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RESEARCH

Validation of grass metabolizable energy prediction equation by Garrido and Mann (1981) for dairy cows in southern Chile

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ABSTRACT

Metabolizable energy (ME) is the most limiting nutrient for milk production in pastoral systems. In Chile, using four wethers and grass cut on six measurement dates, Garrido and Mann (1981) developed an equation to predict grass ME concentration based on content of in vitro digestible organic matter in DM. The application of the Garrido and Mann (1981) equation has been generalized in Chile for all feed ingredients, diets and ruminant species. We hypothesized that ME prediction from this equation has limitations for its use in lactating grass-fed dairy cows. The objective of this study was to validate the ME prediction equation by Garrido and Mann (1981) for lactating grass-fed dairy cows in the autumn and spring seasons. The study was conducted at INIA Remehue (Osorno, Chile) using two sets of eight Holstein Friesian cows, with each group used twice in 21-d experimental periods in autumn and spring, respectively. Fresh grass-only diets with a predominance of perennial ryegrass (*Lolium perenne* L.) were supplied through soiling. In the last 5 d of each period, grass ME concentration was measured by subtracting energy lost in feces, urine and methane from ingested gross energy. A weak linear relationship was obtained between the ME determined in trials and ME predicted from the Garrido and Mann (1981) equation ($R^2 = 0.266$; $P = 0.003$). The ME prediction equation currently in use is inadequate for lactating grass-fed dairy cows. Therefore, new and improved equations for estimating grass ME specific to dairy cows are needed.

Key words: Chile, digestible energy, grazing dairy cow, *Lolium perenne*, metabolizable energy, pasture, perennial ryegrass, prediction.

INTRODUCTION

Temperate pastures constitute the main feed for grazing dairy systems in southern Chile due to their availability and low cost. However, pasture and grass nutrient composition varies throughout the year due to phenological changes within a growth season, that result in structural modifications in the leaf-stem ratio and presence of dead material, which lead to changes in plant nutritive value (Chapman et al., 2014). In general, lactating dairy cows fed grass-only diets may not meet their nutrient requirements, in particular of energy, the most limiting nutrient for milk production in pastoral systems (Kolver, 2003). Therefore, concentrate supplementation is a common practice in pastoral systems to provide nutrients that may be lacking in pasture (Keim and Anrique, 2011). In these systems, an accurate assessment of grass nutrient composition and energy concentration is crucial to provide a diet that covers nutrient requirements, maximizes milk production and maintains adequate body condition.

Energy is an essential nutrient for cattle to cover requirements for maintenance, growth, reproduction and milk production. Dairy cows obtain energy through fermentable carbohydrates, such as dietary starch, sugar, and fiber, and sometimes lipids. Energy feeding systems for dairy cows are used to formulate rations that cover energy requirements and evaluate the energy value of feeds (Weiss, 2010). In these systems, gross energy (GE) is the total amount of energy present in the feed and digestible energy (DE) is equal to GE minus the amount of energy lost in the feces. Metabolizable energy (ME) is equal to DE minus the amount of energy lost as methane and urine, and corresponds to the energy used by the animal to carry out metabolic processes (Weiss, 2010).

In vivo animal trials that measure ME concentration of feeds require confinement of animals in respiration chambers or metabolism stalls to determine GE intake, along with energy excreted in feces, urine, and methane (Doyle et al., 2005). Experimental protocols that measure in vivo digestibility typically require a minimum number of days (3 to 6) and replicates (3 to 4 animals) (Rymer, 2000). Thus, direct feed ME determination is the gold standard in research, but highly complex, expensive and unfeasible for routine diet formulation. A more practical approach is feedstuffs ME prediction from in vitro methods that measure DE (AFRC, 1993; Doyle et al., 2005). In vitro methods simulate the ruminant digestion process using rumen inoculum, with the classical methods of Tilley and Terry (1963) and Van Soest et al. (1966) being the most well-known. These methods are quicker and less expensive than in vivo digestibility studies, although less accurate (Yáñez-Ruiz et al., 2016).

