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Samenvatting NL

Het project "Voeding op Maat" onderzoekt of en hoe het huidige eiwitwaarderingssysteem voor melkvee (het DVE systeem) verbeterd kan worden. In het DVE systeem zijn de afbraak (Kd) en passage (Kp) in en door de pens belangrijke parameters voor het voorspellen van de fermentatie van de verschillende componenten (bijv. Eiwit, Zetmeel (ST), Vezels, Suiker) in de pens. Het voorliggende rapport onderzoekt de passagesnelheid van het potentieel pensfermenteerbare zetmeel (Kp-D-ST) door de pens en de afbraaksnelheid van uitwasbaar zetmeel (Kd-W-ST) in de pens. Dit is gebeurd door verschillende modellen te fitten op een dataset van verteringsproeven. Deze dataset bestond uit gegevens uit de wetenschappelijke literatuur van experimenten waarin voor Europese rantsoenen de pensfermentatie is gemeten van zetmeel. De modellen, die getest werden, onderzochten de invloed van verschillende factoren (voeropname, ruwvoer versus krachtvoer, krachtvoerratio en deeltjesgrootte van zetmeel) op de voorspelling van Kp-D-ST en Kd-W-ST. Uit deze studie bleek dat simpele modellen een betere goodness-of-fit hadden dan meer complexe modellen. Het voorliggende rapport kan als basis dienen voor verdere besluitvorming m.b.t. het actualiseren van het bestaande DVE systeem.

Summary UK

The "Voeding op Maat" project investigates if and how the current protein feed evaluation system for dairy cattle (the DVE system) can be improved. In the DVE system, the degradation (Kd) and passage (Kp) in and through the rumen are important parameters for predicting the fermentation of the different components (e.g. Protein, Starch (ST), Fibers, Sugar) in the rumen. The present report investigates the passage rate of potentially rumen-fermentable starch (Kp-D-ST) through the rumen and the degradation rate of washable starch (Kd-W-ST) in the rumen. This was done by fitting different models to a dataset of digestion experiments. The dataset consisted of data from the scientific literature of experiments in which rumen fermentation of ST was measured for European diets. The tested models examined the influence of different factors (feed intake, forage vs. concentrate, concentrate ratio, and starch particle size) on the prediction of Kp-D-ST and Kd-W-ST. This study found that simple models had a better goodness-of-fit than more complex models. This report can serve as a basis for further decision-making regarding the updating of the existing DVE system.

This report can be downloaded for free at https://doi.org/10.18174/661771 or at <u>www.wur.nl/livestock-research</u> (under Wageningen Livestock Research publications).

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Foreword

The present study 'Prediction of rumen passage rate of dietary starch' was conducted by a collaboration of Wageningen Livestock Research (WLR), the Flanders Research Institute for Agriculture, Fisheries and Food (ILVO) and the foundation CVB as part of the Public Private Partnership "Voeding op Maat" with the aim to reduce nitrogen emission in production animals through improved nutrition. This project was funded by the foundation CVB and the Dutch Ministry of Agriculture, Nature and Food Quality. The authors thank the members of the Ad hoc committee and the technical committee of CVB for their support.

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Summary

The "Voeding op Maat" project investigates whether and how the current protein feed evaluation system for dairy cattle (the DVE/OEB-2007 system) can be improved. In the DVE/OEB-2007 system, the degradation rate in the rumen (Kd) and the passage rate through the rumen (Kp) are important parameters for predicting the fermentation of the different dietary components (e.g. Protein, Starch (ST), Fiber (NDF), sugar) in the rumen. The present report provides prediction formulas to 1) calculate the passage rate of potential rumen fermentable dietary starch (Kp-D-ST; %/h) through the rumen and 2) to calculate the rumen degradation rate of the washout fraction of starch (Kd-W-ST; %/h) (the washout fraction of starch consists of very fine particles that is washed out from the nylon bag after being treated in a washing machine with a wool was program).

