



**HAL**  
open science

# Prediction of the digestible energy content of hay for horses from their chemical composition using the National Institute of Agricultural Research system: new models

W. Martin-Rosset, Donato Andueza, M. Vermorel, Javier Álvarez-Rodríguez

► **To cite this version:**

W. Martin-Rosset, Donato Andueza, M. Vermorel, Javier Álvarez-Rodríguez. Prediction of the digestible energy content of hay for horses from their chemical composition using the National Institute of Agricultural Research system: new models. *Animal - Open Space*, 2024, 3, pp.100075. 10.1016/j.anopes.2024.1000752772-6940/. hal-04815649

**HAL Id: hal-04815649**

<https://hal.inrae.fr/hal-04815649v1>

Submitted on 3 Dec 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



## Research article

# Prediction of the digestible energy content of hay for horses from their chemical composition using the National Institute of Agricultural Research system: new models



W. Martin-Rosset, D. Andueza\*, M. Vermorel

Université Clermont Auvergne, INRAE Vet Agro Sup, UMR Herbivores, 63122 Saint Genès Champanelle, France

## ARTICLE INFO

## Article history:

Received 15 March 2024

Revised 11 July 2024

Accepted 16 July 2024

Handling editor: Javier Álvarez-Rodríguez

## Keywords:

Equine nutrition

Forage

Nutritive value

Proximate composition

Regression equations

## ABSTRACT

Digestible energy (DE) accounts for the major fraction of the energy content of hay fed to equines, but DE measurements are difficult to carry out, and researchers have developed models for predicting the DE content of hays for horses from their chemical composition. Digestible energy varies depending on botanical family, species, and agronomic and climatic conditions. A database of 61 hays harvested in a temperate zone (32 natural grassland hays, 16 grass hays and 13 lucerne hays) was set up from studies carried out at the French National Institute of Agricultural Research (INRAE) or extracted from the literature using a similar methodological corpus, to test the relevance of crude fibre (CF), NDF, ADF, total free reducing sugars (TFRS), CP as potential criteria for the prediction of organic matter digestibility (OMD), energy digestibility (ED) and digestible energy (DE). Models were developed for natural grassland hay, grass hay and lucerne hay. The adjusted cross-validation  $R^2$  values ( $R^2CV$ ) of the models obtained using CF, ADF or NDF as single variables or in combination with CP for the prediction of OMD, ED and DE of natural grassland hay ranged from 0.84 to 0.86, from 0.83 to 0.86 and from 0.82 to 0.84, respectively. For grass hay, the  $R^2CV$  were lower and ranged from 0.65 to 0.71, from 0.63 to 0.71 and from 0.62 to 0.68, for the prediction of OMD, ED and DE, respectively. Finally, for lucerne hay, the  $R^2CV$  ranged from 0.65 to 0.72, from 0.34 to 0.74 and from 0.25 to 0.67, for the prediction of OMD, ED and DE, respectively. The  $R^2CV$  of the prediction models of ED and DE were more heterogeneous for lucerne hay than for grassland and grass hay. The TFRS content of hay was never a significant predictive variable of OMD, ED or DE, either as single variable or combined with any cell wall content criterion. The models obtained in the present study improved those proposed by INRA to date.

© 2024 The Author(s). Published by Elsevier B.V. on behalf of The Animal Consortium. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Reader comments

We invite you to comment on the article on the PubPeer platform by clicking on this link [discuss this article](#).

## Implications

The proportion of forage in the horse's diet ranges from 50 to 95% during the breeding and working periods. However, the energy content of hay is subject to many variations. Nevertheless, end users need to be able to easily find out the digestible energy content of the hay they grow or buy to formulate balanced rations

to meet the energy requirements of horses. This study proposes new models specific to botanical families and natural grasslands for analytical laboratories to accurately predict the digestible energy content of hay using the cell wall criteria that they routinely use.

## Specification table

Subject	Nutrition
Specific subject area	Prediction of organic matter digestibility and energy digestibility of hays in horses from their chemical composition
Type of data	Table

\* Corresponding author.

E-mail address: [donato.andueza@inrae.fr](mailto:donato.andueza@inrae.fr) (D. Andueza).

(continued on next page)

How data were acquired	The digestibility data were obtained with horses housed in metabolic stalls. Chemical analysis was performed with usual laboratory methods: ash, crude fibre according to the Wende method, NDF and acid detergent fibre according to the Van Soest's methodology, Total free reducing sugars using the Somogyi method, CP by the Kjeldhal method and gross energy by adiabatic calorimetry
Data format	Raw, processed and calculated data in Excel and reported results as tables in word
Parameters for data collection	Organic matter digestibility, energy digestibility and digestible energy values of 61 temperate hays (natural grassland hay, grass hay and lucerne hay) were used. Digestibility coefficients were determined in horses. Hays were harvested with good meteorological conditions. Digestibility values were retrieved from the INRAE database and from literature.
Description of data collection	All data were obtained with 5–6 horses of warm blood breeds fed only hay diets. Total faecal collection was performed over at least 5 days after 14 days or 7 days adaptation when using a sequential or a Latin square design, respectively. Hays were supplied between 1.0 and 1.3 times the maintenance energy requirement used by each system and offered twice a day. Hays offered were sampled daily for each horse. The individual horse samples were pooled and mixed to be representative of the whole individual horse period.
Data source location	Institution: INRAE City/Town/Region: Saint-Genès Champanelle/Auvergne-Rhone-Alpes Country: France Latitude and longitude: 45°42'34.985"N, 3°0'48.466"E; 45.709718 – 3.013463
Data accessibility	Repository name: <a href="https://recherche.data.gouv.fr/fr">https://recherche.data.gouv.fr/fr</a> Data identification number: <a href="https://doi.org/10.57745/MXRNGP">https://doi.org/10.57745/MXRNGP</a> Full web address: <a href="https://entrepot.recherche.data.gouv.fr/dataset.xhtml?persistentId=doi:10.57745/MXRNGP">https://entrepot.recherche.data.gouv.fr/dataset.xhtml?persistentId=doi:10.57745/MXRNGP</a>
Related research article	Not applicable