In Chile, in the experimental farm of Universidad Austral de Chile (Valdivia) between September of 1978 and October of 1979, Garrido and Mann (1981) conducted an in vivo experiment using four Romney Marsh wethers to develop an equation to predict grass ME concentration from the content of in vitro digestible organic matter in DM or D-value. A sward of permanent pasture with predominance of *Agrostis* spp., *Holcus lanatus* L., *Bromus unioloides* Kunth, *Lolium multiflorum* Lam., and *Leontodon nudicaulis* auct. was cut on six dates: three in spring, one in summer, one in winter and one in the following spring. Grass in vivo digestibility was determined with animals housed in metabolic crates and fed at maintenance level (5 kg fresh matter d^{-1}) the grass-only diets (n = 24). Metabolizable energy content was estimated by multiplying DE by the factor 0.82 (Blaxter and Wainman, 1961). The resulting linear regression equation to predict ME concentration was (Garrido and Mann, 1981):

$$\text{Predicted ME} = 0.0325 \times D\% + 0.279$$

In Chile, a project conducted to standardize analytical methods for evaluating the nutritive value of animal feeds reported that only two animal nutrition laboratories inform feed ME contents, with both estimating ME from D-value determined in vitro using the Garrido and Mann equation (Valderrama, 2011). In these laboratories, the application of the Garrido and Mann equation has been generalized to all feed ingredients, diets and ruminant species. We hypothesized that the Garrido and Mann equation has limitations for its use in grass-fed dairy cows because, although derived from a study performed with fresh grass, it was conducted with sheep instead of cattle, and where energy losses were not measured in urine and gases, but relied on prediction from DE assuming constant energy losses in methane and urine. The objective of this study was to validate the Garrido and Mann equation used to predict grass ME content with lactating dairy cows in the autumn and spring seasons.

MATERIALS AND METHODS

The study was conducted at the Instituto de Investigaciones Agropecuarias, INIA Remehue dairy farm located in Osorno, Los Lagos Region. The study was approved by INIA's Institutional Animal Care and Use Committee (Reference N° 02/2022).

Two groups of eight multiparous Holstein Friesian cows were used in the study for measuring in vivo ME concentration. At the beginning of the study, the first group of cows had a mean and standard deviation (SD) of 54.1 ± 6.3 d in milk (DIM) and 516 ± 35.1 kg body mass, and the second group of cows had 83.0 ± 4.5 DIM and 488 ± 22.9 kg body mass.

The study was conducted in 2019 and comprised four experimental periods across autumn and spring, representative of the main grazing seasons of many pastoral dairy farms of the southern hemisphere. The first set of cows participated in two periods in autumn (starting on 22 April and 26 May, respectively) and the second set of cows participated in two periods in spring (starting on 10 October and 24 November, respectively). Each period had a duration of 17 d, the first 10 d were for diet and management adaptation with cows housed as a single group, and in the 7 remaining days, cows were individually confined in metabolism stalls and ME measurements were conducted during the final 5 d. There was a gap of 17 and 28 d between experimental periods, in autumn and spring seasons, respectively.

In each period, the diet consisted solely of fresh grass offered *ad libitum* through soiling (cut and carried). The grass was harvested from a paddock of 3.7 ha sown 5 yr earlier with a predominance of ryegrass (*Lolium perenne* L.) and other species such as white clover (*Trifolium repens* L.) and bromus (*Bromus catharticus* Vahl). The grass was cut daily every morning with a motor scythe and offered to the cows once a day allowing for 10% refusals. In addition, each cow received 300 g d^{-1} of a mixture of minerals and vitamins sprinkled on top of the grass (Nutrasal lactancia, Anasac, Santiago, Chile) containing per kilogram: 230 g Ca, 30 g P, 35 g Mg, 40 g Na, 70 g Cl, 20 g S, 50000 IU vitamin A, 20000 IU vitamin D and 1000 IU vitamin E, 18 mg inorganic Co, 2500 mg inorganic Cu, 140 mg inorganic I, 1400 mg inorganic Mn, 4100 mg inorganic Zn, 14 mg inorganic Se, 5 mg organic Co, 50 mg organic Cu, 70 mg organic Mn, 3 mg organic Se and 1160 mg organic Zn.