This was done by fitting various models to a dataset. Initially a dataset was composed from international literature containing 84 studies and a total of 395 treatment means. From the original dataset 7 studies with 29 treatments from European countries (EU) were used. This dataset contained experiments in which the rumen fermentation of starch was determined. This dataset was used to examine the explanatory value of several models developed to explain variation in rumen fermented starch (RFST; kg/cow/d). In these models, the effects of various factors such as type of feed (roughage or concentrate), feed intake level, dietary concentrate ratio and particle size of the starch (course or fine) on Kp-D-ST were estimated in such a way that differences between model predicted RFST and observed RFST were minimized. In all models a Kd-W-ST value was estimated and in some models Kd-W-ST was estimated as a function of Kd-D-ST values of individual feedstuffs. The goodness of fit of the various models tested was done with a leave one out evaluation, where the model was developed on the dataset minus one experiment and the model was then tested on that experiment. This was done repeatedly for all experiments. The model outcomes showed that the simple models containing 1) fixed Kp-D-ST values for roughages and concentrate feedstuffs or a single Kp-D-ST value for both roughages and concentrate feedstuffs and 2) a fixed Kd-W-ST value for both roughages and concentrates had better goodness of fit compared to more complex models that included extra explanatory variables such as feeding level, concentrate ratio, or starch particle size. Also, a simple model with a fixed Kp-D-ST value for feedstuffs with course particles and a fixed Kp-D-ST value for feedstuffs with fine particles performed equally well and resulted in similar Kp-D-ST estimates as a model with fixed Kp-D-ST values which may be explained by the fact that all starch from roughages consists in the form of course particles whereas for concentrate feedstuffs most starch is present in the form of fine particles. In the most simple model, a Kp-D-ST of 0.61 %/h and Kd-W-ST of 29.4 %/h was fitted for both roughage and concentrate feedstuffs.

Besides the evaluation of the new models, also the current DVE/OEB-2007 model from CVB was evaluated for its capacity to explain variation in RFST. It appeared that the present DVE/OEB-2007 performed similar good compared to the in this study newly developed models.

The present report can serve as a basis for further decision making on how to build an improved DVE model.

1 Introduction

Empirical equations that predict nitrogen and energy flow through dairy cows are the foundation of several modern dairy models (NRC, 2001, Van Duinkerken et al., 2011, Noziere et al., 2018). In the DVE/OEB-2007 protein evaluation system for ruminants (Van Duinkerken et al., 2011), each feed has an intestinal digestible protein value (DVE-value) composed of (1) the digestible true protein contributed by feed protein escaping rumen degradation, (2) microbial protein synthesized in the rumen, and (3) a correction for endogenous protein losses in the digestive tract. Estimating ruminal microbial protein synthesis (MPS) relies on estimating nutrient fermentation in the rumen for different feedstuffs/feed classifications fed to cows (Van Duinkerken et al., 2011). The efficiency of MPS is related to the various fractions: S (soluble fraction), W (washout fraction), W-S (insoluble washout fraction), and D (potentially rumen degradable fraction) of the chemical components (e.g., crude protein, starch, NDF, sugars RNSP) and their associated fractional passage rates through the rumen. Precise prediction of starch digestion in the various compartments of the digestive tract is essential for several reasons. Starch is a major source of energy for both the ruminal microbes and the host animal. The energetic efficiency of starch for the host animal is higher when digested in the small intestine as compared with ruminal and hindgut fermentation (Harmon and McLeod, 2001). The current DVE/OEB-2007 system considers two degradable fractions of starch: a slowly degradable fraction (D-starch; D-ST) and a fast degradable washable fraction (W-ST). Each fraction has its fractional degradation rate (Kd) and fractional passage rate (Kp). The Kd values of D-ST (Kd-D-ST; %/h) differ per feedstuff and are determined using the in situ nylon bag incubation technique in the rumen, and the Kd values of W-ST (Kd-W-ST; %/h) are a function of the Kd-D-ST values. The Kp of D-ST (Kp-D-ST; %/h) and the Kp of W-ST values (Kp-W-ST; %/h), on the other hand, are constant values. However, from scientific literature, it can be concluded that the Kp of particles is not fixed but depends on factors such as the feed intake level and the proportion of concentrate in the diet (Noziere et al., 2018). In addition, recent in vitro results indicate that differences between Kd-D-ST and Kd-W-ST may be lower than assumed in the current DVE/OEB-2007 system (De Jonge et al., 2015).

The main objective of this study was to improve the estimation of the amount of rumen fermented starch by establishing equations that predict Kp-D-ST and Kd-W-ST of a given feedstuff. To reach this objective, a dataset of digestion studies was composed in which ruminal starch degradation was measured. Then, based on CVB rumen degradation characteristics of feedstuffs, Kp-D-ST and Kp-W-ST values were estimated using pre-selected equations in such a way that differences between observed rumen fermented starch and model predicted rumen fermented starch were minimized.