## Introduction

Equines are monogastric but essentially herbivorous animals, and forage is their main diet. Therefore, predicting the energy content of forages is of very high concern to accurately meet their requirements. Several models are available to predict the energy

content of feeds and/or forages in terms of digestible energy (DE) (NRC, 2007) or metabolisable energy (ME) (GF, 2014) from their chemical composition. The French Institut National de la Recherche Agronomique (INRA) proposed a stepwise approach to predict the DE, ME and net energy (NE) content of feeds or just of forages (Vermorel and Martin-Rosset, 1997; INRA, 2015). This method has been further improved by using specific equations for predicting DE for natural grassland hay, grass hay or lucerne hay (Martin-Rosset, et al., 2020).

The methods available for cell wall carbohydrate analysis have also been improved over the last few decades. Initially, the criterion used in the INRA system was the crude fibre (CF) content of the feed. However, this criterion has been highly questioned for a long time. Today, the fractionation of cell walls into NDF, ADF and ADL is accepted. The sequential method of analysis of these fractions is now well established (Van Soest et al., 1991) and routinely used by analytical laboratories. These new criteria have been increasingly tested in studies carried out within the framework of the INRA system (Andrieu and Martin-Rosset, 1995; Martin-Rosset et al., 2012). Indeed, it has been noted that the digestion of cell wall content (CWC) in the large intestine depends on the type of fibre feed, namely forages (Moore-Colyer et al., 2002), but they have not yet been included in the INRA 2015 system.

In addition, a new criterion was introduced in the INRA system (Vermorel and Martin-Rosset, 1997), namely, cytoplasmic carbohydrates, currently called total free reducing sugars (TFRSs), because it improved the accuracy of ME and NE predictions. Cytoplasmic carbohydrates contribute to the amount of digestible organic matter (DOM) and DE of forages and can help discriminate between grasses and legumes (Fonnesbeck, 1969; Martin-Rosset et al., 1984). In fact, according to Smith's (1973) classification, the TFRS and the fructan content of forages fed to horses can average 60 and 40%, respectively, of the total non-structural carbohydrates (Longland and Byrd, 2006). Total free reducing sugars are digested and absorbed as glucose in the small intestine (Dyer et al., 2002) whereas fructans are digested in the large intestine (Ince et al., 2005), where they form lactates that are absorbed (Argenzio et al., 1974).

In the INRA system, the DE content of feeds is derived from the gross energy (GE) content of the organic matter multiplied by the ED predicted from the measured OMD. The prediction equations for GE, OMD and ED are specific to the types of forage and feed. The DOM content of forages is therefore also a very important criterion. In the first step of the design of the INRA system, several equations were specifically proposed to predict the OMD from the CF content of natural grassland, grasses and legumes (Martin-Rosset et al., 1994). Therefore, we hypothesised that the use of ADF and NDF as independent variables could lead to more accurate models for predicting the OMD and DE contents of hay. Therefore, the aim of the present study was to design and propose new models for predicting OMD and ED and the DE content of hay using an extended dataset of hay extracted from INRAE studies and from the recent literature, classified by botanical family, as well as updated analytical methods already available to analytical laboratories.

## Materials and methods

*Dataset designed for updating the prediction of the digestible energy content of hay*

This dataset was designed to update the INRA (2015) models to predict the OMD, ED and DE values of hay whose chemical composition and *in vivo* digestibility coefficients were experimentally determined in accordance with a precise methodological corpus

(Martin-Rosset et al., 2020). Only hay harvested in the temperate zone were studied: pure grasses (bromegrass, timothy, orchardgrass, ryegrass), natural grasslands and lucernes. Data were extracted only from INRA studies for natural grasslands and from the literature and INRA studies for grasses or lucernes. The natural grassland hay was harvested in good climatic conditions at the 1st cycle, with early or late cuts (66%), or at the 2nd cycle (34%). The grass hay was harvested in the 1st cycle, with early or late cuts (50%), or in the 2nd cycle (50%). The lucerne hay was harvested in the 1st cycle (15%), 2nd cycle (54%) or 3rd cycle (31%). The dataset (n = 61 hays) was composed of 32 natural grassland hays from INRA, 16 grass hays (11 from the literature and 5 from INRA), 13 lucerne hays (7 from the literature and 6 from INRA). The natural grassland hay consisted mainly of grasses. The botanical composition was not determined but they were included in the grassland types reported by Andueza et al. (2016) in the same area.

