

***In vivo* grass digestibility prediction from biochemical criteria and the sum of temperatures at cutting**

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Abstract

In vivo digestibility (OMd) of forages is highly dependent on plant maturity stage at cutting, which is linked to the sum of temperatures (ST). In this study, we aimed to test if combining biochemical criteria, such as chemical composition and *in vitro* enzymatic digestibility, and ST improves the prediction of digestibility of hays harvested in three different locations (Massif Central and Jura in France and Fribourg foothills in Switzerland). *In vivo* OMd on sheep, chemical composition, *in vitro* enzymatic digestibility and ST were measured on 32 multispecies grassland hays harvested from 2015 to 2017. Spearman's correlations indicated that ST was negatively linked with OMd of hays harvested during the first vegetation cycle, whereas *in vitro* enzymatic digestibility was the biochemical criteria most strongly related to *in vivo* OMd. The best prediction models of *in vivo* OMd using biochemical criteria were obtained with *in vitro* enzymatic digestibility and crude protein ($R^2=0.884$; RMSE=0.0173), and the addition of ST slightly improved the model ($R^2=0.910$; RMSE=0.0153).

Keywords: hay, *in vivo* digestibility, *in vitro* enzymatic digestibility, sum of temperatures

Introduction

The determination of feed value of forage, especially organic matter digestibility (OMd), has an important role in the context of ruminant feeding, and it needs to be predicted precisely. The plant maturity stage at cutting highly influences OMd (Buxton, 1996). The sum of temperatures (ST; in growing degree-days) is positively linked to plant maturity stage at cutting, and negatively to chemical composition and *in vitro* enzymatic digestibility (Michaud *et al.*, 2012). Chemical composition and/or *in vitro* digestibility are commonly used to predict *in vivo* OMd in most ruminant feeding systems. In a 32-year survey of *in vivo* OMd of hays harvested at the same location in Massif Central (France), it was shown that ST explained part of the variability in OMd that chemical components did not (Deroche *et al.*, 2020). In this study, we aimed to test if combining biochemical criteria and ST can improve the prediction of digestibility of hays harvested in different locations.

Materials and methods

A collection of 32 hays harvested on multispecies grasslands was used in the study. Twenty-six hays were harvested during the first vegetation cycle: two in 2015 and four in 2017 in Fribourg foothills (Switzerland), 8 in 2017 in Jura (France) and 12 in Massif Central (France) (4 each year between 2015 and 2017). In addition, six regrowth hays were used, including two regrowth hays cut during the third vegetation cycle in 2015 in Fribourg foothills (Switzerland) and four regrowth hays cut during the second vegetation cycle in 2017 in Massif Central (France). The *in vivo* OMd of the 32 hays was measured on sheep at INRA according the method described by Demarquilly *et al.* (1995). The chemical composition of the hays offered to the sheep was analysed for determination of crude protein (CP), total soluble carbohydrates (TSC), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents, and the *in vitro* enzymatic dry matter (DM) digestibility (eDMd) was analysed according to Aufrère *et al.* (2007). The ST at cutting was calculated according to Theau and Zerourou (2008) by accumulation of mean daily air temperatures comprising between 0 to 18 °C from 1 February to the cutting date for hays harvested

during the 1st vegetation cycle, and from the previous cutting date to the regrowth cutting date for hays harvested during 2nd and 3rd vegetation cycles. Correlations between OMD and biochemical criteria and ST were assessed using Spearman's correlation (SAS 5.1). Stepwise linear regression was used to predict OMD by (1) chemical components, (2) *in vitro* enzymatic digestibility, (3) chemical components and *in vitro* enzymatic digestibility, and (4) biochemical criteria and ST. The effect of ST was nested within the vegetation cycle (1st cycle and 2nd or 3rd cycles). Stability and validity of each model was checked by a leave one out cross validation (R software – package lm).

