic production of the major VFA were summarized by Hungate (1966). Since then, the ability of some microbes to produce acetate and propionate together has been appreciated and the overall stoichiometry modified (Wolin *et al*. 1997). All modelling of fermentation stoichiometry uses the availability of hydrogen (2H; being H_2 or protons on reduced cofactors) to link VFA and MPR. MPR is normally assumed to utilize all excess 2H arising from fermentation according to the following reaction: $CO_2 + 8H \rightarrow CH_4 + 2H_2O$. Conversely, if MPR is known, stoichiometry can be used to calculate the quantity of VFA or of VFA energy produced (Ørskov *et al*. 1968).

Recovery of 2H in VFA, methane and cells is typically 0·85–0·95 of that anticipated from stoichiometry in *in vitro* studies so the stoichiometry itself is a source of error, being an incomplete description of the fermentation process and end-products. Discrepancies in stoichiometry become greater in the hindgut (Demeyer & De Graeve 1991) and there is a lower methane:VFA yield in the hindgut than in the rumen (Immig 1996). Additional issues that could potentially cause stoichiometry to differ from that assumed include variations in microbial growth efficiency, differences between VFA in the relationship between concentration and absorption rate, and failure to include all 2H sinks.

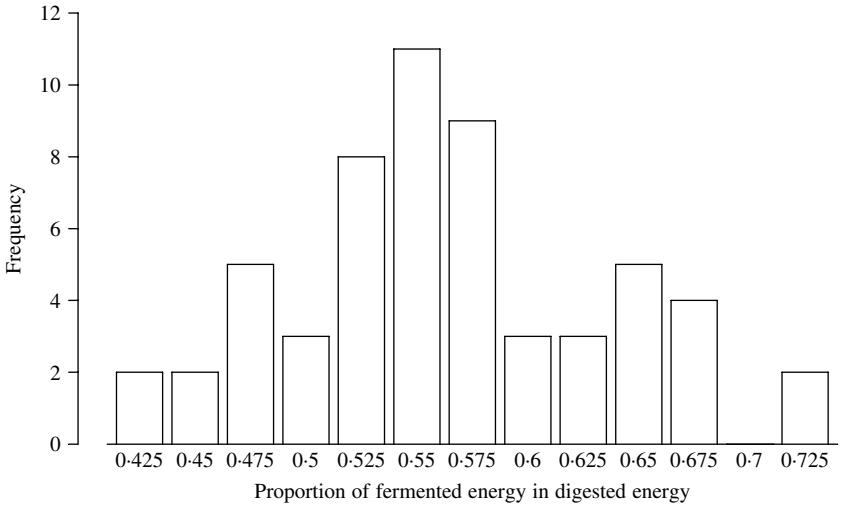

Fig. 9. Frequency distribution of the proportion of DE fermented and lost from the rumen by individual sheep ingesting a single cultivar of fresh ryegrass. The amount of energy fermented was calculated from the data of Armstrong (1964) using the procedure of Ørskov *et al.* (1968) from the mean MPR and the mean VFA proportions for each sheep over three measurement periods.

Energy loss through fermentation v. *digestion*

Even if a revised stoichiometry were developed to allow precise estimation of energy partitioning into VFA, cells and methane, it could only be used to quantify the energy made available in the gut through fermentation. It could not be reasonably expected to predict the energy liberated by mammalian digestion within the abomasum and intestines. There need be no biological or mathematical connections between the magnitude of fermentation and of mammalian digestion in the gut. Information relating to the partitioning of energy release between these two processes is reviewed below.

While there have been few studies on the contributions of mammalian digestion and fermentation to whole tract digestion (Ørskov *et al.* 1968), assessments of variance in the proportions of the diet disappearing from the forestomachs relative to the intestines (small and large) abound. Results of these studies are summarized in Table 3, but more exhaustive reviews focusing on starch and protein digestion have been prepared by Merchen *et al.* (1997) and Wanderley *et al.* (1987). Importantly, the CV across studies is of the order of 0·10 (Table 3), indicating that an assumption of a constant partitioning between forestomach and intestinal digestion is inappropriate in seeking to develop accurate estimates of MEI for individual animals.

Level of intake of a single species of fresh pasture has little effect on the proportions of organic matter apparently digested proximal to the abomasum; however, there are large differences in site of digestion between pasture species (Beever *et al.* 1985) and variation due to the maturity of the forage (Beever *et al.* 1972). The existence and magnitude of these sources of variation imply that the proportions of fermented and mammalian-digested energy are likely to vary in grazing animals. To investigate further the between-sheep variance in the proportion of DE fermented on a single fresh forage (ryegrass), the stoichiometric procedures of Ørskov *et al.* (1968) were applied to the data of Armstrong (1964). The proportion of DE apparently released through fermentation was highly variable as shown in Fig. 9. The implication of the variation displayed is that while a measure of MPR and stoichiometry using VFA molar proportions may provide accurate prediction of ME arising from fermentation and an approximate prediction of MEI (model 4), it is unrealistic to expect further refining of this approach to provide a quantitative estimate of total DE or MEI, as ME that does not arise from fermentation is an unknown proportion of MEI. Another constraint to accurate prediction of MEI is the variation in daily feed intake as initially reviewed. Since methane production persists for at least 48 h after feeding, MPR as measured will reflect not only DMI on a given day, but also an unknown production of methane from the days before, when intake may have been different.

CONCLUSIONS

Re-assessment of published studies has shown that digestive parameters such as those provided by the

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new faecal NIRS and ERUCT methods, together with ruminal sampling, can be used to predict MEI from ME_f. Realization of this accuracy in field-use will be lower due to the additional error in estimating DM digestibility from faecal NIRS and errors in measuring MPR by ERUCT (Grainger *et al*. 2007), which were not incurred in animal house and respiration chamber measures used for model development and testing. The value of MPR as a predictive tool for MEI can be increased by measurement of rumen VFA proportions and calculation of ME arising from fermentation (data of Itabashi *et al*. 1984) (Figs 4, 6 and 8), but variation in the proportion of MEI obtained from fermentation rather than mammalian digestion will limit the accuracy of this approach. Future efforts to ascertain MEI from measurement of animal parameters may benefit from ongoing improvement in predicting intake from faecal NIRS, and by measurement of CO_2 production using the ERUCT technique from which to derive energy expenditure.

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