#### Methodological corpus for *in vivo* experiments and chemical analysis

The main points of the methodological corpus, fully described in a previous publication (Martin-Rosset et al., 2020), are briefly recalled as follows: All data were obtained with 5–6 horses of warmblood breeds fed a hay-only diet and with total faecal collection. Hay provided between 1.0 and 1.3 times the maintenance energy requirement and was offered twice daily. The hay offered was sampled daily for each horse. Individual horse samples were pooled and mixed to be representative of the entire individual horse period. Refusals, if any, were identified and sampled accordingly. Faeces were collected daily from each horse and pooled accordingly. Hay samples were stored at ambient temperature (15–25 °C), and faecal samples were stored at –20 °C. Hay and faeces were analysed on oven-dried samples according to the same official or published and recognised methods for DM, ash for calculation of organic matter, CF by the Weende method, NDF and ADF by the Van Soests methods (Goering and Van Soest 1970; Van Soest et al., 1991), nitrogen by the macro-Kjeldahl method and multiplied by 6.25 to calculate CP, TFRS by the method of Somogyi (1952) and GE determined by adiabatic calorimetry. The *in vivo* digestibility coefficients of OMD and ED were all provided.

#### Statistical analysis

The dataset was characterised by mean, SD, minimum and maximum values. This statistical analysis of the dataset was also calcu-

lated separately for natural grassland, grass and lucerne. Linear regression models to predict OMD, ED and DE from their chemical composition were separately computed for natural grassland, grass and lucerne hay. The regression models were evaluated by leave-one-out cross-validation. All statistical analyses were performed using the Statistical Analysis System statistics package (SAS, 1998) and GNU-project R software version R 4.3.0 (R Foundation for Statistical Computing, <https://www.R-project.org>).

## Results

### Chemical composition and digestion coefficients of hay

The mean and SD values of the chemical composition, digestibility coefficients and energy contents of the natural grassland, grass and lucerne hay are given in Table 1. The minimum and maximum CP, CF, ADF and NDF contents of the natural grassland hay ranged from 55 to 214, 235 to 386, 264 to 403, and 527 to 687 g/kg DM, respectively; the OMD and ED contents ranged from 41.6 to 63.2% and 39.0 to 60.2% respectively; and DE ranged from 6.89 to 11.25 MJ/kg DM. The minimum and maximum CP, CF, ADF and NDF contents of grass hay ranged from 83 to 184, 257 to 431, 288 to 450, 571 to 782 g/kg DM, respectively; OMD ranged from 41.5 to 64.9%; ED ranged from 38.1 to 60.3%; and DE ranged from 7.02 to 11.22 MJ/kg DM. The minimum and maximum CP, CF, ADF and NDF contents of the lucerne hay ranged from 114 to 209, 281 to 441, 321 to 402 and 424 to 587 g/kg DM, respectively; OMD and ED ranged from 51.0 to 63.9% and 46.7 to 59.6%, respectively; and DE ranged from 8.08 to 11.06 MJ/kg DM.

### Prediction of the organic matter digestibility, energy digestibility coefficients and digestible energy content of hay

All the models developed to predict OMD were significant regardless of the CWC criterion used for grassland hay, as the adjusted  $R^2$  in cross-validation ( $R^2_{CV}$ ) ranged from 0.84 to 0.86, but NDF was associated with CP. For grass hay, the adjusted  $R^2_{CV}$  ranged from 0.65 to 0.71, but all the CWC criteria were associated with CP. The models obtained for lucerne hay were also significant, with adjusted  $R^2_{CV}$  values ranging from 0.65 to 0.72, but NDF was associated with CP (Table 2).

The models used to predict the ED content of grassland hay were all significant. Neutral detergent fibre was associated with CP. The adjusted  $R^2_{CV}$  ranged from 0.83 to 0.86. For grass hay,

**Table 1**

Mean chemical composition (g/kg DM) and energy content (MJ/kg DM) assayed or mean digestion coefficients (%) determined *in vivo* in horses for the natural grassland, grass and lucerne hay of the dataset.

Chemical composition	Natural grassland hay 32		Grass hay 16		Lucerne hay 13	
	Mean	SD	Mean	SD	Mean	SD
OM	913	9.1	919	18.4	917	9.2
CP	117	44.5	130	35.1	167	25.1
CF	318	44.5	342	47.9	327	45.8
NDF	618	49.4	675	60.7	517	41.4
ADF	344	44.7	367	47.7	342	30.8
TFRS	78	28.1	78	26.5	31	10.9
Digestion coefficients						
DMD	51	7.2	51	6.1	60	4.0
OMD	52	6.8	52	6.2	58	4.1
ED	48	6.3	47.6	5.7	53	4.8
Energy content						
GE	18.3	0.37	18.6	0.37	18.6	0.19
DE	8.8	1.23	8.9	0.98	9.9	1.09

OM: organic matter, CF: crude fibre, TFRS: total free reducing sugars, DMD: dry matter digestibility, OMD: organic matter digestibility, ED: energy digestibility, GE: gross energy, DE: digestible energy.

the adjusted  $R^2CV$  ranged from 0.63 to 0.71 when CF or a CWC criterion was associated with CP. In contrast, ED of lucerne hay was predicted with a significant model using CF; the adjusted  $R^2CV$  was 0.74. In addition, the model using ADF was significantly associated with CP (adjusted  $R^2CV$  was 0.72). The model using NDF + CP as independent variables was not significant ( $P > 0.05$ ; Table 3).

The DE content of natural grassland hay could only be predicted with CF: the adjusted  $R^2CV$  was 0.83, whereas ADF and NDF were associated with CP (the adjusted  $R^2CV$  was 0.82 and 0.84, respectively). Crude fibre and all CWC criteria should be associated with CP to predict the DE content of grass hay: adjusted  $R^2CV$  values were 0.62 and 0.68 for CF and NDF, respectively, and the model with ADF was significant; the adjusted  $R^2CV$  was 0.57, and the RMSE in cross-validation (RMSECV) was 1.260 MJ/kg DM. The DE content of lucerne hay was only significantly predicted by CF, and the adjusted  $R^2CV$  was 0.55. Acid detergent fibre was associated with CP to significantly predict DE, and the adjusted  $R^2CV$  was 0.67. The model including NDF and CP as independent variables was not significant, the adjusted  $R^2CV$  value was 0.25, and the RMSECV was 0.880 MJ/ DM (Table 4).