During measurement periods, the amount of grass offered and refused was recorded daily and one representative sample of the grass offered was taken daily. Samples of the pasture refused per cow were sampled daily and composed per cow per period. Cows were milked twice daily, at approximately 08:00 and 17:00 h and milk yield was recorded. In each period, body mass was recorded 1 d prior to entering the metabolism stalls and body condition was recorded on a 5-point score by two trained personnel on the final day before leaving the metabolism stalls.

Individual milk samples were collected at each milking for the 5 d measurement period and composed per cow d^{-1} proportional to production, preserved with 2-bromo-2-nitro-1,3-propanediol (bronopol) and analyzed for milk fat, protein and urea concentration using a milk infrared analyzer (Milkoscan 7 RM, Foss, Hillerød, Denmark). Also, a composite sample per cow per period was composed to determine GE content.

Total collection of urine was carried out by attaching a hose to artificial leather patches glued to the cow vulva using velcro, the urine was acidified to $pH < 3$ adding sulfuric acid to avoid ammonium volatilization (Muñoz et al., 2019). All feces produced on an individual and daily basis were collected in trays covered with plastic. Total individual daily urine and feces production was recorded, sampled daily, and composed per animal per period for N and GE determination.

Methane was measured daily for each 5 d measurement period using the sulfur hexafluoride (SF_6) tracer technique (Muñoz et al., 2019). In each season, at the beginning of Period 1 all cattle were dosed with a brass permeation tube containing SF_6 gas (NIWA, Auckland, New Zealand) with known release rates ranging from 4.13-4.48 and 4.62-4.86 mg d^{-1} in autumn and spring, respectively. For the collection of gas samples, 12 evacuated PVC collars were used, eight sampling air from around the cow's muzzle carried through nylon tubing placed on a halter with the air flow restricted by a crimped capillary tube, and four sampling background gas emissions from the environment. After collection, samples were pressurized to around 20 kPa with N, and subsamples stored in pre-evacuated glass vials with double-sided septa. Each sample was analyzed in duplicate using a gas chromatograph (Clarus 600, Perkin Elmer, Waltham, Massachusetts, USA). A flame ionization detector with a Carboxen 1010 plot column (15 m × 0.32 mm ID; Supelco, Sigma-Aldrich, St. Louis, Missouri, USA) was used for methane concentration, and an electron capture detector with an Elite-GC GS Molesieve column (30 m × 0.53 mm ID × 50 μm film thickness, Perkin Elmer) was used for SF_6 concentration. Daily methane production was calculated from collected SF_6 and CH_4 concentrations in the collars discounting background concentrations, and the SF_6 release rate form the permeation tubes.

Grass and feces samples were oven dried at 60 °C for 48 h and ground through a 1 mm sieve in a Wiley mill, prior to chemical analysis. The concentration of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and ether extract (EE) were determined for all grass, refusals and fecal samples, based on AOAC International (1995) procedures. Gross energy concentrations of dry grass and feces, and of freeze-dried samples of milk and urine, were determined in the Animal Nutrition Laboratory of the Austral University of Chile by means of oxygen bomb calorimetry (Bateman, 1970).

In vitro digestibility of the dry grass samples was determined based on the two-stage technique of Tilley and Terry (1963) and involved incubation with rumen inoculum from two fistulated cows followed by incubation with acid pepsin. Metabolizable energy was estimated from D-value determined by in vitro digestibility using the Garrido and Mann (1981) equation.

For each period, the data collected per cow on a daily basis (intakes and refusals, milk production and composition, feces and urine outputs, methane production) and the daily digestibility and metabolizability calculations was averaged to obtain one average value per cow and period prior to statistical analyses. During the study, the observed ME data for one cow was missed in period 1 of spring because of technical difficulties with the equipment for measuring urine output, thus only 31 observations were included in the analyses.

A linear regression was used to compare the relationship between in vivo measured ME for each cow in each period and predicted ME from Garrido and Mann (1981). The analysis was performed using JMP 13.2.1 (SAS Institute, Cary, North Carolina, USA).

RESULTS AND DISCUSSION

Grass chemical composition in both periods of each season is reported in Table 1. In autumn, the advancement of the season resulted in numerically higher CP, while in spring the opposite was observed. The content of NDF and ADF was numerically higher in autumn than in spring. The content of EE and GE was numerically lower in the second spring period compared with the other periods. In vitro digestibility was numerically higher in the first spring period than in the other periods.