2 Materials and Methods

2.1 Dataset

Literature research on digestibility trials was carried out in 2018, in which rumen digestibility of starch was recorded. This was carried out using the search terms "digestibility or digestion", "rumen", "dairy cattle or dairy cows". Only those studies were included in the meta-analysis dataset that: 1) were based on dairy cattle, 2) had recorded the dry matter intake, and 3) where for the various starch-containing feedstuffs that were included in the diet, in situ rumen degradation characteristics of starch could be predicted using CVB information. The literature research resulted in an original dataset with 84 studies from European countries (EU) and North America (NA) and included a total number of 395 observations (treatment means). A first selection was made by choosing only EU studies, because of the difference in nutritional characteristics between EU and NA diets, and so from the original dataset, 11 studies (44 observations) were retained. One study was left out because of negative values for starch degradation in the rumen and 3 studies were left out because there were no values for body weight (BW). The remaining 7 studies (29 observations) were used to develop equations for predicting Kp-D-ST and Kd-W-ST of a given feedstuff. The Kd-D-ST of individual feedstuffs in diets included in the dataset were set equal to currently used CVB values. The Kp-W-ST was calculated as Kp-fluid minus 3%/h, with Kp-fluid calculated with the INRA equation (Noziere et al., 2018): Kp-fluid (%/h)=5.35+2.18 x DMI (% of BW) - $3.71 \times CL^2$

Where BW = body weight, DMI is dry matter intake and CL is the concentrate ratio in the diet. The reduction of the Kp-fluid with 3%/h for the calculation of Kp-W-ST is done because it is assumed in the current DVE/OEB system that the passage rate of small particles is in between the passage rate of D-ST (6.00%/h) and rumen fluid (11%/h). An alternative way for calculating Kp-W-ST is to calculate it as the real average between the Kp-fluid, which is calculated as described above, and the predicted Kp-D-ST. This alternative approach has also been investigated in the current study.

A summary of the used EU-dataset is given in Table 1.

Animal characteristics	n	Mean	Std Dev	Min	Max
BW (kg)	29	624	20.8	599	658
Milk (kg/d)	22	23.5	7.66	13.0	33.2
DMI (kg/d)	29	17.0	3.47	9.8	21.6
DMI (%BW)	29	2.7	0.56	1.6	3.6
Diet characteristics					
Concentrate (% in DM)	29	44.3	17.06	17.0	65.0
OM (g/kg DM)	29	926	10.3	909	950
CP (g/kg DM)	29	174	37.1	134	293
NDF (g/kg DM)	29	382	61.3	287	557
Starch (g/kg DM)	29	180	66.6	39	268
Digestibility characteristics					
Total tract digestible starch (%)	23	97.9	2.31	90.4	100
Ruminally degradable starch (%)	29	87.9	5.8	71.3	95.4
Ruminally degradable starch (g/kg DM)	29	160	61.8	33	249

Table 1Overview of animal, diet, and starch digestibility characteristics for the
European dataset.

2.2 The DVE/OEB-2007 system

In the current DVE/OEB-2007 system, Kp-D-ST and Kp-W-ST have fixed values of 6.00%/h and 8.00%/h, respectively. Starch in roughages is assumed to behave like starch in concentrates, so that it is assumed that the Kp-D-ST of 6.00%/h is equal for roughages and concentrates. Further, the Kp-W-ST was assumed as being the average between the Kp of concentrate particles (6.00%/h) and the Kp of soluble nutrients (11.00%/h) (Van Duinkerken et al., 2011).

The Kd-W-ST is dependent on Kd-D-ST as follows: $\mbox{Kd-W-ST}\ (\%/h) = 2 \ x \ \mbox{Kd-D-ST}\ (\%/h) + 37.5$

The amount of starch fermented in the rumen (RFST; kg/cow/d) for a given feedstuff (RFST_i; kg/cow/d) is calculated/estimated by a function based on the two fractions (%D-ST and %W-ST) and the values of the fractional degradation rate (Kd, %/h) and fractional passage rate (Kp; %/h) of the feedstuff:

RFST_i (kg/d)=ST_i (kg/d) × [(D-ST(%) × Kd-D-ST (%/h))/(Kd-D-ST (%/h)+Kp-D-ST (%/h)) + (W-ST(%) × Kd-W-ST(%/h))/(Kd-W-ST (%/h)+Kp-W-ST(%/h))]

Where D-ST, W-ST, and Kd-D-ST for individual feedstuffs are the currently used CVB values and ST_i is the starch intake (kg/d) from the feedstuff i. The total dietary amount of RFST is calculated as the sum of the individual RFST_i amounts.