Results and discussion

The *in vivo* OMD of the herbage from 32 grasslands conserved as hays ranged from 0.539 to 0.725 (Table 1). *In vivo* OMD was highly correlated with *in vitro* enzymatic digestibility and NDF content and to a lesser extent with the other chemical components. The *in vivo* OMD was negatively correlated with ST for the 26 hays harvested during the first vegetation cycle, in accordance with Michaud *et al.* (2012). The positive correlation between ST and *in vivo* OMD on regrowth hays might be explained by the presence of leafy species (e.g. *Taraxacum* sp.) whose digestibility remains high in advanced maturity stages (Schubiger *et al.*, 2001). The *in vitro* enzymatic digestibility confirmed to be a very good predictor of *in vivo* OMD ($R^2=0.871$), better than the combination of CP and ADF contents ($R^2=0.786$) (Table 2) in accordance with Aufrère *et al.* (2007). The prediction of *in vivo* OMD by *in vitro* eDMd was slightly improved by the addition of the CP content in the model ($R^2=0.884$) or by the addition of CP content and ST ($R^2=0.910$; $RMSE=1.531$), which was the best model established on our dataset (Table 2). The negative coefficient of ST in the prediction model for hays harvested during the first vegetation cycle is consistent with literature (Michaud *et al.*, 2012), and with previous study (Deroche *et al.*, 2020).

Table 1. Descriptive statistics of the variables measured on the 32 multispecies grassland hays and Spearman's correlation between *in vivo* OMD and other criteria.

Variables ¹	n	Mean	SD	Minimum	Maximum	Correlation with OMD ²
<i>In vivo</i> OMD (g g ⁻¹)	32	0.642	0.051	0.539	0.725	-
Crude protein (g kg ⁻¹ DM)	32	107	21.1	77.5	161	0.81***
Neutral detergent fibre (g kg ⁻¹ DM)	32	572	72.0	430	678	-0.93***
Acid detergent fibre (g kg ⁻¹ DM)	32	303	43.1	228	376	-0.90***
Total soluble carbohydrate (g kg ⁻¹ DM)	32	159	53.8	104	308	0.80***
eDMd (g g ⁻¹)	32	0.607	0.100	0.444	0.770	0.95***
ST_1 st cycle hay (degree.day)	26	914	177	634	1325	-0.73***
ST_regrowth hay (degree.day)	6	1012	168	765	1295	0.85*

¹ DM = dry matter; eDMd = enzymatic dry matter digestibility; OMD = organic matter digestibility; ST = sum of temperatures; ST_1st cycle hay or ST_regrowth hay = ST is analysed within vegetation cycle.

² Significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; ns = non-significant.

Table 2. *In vivo* hay OMD prediction models (n=32), and cross validation with the leave one out method.¹

Model components	Calibration		Cross validation
	RMSE	R ²	RMSPE
$0.767 - 7.087 \times 10^{-4} \times \text{ADF (g kg}^{-1} \text{ DM)} + 8.431 \times 10^{-4} \times \text{CP (g kg}^{-1} \text{ DM)}$	0.0235	0.786	0.0246
$0.354 + 0.475 \times \text{eDMd (g g}^{-1})$	0.0186	0.871	0.0194
$0.349 + 0.388 \times \text{eDMd (g g}^{-1}) + 0.054 \times \text{CP (g kg}^{-1} \text{ DM)}$	0.0173	0.884	0.0181
$0.371 + 0.316 \times \text{eDMd (g g}^{-1}) + 1.041 \times 10^{-3} \times \text{CP (g kg}^{-1} \text{ DM)} - 2.94 \times 10^{-5} \times \text{ST}_{1^{\text{st}} \text{ cycle hay (degree.days)} - 5.025 \times 10^{-5} \times \text{ST}_{\text{regrowth hay (degree.days)}}$	0.0153	0.910	0.0166

¹ ADF = acid detergent fibre; CP = crude protein; DM = dry matter; OMD = organic matter digestibility; RMSE = root mean square error; RMSPE = root mean square of prediction error.

Conclusions

This study confirmed on hays from various origins that the *in vitro* enzymatic dry matter digestibility is a powerful criterion to predict *in vivo* digestibility. Agro-climatic criteria such as ST, which can be easily obtained from weather stations, are also correlated with *in vivo* digestibility of grass. Nevertheless, when combined with *in vitro* digestibility they improve only slightly the predictions of *in vivo* digestibility of grass.

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