The CP content of hay was always related to OMD, ED and DE in the three botanical groups. The adjusted  $R^2CV$  values ranged from 0.59 to 0.66 for grassland hay, 0.58 to 0.65 for grass hay and 0.47 to 0.57 for lucerne hay, respectively, according to the three previous criteria. The average TFRS contents of grassland, grass and lucerne hay were 78, 59 and 51 g/kg DM, respectively. However, TFRS was never a significant independent variable for predicting OMD, ED and DE for the three botanical groups.

### Author's point of views

*Comparison of the new models with the previous models developed by the French National Institute for Agricultural Research*

The new prediction models of OMD of natural grassland, grass and lucerne hay are all more accurate than the previously developed models. The regression coefficients of the models obtained to predict OMD with CF used as a single variable in the present study are all higher ( $R^2 = 0.86, 0.65$  and  $0.68$ , respectively) than those obtained in the previous models ( $R^2 = 0.51, 0.18$  and  $0.44$ ,

**Table 2**

Linear regression between organic matter digestibility (%) in horses and the chemical composition (g/kg DM) of natural grassland, grass and lucerne hay.

	RMSE	Adj $R^2$	RMSECV	$R^2CV$	Min and Max
Natural grassland hay (n = 32)					
OMD = $94.67 - 0.134 \times CF$	2.37	0.87	2.41	0.86	235<CF<385
OMD = $99.31 - 0.138 \times ADF$	2.55	0.85	2.60	0.84	264<ADF<403
OMD = $110.75 + 0.028 \times CP - 0.101 \times NDF$	2.42	0.86	2.50	0.86	527<NDF<687 55<CP<200
Grass hay (n = 16)					
OMD = $54.40 + 0.102 \times CP - 0.046 \times CF$	3.52	0.68	3.80	0.65	257<CF<431 83<CP<192
OMD = $61.79 + 0.088 \times CP - 0.058 \times ADF$	3.36	0.71	3.50	0.71	288<ADF<450 83<CP<192
OMD = $69.40 + 0.091 \times CP - 0.043 \times NDF$	3.40	0.70	3.90	0.67	571<NDF<782 83<CP<192
Lucerne hay (n = 13)					
OMD = $82.13 - 0.074 \times CF$	2.28	0.68	2.60	0.65	281<CF<441
OMD = $96.44 - 0.112 \times ADF$	2.13	0.72	2.20	0.72	304<ADF<402
OMD = $78.41 + 0.073 \times CP - 0.063 \times NDF$	1.94	0.77	2.47	0.67	479<NDF<587 114<CP<209

OMD: organic matter digestibility, CF: crude fibre, RMSECV: root mean square error in cross-validation, Adj  $R^2$ : adjusted coefficient of determination,  $R^2CV$ : coefficient of determination in cross-validation, Min: minimum value, Max: maximum value.

**Table 3**

Linear regression between the energy digestibility (%) in horses and the chemical composition (g/kg DM) of natural grassland, grass and lucerne hay.

	RMSE	Adj $R^2$	RMSECV	$R^2CV$	Min and Max
Natural grassland hay (n = 32)					
ED = $87.76 - 0.125 \times CF$	2.29	0.86	2.40	0.86	235<CF<385
ED = $91.88 - 0.127 \times ADF$	2.51	0.83	2.60	0.83	264<ADF<403
ED = $102.10 + 0.027 \times CP - 0.09 \times NDF$	2.37	0.85	3.00	0.85	527<NDF<687 55<CP<200
Grass hay (n = 16)					
ED = $50.29 + 0.096 \times CP - 0.044 \times CF$	3.24	0.70	3.80	0.63	257<CF<431 83<CP<192
ED = $56.09 + 0.085 \times CP - 0.053 \times ADF$	3.13	0.71	3.2	0.71	288<ADF<450 83<CP<192
ED = $63.21 + 0.088 \times CP - 0.040 \times NDF$	3.17	0.71	4.40	0.71	571<NDF<782 83<CP<192
Lucerne hay (n = 13)					
ED = $81.73 - 0.087 \times CF$	2.33	0.74	2.35	0.74	281<CF<441
ED = $68.20 + 0.076 \times CP - 0.080 \times ADF$	2.43	0.72	3.00	0.72	304<ADF<402 114<CP<209
ED = $66.01 + 0.104 \times CP - 0.058 \times NDF$	2.23	0.76	4.10	0.34	479<NDF<587 114<CP<209

ED: energy digestibility, CF: crude fibre, RMSECV: root mean square error in cross-validation, Adj  $R^2$ : adjusted coefficient of determination,  $R^2CV$ : coefficient of determination in cross-validation, Min: minimum value, max: maximum value.



**Table 4**

Linear regression between the digestible energy content (MJ/kg DM) of hay in horses and the chemical composition (g/kg DM) of natural grassland, grass and lucerne hay.