2.3 Equation development and evaluation

2.3.1 Optimization procedure

The main objective of the optimization procedure was to improve the estimation of individual and dietary RFST (calculated as described for the current DVE/OEB-2007 system) by establishing formulas that predict the Kp-D-ST of a given feedstuff and are based on dietary characteristics (e.g., concentrate level, type of starch (coarse or ground), and feed intake level). And by predicting Kd-W-ST of a given feedstuff either as constant value or dependent on the feed specific Kd-D-ST values of CVB, like in the current DVE/OEB system. However, there are no direct measurements of Kp-D-ST and Kd-W-ST (for individual feedstuffs) available in the dataset to compare the predicted Kp-D-ST and Kd-W-ST values. Therefore, the RFST of the diet that is calculated based on the model estimated Kp-D-ST and Kd-W-ST values is compared with the observed RFST of the diet in the dataset instead. The difference (residual error) between the estimated RFST (the result of the function) and the observed RFST was calculated. The model parameters in the models estimating Kp-D-ST and Kd-W-ST was calculated.

This minimization was done by using the "nlminb" optimization method in the "opm" function in the "optimx" package of the software program R (version 4.0.3). The "opm" function is a general-purpose optimization function that compares multiple optimization functions based on different optimization methods (e.g., "Nelder-Mead", "nlminb", "BFGS (a quasi-Newton method)", "nmkb"). The "nlminb" method was chosen based on preliminary optimization tests.

In a first approach, the Kp-D-ST-values were optimized for the roughages and concentrates separately. In a second approach, the Kp-D-ST-values were optimized without differentiation between roughages and concentrates. The Kp-D-ST-values were optimized for feedstuffs with coarse and finely ground starch separately in the third approach. The Kd-W-ST values were assumed to be equal for roughages and concentrates, and equal for coarse and finely ground starch.

The Kp-D-ST - and Kd-W-ST-values were estimated using the following models:

The first approach (separate Kp-D-ST for roughages and concentrates)

Model 1a: Kp-D-ST-roughages $(\%/h) = INT_r$ Kp-D-ST-concentrates $(\%/h) = INT_c$ Kd-W-ST (%/h) = INT_w Model 2a: Kp-D-ST-roughages (%/h) = $INT_r + B_0 \times FL$ Kp-D-ST-concentrates (%/h) = $INT_c + B_1 \times FL$ Kd-W-ST (%/h) = INT_w Model 3a: Kp-D-ST-roughages (%/h) = $INT_r + B_2 \times CL$ Kp-D-ST-concentrates (%/h) = $INT_c + B_3 \times CL$ Kd-W-ST = INTwModel 4a: Kp-D-ST-roughages (%/h) = $INT_r + B_0 \times FL + B_2 \times CL$ Kp-D-ST-concentrates (%/h) = $INT_c + B_1 \times FL + B_3 \times CL$ Kd-W-ST (%/h) = INT_w Model 5a: Kp-D-ST-roughages (%/h) = $INT_r + B_4 \times COR$ Kp-D-ST-concentrates $(\%/h) = INT_c + B_5 \times COR$ Kd-W-ST $(\%/h) = INT_w$ Model 6a: Kp-D-ST-roughages $(\%/h) = INT_r$ Kp-D-ST-concentrates (%/h) = INT_c Kd-W-ST (%/h) = $INT_w + B_6 \times Kd-D-ST$ Where: INTr is the intercept value for the Kp-D-ST equation for roughage feedstuffs; INT_c is the intercept value for the Kp-D-ST equation for concentrate feedstuffs; INT_w is the intercept value for the Kp-W-ST value; FL (%BW) is the feeding level calculated as a percentage of the body weight (BW; kg): FL (% of BW) = (DMI (kg/d)) / (BW (kg)) x 100 CL (ratio) is the concentrate level in the diet calculated as a ratio: $CL = \Sigma concentrate intake (kg DM/d)/DMI (kg/d)$ COR (ratio) is the ratio of coarse starch to total amount of starch in the diet. Kd-D-ST is the current CVB Kd-D-ST value (%/h). B₀ – B₆ are regression coefficients for FL, CL, COR and Kd-D-ST.