Several factors affect plant nutritive content, including climate, cultivar, soil type, grazing management, and most importantly, maturity (Palladino et al., 2009). Maturity, often determined by the leaf:stem ratio, is influenced by regrowth days, when for example, NDF linearly increases from 44% to 53% between 6 to 30 d regrowth (Chilibroste et al., 2000). This can help to explain the differences observed between autumn and spring with the advance of the season, where a regrowth period of 17 d in autumn improved plant nutritive content, whereas a regrowth period of 28 d in spring decreased its nutritive value. The numerically lower GE observed towards the end of spring is likely related to the lower EE and higher ash content. The lipid fraction of plants can represent between 0.3% to 10% of the plant on a DM basis (Elgersma et al., 2006). Growth stage, regrowth days, maturity, season, temperature and light intensity, cultivar, and N fertilization rate are all factors that influence total lipid concentration; when highest lipid contents are common in younger plants with reduced regrowth days, during vegetative growth stage, in spring and autumn (Dewhurst et al., 2001; Elgersma et al., 2006; Palladino et al., 2009), or when high N application rates are used (Elgersma et al., 2005). Importantly, fatty acid content in grasses rapidly declines as the duration of the regrowth period increases (Dewhurst et al., 2001; Elgersma et al., 2006).

Descriptive statistics for grass in vivo observed ME content and predicted ME from the Garrido and Mann equation is presented in Table 2. Grass observed ME content ranged from 5.6 to 11.0 MJ kg^{-1} DM (mean ± SD: 9.2 ± 1.28 MJ kg^{-1} DM), and predicted ME content ranged from 8.96 to 11.2 MJ kg^{-1} DM (9.76 ± 0.92 MJ kg^{-1} DM). Predicted ME ranged from 88% to 160% of in vivo observed ME (107 ± 15.3%).

The relationship between grass observed ME and grass predicted ME values from the Garrido and Mann equation is shown in Figure 1. The regression equation with observed ME concentration as the dependent variable and predicted ME concentration as the independent variable was:

$$\text{Observed ME} = 0.7359 \times \text{Predicted ME} + 2.0337$$

where predicted and observed ME concentrations are expressed as MJ kg^{-1} DM; $R^2 = 0.266$, root mean square error = 1.12, $P = 0.003$, 95% confidence intervals: intercept [-2.493, 6.561] and slope [0.272, 1.200]; slope was not different from unity ($P = 0.26$).

When assessing goodness-of-fit, the proportion of the variation explained by the model was 0.266. Thus, only approximately 27% of variation in observed ME was explained by its linear relationship with predicted ME and the remaining 73% was unexplained by the relationship, indicating that the line was a poor fit in the specified range. Variation in ME content results from variation in the composition of feed ingested (which is expected to be greater than the composition of feed offered, as individual cows can differ in feed selection) and individual animals digestive physiology. It is noted that the level of dispersion observed in the Autumn 1 period is especially high, and we do not know the reason.

When assessing the regression slope, observed pasture ME concentration increased on average by 0.74 MJ kg^{-1} DM for every 1 MJ kg^{-1} DM increase of predicted ME concentration. Given the high levels of unexplained variation observed, the precision of this prediction will likely be compromised. Nevertheless, looking at the plotted regression line (Figure 1), the Garrido and Mann equation appears to over-predict pasture ME concentration, particularly at the higher end. It is acknowledged that the validation carried out in the present study, although used a higher number of observations than the Garrido and Mann study, limited the predicted ME values per period by combining daily the grass samples for all animals before in vitro analysis.

Table 1. Grass mean chemical composition in the two periods and two seasons of the study.