	RMSE	Adj R <sup>2</sup>	RMSECV	R <sup>2</sup> CV	Min and Max
Natural grassland hay (n = 32)					
DE = 16.640 - 0.025 × CF	0.50	0.83	0.50	0.83	235<CF<385
DE = 13.956 + 0.009 × CP - 0.018 × ADF	0.51	0.82	0.51	0.82	264<ADF<403 55<CP<200
DE = 17.188 + 0.009 × CP - 0.015 × NDF	0.49	0.84	0.49	0.84	527<NDF<687 55<CP<200
Grass hay (n = 16)					
DE = 10.273 + 0.015 × CP - 0.010 × CF	0.63	0.66	0.65	0.62	257<CF<431 83<CP<192
DE = 11.704 + 0.012 × CP - 0.012 × ADF	0.61	0.69	1.26	0.57	288<ADF<450 83<CP<192
DE = 13.247 + 0.013 × CP - 0.009 × NDF	0.61	0.68	0.68	0.68	571<NDF<782 83<CP<192
Lucerne hay (n = 13)					
DE = 14.990 - 0.015 × CF	0.47	0.69	0.50	0.55	281<CF<441
DE = 12.640 + 0.013 × CP - 0.014 × ADF	0.49	0.67	0.54	0.67	304<ADF<402 114<CP<209
DE = 12.909 + 0.018 × CP - 0.011 × NDF	0.41	0.76	0.88	0.25	479<NDF<587 114<CP<209

DE: digestible energy, CF: crude fibre, RMSECV: root mean square error in cross-validation, Adj R<sup>2</sup>: adjusted coefficient of determination, R<sup>2</sup>CV: coefficient of determination in cross-validation, Min: minimum value, Max: maximum value.

respectively) (INRA, 1984, 2015). The optimal models identified in this study for natural grassland hay, grass hay, and lucerne hay were characterised by RMSECV values of 2.41, 3.50, and 2.20, respectively. These errors are responsible for variations between 0.03 and 0.06 in the net energy content (UFC)/kg DM of the hay (INRA, 2015). These variations result in a discrepancy of 0.11 for the amount of natural meadow hay, 0.15 for grass hay and 0.09 for lucerne hay to be fed to the animals to meet the nutritional energy requirements for the maintenance of a 500 kg horse. As already well known, the intake capacity of horse can vary according to the nutritional value of forages (INRA, 2015). Therefore, from a practical point of view, the prediction errors of OMD of hay can be managed by intake amount regulation by horse itself.

The regression statistics of the model used to predict ED of each different botanical family of hay are lower with CF used as a single variable in the present study than with the model used in the previous INRA system (INRA, 1984, 2015), adjusted R<sup>2</sup> = 0.70 to 0.86 vs. 0.91, respectively. This is because the new models were developed with forage-only diets whereas the previous model was computed for all kinds of feeds and forages with a specific correction factor for forages. The new models were specifically elaborated with hay of the three main botanical groups of hay in the present study.

The present study supports the initial hypothesis stated previously in the INRA system (INRA, 1984) to calculate different models to predict the digestion coefficients of forages according to the botanical family. This option has been further maintained (INRA, 2015) and updated in the present study. The CP content was significant as an independent variable; however, there is no outstanding model regarding the CWC criteria. Similar results have been reported by INRA (2007) in ruminants, particularly for leguminous forages.

#### Comparison of the new models with other models

The predictive models of DE proposed by Fonnebeck (1981), Zeyner and Kienzle (2002) or Hansen and Lawrence (2017) using data files including feeds and diets cannot be compared with the new INRA models established with forage-only diets. In contrast,

Fonnebeck et al. (1967, 1968) determined the OMD of 12 hay-only diets composed of 9 grass hays and 3 legume hays using a similar methodological corpus to that used in the present study. The range of OMD (%) of hay was 44.5 to 49.9 for grass and 51.0 to 59.9 for legumes. No predictive models of DE were established, but Fonnebeck (1969) studied the relationship between the apparent digestibility of different fractions of forages: cellular content, CWC and hemicelluloses, soluble carbohydrates and their respective content relative to DM content. This author clearly showed that grasses and legumes were two different populations. The correlation coefficients ranged from 0.85 to 0.99, whereas the range of the content in g/kg DM was 450 to 800 for CWC, 70 to 320 for hemicellulose, 270 to 390 for cellulose, 230 to 530 for cellular content and 80 to 320 for soluble carbohydrates. The same pattern was observed in ruminants by Van Soest (1967). Thus, the specialised models proposed for grass and lucerne hay in the present study are quite original and relevant compared with those in the literature. For predicting OMD, the R<sup>2</sup>CV of the models developed from ADF and CP values and from NDF and CP values were 0.71 and 0.67, respectively, for grass hay and 0.72 and 0.67, respectively, for lucerne hay. In the present study, the RMSECV of the prediction of OMD for grass hay ranged from 3.50 to 3.90% whereas RMSECV of the prediction of OMD for lucerne hay ranged from 2.20 to 2.60% units. The number of data points for these types of forages, n = 16 and n = 13 for grass hay and lucerne hay, respectively, was lower than the data used for calculating the grassland model (n = 32). Indeed, the effects of species, vegetation stage, agronomic and climatic conditions and their interactions were very heavy with respect to the data number for each botanical family.

*Effect of the cell wall content and the intrinsic chemical composition of cell wall content on the prediction of organic matter digestibility, energy digestibility and digestible energy content depending on the botanical groups*

Three criteria, CF, ADF and NDF, were used in the present study according to systems implemented worldwide to characterise the CWC content of hay and to predict the OMD, ED and DE content of hay. The accuracy of the prediction depends on the specific

chemical composition of these criteria according to the botanical groups (Fonnesbeck 1968, 1969). Crude fibre and ADF are, on average, characterised by high cellulose and lignin levels even though they are not pure lignocellulose. Crude fibre, NDF and ADF are contaminated to various degrees by protein residues. The hemicellulose content of NDF is higher than that of CF and ADF, but these values have some degree of contamination by hemicelluloses. Indeed, CF is an overestimation of the cellulose content of forages and NDF is an overestimation of CWC.

In the present study, no CWC criterion was more relevant than the others when all the best models were considered, with or without CP as an independent variable. The adjusted  $R^2$  values of the models used for predicting the OMD and ED of grass or lucerne hay were slightly lower with CF than with ADF or NDF. The respective contents of the different residues, such as protein, hemicelluloses, pectic substances and lignin, of CF, ADF and NDF might explain the observed differences as mentioned above. The adjusted  $R^2$  of the models used for predicting DE were all lower regardless of the CWC criterion used for grass and lucerne hay than the adjusted  $R^2$  obtained for the digestion coefficients. In addition, there were strong differences in adjusted  $R^2$  between the DE models according to the CWC criterion used depending on whether they were associated with CP for grass hay and lucerne hay. The RMSEs of the different models used for predicting the DE content of grass hay were all high with the CF + CP and NDF + CP models and even higher with the ADF + CP model. These observations are consistent with those of a study carried out by Lindberg and Ragnarsson (2010) who compared the relevance of ADF or NDF to predict ED and DE in horses fed forages only-diets (haylages). The authors concluded that the regression coefficients of the ADF or NDF-specific models were both low and non-significant:  $R^2$  values ranged from 0.48 to 0.60 for ED and from 0.53 to 0.59 for DE.

However, it should also be added that NDF would underestimate the digestion of dietary fibre in the large intestine of horses compared with that of the non-starch polysaccharides, a new concept derived from a new determination of the structural cell wall polysaccharides of the plant (Hoffman et al., 2001), to predict the potentially energy-yielding fraction of dietary fibre (Longland et al., 1997). Non-starch polysaccharides include fructans, pectins, gums, mucilages,  $\beta$ -glucans, hemicelluloses, and cellulose but do not include some lignin contrary to NDF. This might explain the higher losses of non-starch polysaccharides (30 and 34%) of the CWC polyoxides (arabinose, mannose, galactose, rhamnose and xylose) in the large intestine, as measured in fistulated equines by Moore-Colyer et al. (2002) and Brøkner et al. (2012).

*Effect of the cellular protein and soluble carbohydrate content on the prediction of the digestion coefficients and the digestible energy content of hay of the three botanical groups*

In the temperate zone, the CWC content is 1.4 times higher in grass hay (70%) than in legume hay (50%). The cellulose content of CWC is similar in grasses (29%) and legumes (24%), whereas the hemicellulose content is three times higher and the lignin content is two times lower in grasses (25 and 4% of DM) than in legumes (7 and 9%) (Fonnesbeck, 1968). Consequently, the apparent amount of CWC is, on average, 20% units higher in grasses than in legumes (Van Soest, 1967; Fonnesbeck, 1968, 1969).

In the present study, the protein content of forages was significantly correlated with OMD, ED and consequently DE ( $r > 0.70$ ). The utilisation of CP as a single variable is questionable. Fonnesbeck (1969) showed that the amount of digestible protein might be more relevant because there was a high correlation coefficient ( $r = 0.88$ ) between the amount of digestible protein and the protein content of forages. As a result, the amount of digestible protein might be a single predictor more relevant than the CP con-

tent for predicting OMD and ED. However, in practice, it is relatively easy to use CP as an independent variable in multiple linear regression models in which CWC criteria are also used (Fonnesbeck, 1968).

The water-soluble carbohydrate content of forages is of high concern to evaluate and predict their energy value for horses, as they are either digested in the small intestine (monomers) or fermented (fructans) in the large intestine. Water-soluble carbohydrates include total sugars (glucose, fructose so-called free reducing sugars). The addition of sucrose leads to the TFRS and fructans according to Smith (1973). Water-soluble carbohydrates are included in the TNSC since a new representation of the carbohydrate fractions, structural and non-structural carbohydrates (the latter also includes starch), was proposed by Hoffman et al. (2001). It is well established that the TNSC and water-soluble carbohydrate contents and the TFRS/fructan ratio of green forages change with the botanical group, species and vegetation stage, time of day and environmental factors (Vikajärvi et al., 2012). The content of TFRS is, on average, 30% higher in grasses than in legumes (Jarrige, 1981). In the first cycle, close to blooming or flowering, the TFRS content ranges from 3 to 5% of DM in legumes, from 4 to 10% in grasses and from 4 to 8% in natural grasslands. The TFRS content decreases during the second cycle (Jarrige, 1981). In the present study, it ranged from 3 to 13% of DM in grassland hay, from 4 to 11% in grass hay and from 3 to 7% in lucerne hay. However, the TFRS content was not a significant criterion to predict the digestion coefficients OMD and ED and consequently, the DE content of the three botanic groups of hay whereas it is established that TFRS was included in the prediction of ME and NE of forages (Vermorel and Martin-Rosset, 1997). Unfortunately, the fructan content of the hays used in the present study was not determined, whereas the content may be very high (Longland and Byrd, 2006).