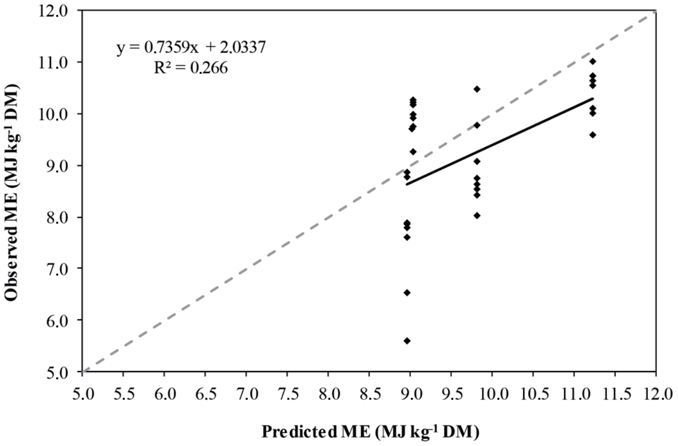
	Autumn		Spring	
	1	2	1	2
Dry matter (DM), g kg^{-1} fresh matter	152.6	95.7	129.3	116.0
Crude protein, g kg^{-1} DM	176.6	209.9	243.5	222.1
Ether extract, g kg^{-1} DM	25.8	25.6	23.4	15.8
Neutral detergent fiber, g kg^{-1} DM	629.7	573.1	497.6	478.5
Acid detergent fiber, g kg^{-1} DM	310.9	319.2	267.7	297.5
Ash, g kg^{-1} DM	115.1	134.7	134.4	171.3
Gross energy, MJ kg^{-1} DM	18.3	18.3	18.1	17.2
In vitro digestibility, g kg^{-1} DM	659.5	681.9	857.4	746.7
D value, g kg^{-1} DM	572.8	578.2	739.5	601.6

D value: Content of digestible organic matter in DM.

Table 2. Descriptive statistics for grass observed metabolizable energy (ME) (MJ kg^{-1} DM) and predicted ME from the Garrido and Mann (1981) equation.

Season and period	Grass ME values	n	Minimum	Maximum	Mean	Standard deviation
Autumn 1	Observed ME	8	5.60	8.88	7.62	1.09
	Predicted ME	8	8.96	8.96	8.96	0.00
	Predicted ME/Observed ME	-	1.01	1.60	1.20	0.20
Autumn 2	Observed ME	8	9.26	10.28	9.91	0.34
	Predicted ME	8	9.02	9.03	9.03	0.00
	Predicted ME/Observed ME	-	0.88	0.97	0.91	0.03
Spring 1	Observed ME	7	9.60	11.01	10.38	0.49
	Predicted ME	8	11.22	11.22	11.22	0.00
	Predicted ME/Observed ME	-	1.02	1.17	1.08	0.05
Spring 2	Observed ME	8	8.02	10.48	8.96	0.80
	Predicted ME	8	9.82	9.82	9.82	0.00
	Predicted ME/Observed ME	-	0.94	1.22	1.10	0.09
Overall	Observed ME	31	5.60	11.0	9.18	1.28
	Predicted ME	32	8.96	11.2	9.76	0.92
	Predicted ME/Observed ME	-	0.88	1.60	1.07	0.15

Figure 1. Relationship between the predicted metabolizable energy (ME) from the Garrido and Mann (1981) equation and the observed ME content of pasture. The black line represents the fitted regression line for the relationship between predicted and observed ME values and the grey dotted line represents the identity line (y = x).



Ideally, a regression between two methods should have an intercept of zero and a slope of one. In this study, the relationship between observed ME and predicted ME values resulted in a 95% confidence interval for the intercept and slope that contained zero and one, respectively (i.e., one was within the limits of the slope interval and zero was within the limits of the intercept interval), indicating nonsignificant intercept or slope systematic bias of the Garrido and Mann (1981) prediction (Zady, 2000). That said, the mid points of the intercept and slope 95% confidence intervals were not close to the theoretically expected values of zero and one, respectively.

These results indicate that the current prediction of ME content using the Garrido and Mann equation is inadequate for grass-fed dairy cows. The Garrido and Mann study was a digestibility study conducted using four Romney Marsh wethers fed a grass-only diet at maintenance level. The resulting equation is currently extrapolated to lactating dairy cows fed *ad libitum* diets that contain concentrate and forage ingredients that are different from grass, including partial and total mixed diets. The efficacy of sheep as a digestibility model for cattle has been previously questioned (Chishti et al., 2019). In vivo digestibility studies are usually conducted using mature sheep at maintenance to remove effects of intake, physiological state and animal species (Doyle et al., 2005). However, compared with sheep, cows typically have greater intakes and higher rumen capacity in relation to their body mass (Playne, 1978). In addition, compared with non-lactating animals fed at maintenance, lactating dairy cows have higher intakes and rates of passage through their digestive system (Yan et al., 2004; Doyle et al., 2005). These differences can have important implications when formulating diets for lactating dairy cows using pasture ME values derived from digestibility studies using sheep.