*Adaptive ability of these models to predict the energy content of hays for rationing all the types of horses fed with only-hay diets or mixed diets*

The models proposed in the present study make it possible to calculate the energy content of natural grassland hay, grass hay or lucerne hay obtained under temperate conditions. From these values and the estimation of the animals' energy requirements (INRA, 2015), the amount of feed to be given to the animal can be determined. These models can be used for all types of horses, whether hay is the sole diet or hay is supplemented with concentrates. Indeed, several studies (Martin-Rosset and Dulphy, 1987; Martin-Rosset et al., 1990; INRA, 2015) have shown that the intake capacity of the horse is directly related to the energy requirements of the animal, without any influence of the fibre content or CWC content of the forage. These authors also showed that, unlike in ruminants, there is no forage-concentrate interaction in equine diets.

## Conclusions

The DE content of hay harvested in the temperate zone can be predicted more accurately with the new models specific to botanical families than with other existing models dedicated only to hay. These new models use ADF or NDF as representative of the CWC of hay, in association with CP, but models based on the CF content were also proposed. These chemical components are assayed with standardised analytical methods that are routinely implemented by analytical laboratories. The new models are available to either directly predict the DE content of hay for field applications or pre-

dict the digestion coefficients to be used in the INRA system for subsequently predicting the DE, ME and NE contents of hay-

These methods are likely useful to describe the variations in the CWC content and simultaneously the variation in subfibre fractions (pentosanes, pectic substances, etc.) and in carbohydrate fractions such as soluble (fructans) and semisoluble (starch) fractions. The contents of these fractions in forages were used to first improve the understanding of the effects of age, such as stage of growth, botanical group, species of the forage and the agronomic and climatic conditions on the digestion of forages in horses. Second, it is necessary to improve the prediction of the digestibility of organic matter in the scope of the new scheme of the utilisation of the different carbohydrate fractions in the horse.

### Peer Review Summary

Peer Review Summary for this article (<https://doi.org/10.1016/j.anopes.2024.100075>) can be found at the foot of the online page, in Appendix A.

### Ethics approval

Not applicable.

### Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

### Authors OIRCD

**Donato Andueza:** <https://orcid.org/0000-0002-1839-4461>.

### Author contributions

**William Martin-Rosset:** Conceptualisation, data curation, formal analysis, investigation, methodology, resources, supervision, validation, writing – original draft, writing – review and editing. **Donato Andueza:** Conceptualisation, formal analysis, investigation, methodology, resources, validation, writing – original draft, writing – review and editing. **Michel Vermorel:** Conceptualisation, data curation, formal analysis, investigation, methodology, resources, supervision, validation, writing – original draft, writing – review and editing.

### Declarations of interest

None.

### Acknowledgments

None.

### Financial support statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### References

Andrieu, J., Martin-Rosset, W., 1995. Chemical, biological and physical (NIRS) methods for predicting organic matter digestibility of forages in horse. In: Proceedings of the 14th Equine Nutrition and Physiology Symposium, January 19-21, Ontario, Canada, pp. 76-77.

Andueza, D., Rodrigues, A.M., Picard, F., Rossignol, N., Baumont, R., Cecato, U., Farrugia, A., 2016. Relationship between composition, biomass and feed value of forage issued from permanent grassland over the first growth cycle. *Grass and Forage Science* 71, 366–378.

Argenzio, R.A., Southworth, M., Stevens, C.E., 1974. Sites of organic acid production and absorption in equine gastrointestinal tract. *American Journal of Physiology* 226, 1043–1050.

Brøkner, C., Austbø, D., Næsset, J.A., Bach Knudsen, K.E.B., Tauson, A.H., 2012. Equine pre-caecal and total tract digestibility of individual carbohydrate fractions and their effect on caecal pH response. *Archives of Animal Nutrition* 66, 490–506.

Dyer, J., Fernandez-Castano, E., Merediz, K.S.H., Salmon, J.C., Proudman, G.B., Shirazi-Beechey, S.P., 2002. Molecular characterization of carbohydrate digestion and absorption in equine small intestine. *Equine Veterinary Journal* 34, 349–358.

Fonnesbeck, P.V., 1968. Digestion of soluble and fibrous carbohydrate of forages by horses. *Journal of Animal Science* 27, 1336–1344.

Fonnesbeck, P.V., 1969. Partitioning of the nutrients of forage for horses. *Journal of Animal Science* 28, 624–633.

Fonnesbeck, P.V., 1981. Estimating digestible energy and TDN for horses with chemical analysis of feeds. *Journal of Animal Science* 241, 290.

Fonnesbeck, P.V., Lydman, R.K., Vander Noot, G.W., Symons, L.D., 1967. Digestibility of the proximate nutrients of forages by horses. *Journal of Animal Science* 26, 1039–1045.

Gesellschaft für Ernährungsphysiologie (GfE), 2014. *Empfehlungen zur energie- und Nährstoffversorgung von Pferden*. DLG Verlag GmbH, Frankfurt am Main, Deutschland.

Goering, H.K., Van Soest, P.J., 1970. Forage fiber analyses apparatus, reagents, procedures, and some applications. USDA-ARS, Agricultural Handbook No. 379, pp. 1–20. US Government Printing Office, Washington, DC, USA.

Hansen, L., Lawrence, L.M., 2017. Composition factors predicting forage digestibility by horses. *Journal Equine Veterinary Science* 58, 97–102.

Hoffman, R.M., Wilson, J.A., Kronfeld, D.S., Cooper, W.L., Lawrence, L.A., Sklan, D., Harris, P.A., 2001. Hydrolyzable carbohydrates in pasture, hay, and horse feeds: direct assay and seasonal variation. *Journal of Animal Science* 2001 (79), 500–506.

Ince, J., Longland, A., Moore-Colyer, M., Harris, P., 2005. Effect of high or lower water-soluble carbohydrate perennial ryegrass (*Lolium perenne*) on lactate and pH with equine hind-gut microbial inoculum. *Proceedings of the Equine Science Society*, May 31-June 3, Tucson, AZ, USA, p. 149.

Institut National Recherche Agronomique (INRA) 1984. *Le Cheval* (eds. R Jarrige and W Martin-Rosset), INRA Editions, Versailles, France.

Institut National Recherche Agronomique (INRA) 2007. *Alimentation des bovins, ovins et caprins – Besoins des animaux – Valeurs des aliments – Tables INRA 2007*. Editions Quae, Versailles, France.

Institut National Recherche Agronomique (INRA) 2015. *Equine nutrition, INRA nutrient requirements, recommended allowances and feed tables*. (ed. W Martin-Rosset), Wageningen Academic publishers, Wageningen, The Netherlands.

Jarrige, R., 1981. Les constituants glucidiques des fourrages : variations, digestibilité et dosage. In: *Prévision de la valeur nutritive des aliments des ruminants. Tables de la prévision de la valeur alimentaire des fourrages*. (ed. Demarquilly C.), INRA Editions, Versailles, France, pp. 13–40.

Lindberg, J.E., Ragnarsson, S., 2010. Prediction of energy digestibility and energy content in forages fed to horses. In *Energy and Protein Metabolism and Nutrition*. EAAP publication N° 127 (ed. Crovetto, M.) Wageningen Academic Publishers, Wageningen, The Netherlands, pp 637–638.

Longland, A., Byrd, B., 2006. Nonstructural carbohydrates and equine laminitis. *Journal Nutrition* 136 (suppl. 7), 2099S – 2012S.

Longland, A., Moore-Colyer, M., Hyslop, J.J., Dhanoa, M.S., 1997. Comparison of the *in sacco* degradation of the non-starch polysaccharide and neutral fibre fraction of four sources of dietary fibre by ponies. *Proceedings of the 15th Equine Nutrition Physiology Society*, May 28-31, Fort Worth, TX, USA, pp. 120–121.

Martin-Rosset, W., Andrieu, J., Vermorel, M., Dulphy, J.P., 1984. Valeur des aliments pour le cheval. In: Jarrige, R., Martin-Rosset, W. (Eds.), *Le Cheval: Reproduction, Alimentation, Exploitation*. INRA Editions, Versailles, France, pp. 209–238.

Martin-Rosset, W., Vermorel, M., Doreau, M., Tisserand, J.L., Andrieu, J., 1994. The French horse feed evaluation systems and recommended allowances for energy and protein. *Livestock Production Science* 40, 37–56.

Martin-Rosset, W., Andrieu, J., Jestin, M., Macheboeuf, D., Andueza, D., 2012. Prediction of organic matter digestibility of forages in horses using different chemical, biological and physical methods. In: Saastamoinen, M., Fradinho, M.J., Santos, A.S., Miraglia, N. (Eds.), *Forage and Grazing in Horse Nutrition EAAP Publication No 132*. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 83–96.

Martin-Rosset, W., Dulphy, J.P., 1987. Digestibility interactions between forages and concentrates in horses: influence of feeding level – comparison with sheep. *Livestock Production Science* 17, 263–276.

Martin-Rosset, W., Doreau, M., Boulot, S., Miraglia, N., 1990. Influence of level of feeding and physiological state on diet digestibility in light and heavy breed horses. *Livestock Production Science* 25, 257–264.

Martin-Rosset, W., Andueza, D., Vermorel, M., 2020. Comparison of three systems for predicting the digestible energy value of natural grassland and lucerne hays for horses. *Animal* 14, 1413–1421.

Moore-Colyer, M.J.S., Hyslop, J.J., Longland, A.C., Cuddeford, D., 2002. The mobile bag technique as a method for determining the degradation of four botanically



- diverse fibrous feedstuffs in the small intestine and total digestive tract of ponies. *British Journal of Nutrition* 88, 729–740.
- National Research Council (NRC), 2007. Nutrient requirements of horses, 6<sup>th</sup> revised edition. National Academy Press, Washington, DC, USA.
- Smith, D., 1973. The nonstructural carbohydrates. In: Butler, G.W., Bailey, R.W. (Eds.), *Chemistry and Biochemistry of Herbage*. Academic Press, London, UK, pp. 105–155.
- Somogyi, M., 1952. Notes on sugar determination. *Journal of Biological Chemistry* 195, 19–23.
- Statistical Analysis Systems Institute, 1998. SAS user's guide, version 6.12, SAS Institute Inc., Cary NC, USA.
- Van Soest, P.J., 1967. Development of a comprehensive system of feed analyses and its application to forages. *Journal of Animal Science* 26, 119–128.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74, 3583–3597.
- Vermorel, M., Martin-Rosset, W., 1997. Concepts, scientific basis structure and validation of the French horse net energy system (UFC). *Livestock Production Science* 47, 261–275.
- Vikajärvi, M.P., Saarijärvi, K., Rinne, M., Saastamoinen, M., 2012. Grass physiology and its relation to nutritive value in feeding horses. In: Saastamoinen, M., Fradinho, M.J., Santos, A.S., Miraglia, N. (Eds.), *Forage and Grazing in Horse Nutrition EAAP Publication No 132*. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 17–44.
- Zeyner, A., Kienzle, H., 2002. A method to estimate digestible energy in horse feed. *Journal of Nutrition* 132, 1771S–1773S.