An additional consideration that may have limited the prediction accuracy of the Garrido and Mann equation is the fact that their study did not actually measure urine energy excretion or methane emissions. Instead, they relied on ME prediction from DE assuming constant losses of DE in methane and urine (DE × 0.82; Blaxter and Wainman, 1961). In the present study, we actually measured energy losses in urine and methane through total collection and the SF_6 technique, respectively; a methodology to measure methane production was not available in Chile when Garrido and Mann conducted their study (1981). Including these measurements may have increased the accuracy of ME prediction by Garrido and Mann. It has been previously reported that losses of energy in methane and urine are variable across diets (Fuller et al., 2020), varying with level of intake and diet nutrient composition (Hynes et al., 2016; Hales et al., 2022). In our study, mean ME concentration was 0.79 ± 0.04 of the observed mean DE concentration.

Several technological advances have occurred with cattle and forage genetics since the time of the Garrido and Mann study (1981). These include improved productivity from forage species and increased forage quality achieved by better pasture management (Knapp et al., 2014) and increased dairy feed efficiency through increased milk production and lower proportion of feed intake being used for maintenance (VandeHaar et al., 2016). These different production settings (present-day cow and forage genetics, among others) may contribute to different animal digestive efficiencies of forages than in the 1980 decade and differing animal energy utilization efficiencies. These differences, especially those related to grass changes, may contribute to potential error when predicting grass ME contents from Garrido and Mann.

Grazed grass constitutes the main feedstuff of dairy cows from the southern region of Chile in spring and autumn seasons. The observed pasture ME content measured in vivo in the present study for the autumn and spring seasons (mean 8.8 and 9.0 MJ kg^{-1} DM, respectively) was lower than reference values for autumn and spring pastures for the south of Chile reported by local nutrition laboratories using the Garrido and Mann equation (mean 11.3 and 11.5 MJ kg^{-1} DM, respectively; Anrique et al., 2008). One of the advantages of the Garrido and Mann equation is that it was derived from studies with fresh grass diets, as opposed to the majority of works on feed ME prediction that have been performed with diets based on conserved forages and concentrates (Stergiadis et al., 2015). Paradoxically, its application locally has been generalized for all dietary ingredients, types and ruminant species.

Pastoral systems are challenging due to seasonal changes in pasture and nutrient composition that decrease the consistency of animal nutrient intake between days and across seasons (Wilkinson et al., 2020). In these systems, it is well known that energy is the most limiting nutrient for dairy cows (Kolver, 2003). Therefore, an inadequate assessment of grass ME can have a profound impact on milk production and dairy farm profits (Stergiadis et al., 2015), especially in farms that offer concentrate supplementation. Dairy cow supplementation on pasture provides nutrients for maintenance and production that are lacking from the forage diet, and concentrates are formulated considering grass energy content to supply the energy requirements that are lacking (Bargo et al., 2003). If grass energy content is not assessed correctly, an underestimation would increase production cost due to over-supply of supplements and an overestimation could compromise productivity due to insufficient supplements to cover cow nutrient requirements (Stergiadis et al., 2015).

This study highlights the need for developing new equations with improved prediction accuracy of grass ME content that are readily available for dairy farmers, nutrition consultants and researchers. These equations should be robust, practical and specific to the dietary ingredient, diet type and ruminant species to which it will be applied. Our group is currently gathering data towards this goal. In addition, it would be of interest to generate prediction models based on near infrared spectroscopy, further increasing the convenience of this information for the dairy industry.

CONCLUSIONS

The equation currently in use to predict metabolizable energy (ME) concentration is inadequate for lactating grass-fed dairy cows. The most important problem revealed by our validation for the Garrido and Mann prediction was the high random error shown by the low R^2. A poor prediction of grass ME concentration can decrease milk production and distort concentrate supplementation efforts, which can have a profound effect on dairy farm profits, especially in pastoral systems. This study highlights the need to generate new and improved grass ME concentration prediction equations for lactating dairy cows.

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