Ground starch was defined as: sum of starch from all sources of starch that were reported to be finely ground.

Coarse starch was defined as: sum of starch from all sources of starch that were processed by a method other than fine grinding (cracking, steam flaking, coarse grinding etc.) and starch from corn silage

The second approach (without separating roughage and concentrate starch)

Model 1b: Kp-D-ST(%/h) = INTKd-W-ST (%/h) = INT_w Model 2b: $Kp-D-ST(\%/h) = INT + B_7 \times FL$ $Kd-W-ST(\%/h) = INT_w$ Model 3b: $Kp-D-ST(\%/h) = INT + B_8 \times CL$ Kd-W-ST (%/h) = INT_w Model 4b: $Kp-D-ST(\%/h) = INT + B_7 \times FL + B_8 \times CL$ Kd-W-ST (%/h) = INT_w Model 5b: $Kp-D-ST(\%/h) = INT + B_9 \times COR$ $Kd-W-ST(\%/h) = INT_w$ Model 6b: Kp-D-ST(%/h) = INTKd-W-ST (%/h) = $INT_w + B_{10} \times Kd-D-ST$

Where: INT is the intercept value for the Kp -D-ST equation for all feedstuffs (%/h). $B_7 - B_{10}$ are regression coefficients for FL, CL, COR and Kd-D-ST.

The third approach (separate Kp-D-ST for coarse and finely ground starch)

Model 1c: Kp-D-ST-coarse (%/h) = INT_{cor} Kp-D-ST-ground (%/h) = INT_{grd} Kd-W-ST (%/h) = INT_w

Model 4c: Kp-D-ST-coarse (%/h) = $INT_{cor} + B_{11} \times FL + B_{13} \times CL$ Kp-D-ST-ground (%/h) = $INT_{grd} + B_{12} \times FL + B_{14} \times CL$ Kd-W-ST (%/h) = INT_w

Model 6c: Kp-D-ST-coarse (%/h) = INT_{cor} Kp-D-ST-ground (%/h) = INT_{grd} Kd-W-ST (%/h) = $INT_w + B_{15} \times Kd$ -D-ST

Where:

 INT_{cor} is the intercept value for the Kp -D-ST equation for feedstuffs with coarse starch; INT_{grd} is the intercept value for the Kp-D-ST equation for feedstuffs with finely ground starch; $B_{11} - B_{15}$ are regression coefficients for FL, CL, and Kd-D-ST.

2.3.2 Weighted averages of (estimated) Kp- and Kd-values

Weighted average values of the (estimated) Kp- and Kd-values were calculated by first multiplying the values for the individual feedstuffs with their relative starch amount in the diet. Next, the weighted values were summed up per diet. These diet values were then weighted according to the dietary starch amount relative to the average starch amount in the dataset. Finally, these weighted values were averaged.

2.3.3 Cross-validation and goodness-of-fit evaluation of the models

Cross-validation was carried out by optimizing the model on a training set of all data, excluding one study (leave one cluster out method). Then the fitted model was used to predict the RFST of the study's observations that were excluded (validation set). In this way, for all individual studies, the RFST was predicted instead of being estimated.

For evaluating the different models, the goodness of fit of the predicted RFST on the diet level, based on the cross-validation, was compared to observed values using two methods as described by Ellis et al. (2010). The first method consisted of calculating the mean square prediction error (MSPE) as follows:

 $\text{MSPE} = \sum_{i=1}^{n} (Oi - Pi)^2 / n$

Where n is the total number of observations, O_j is the observed RFST on diet level (of diet j), and P_j is the predicted RFST on diet level (of diet j). Thus, the square root of the MSPE (RMSPE), expressed as a percentage of the observed mean, estimates the overall relative prediction error. The RMSPE was further decomposed into error due to overall bias (ECT), error due to deviation of the regression slope from unity (ER), and error due to the disturbance (random error) (ED) (Bibby and Toutenburg, 1977). The second method consisted of calculating the concordance correlation coefficients (CCC) according to Lawrence and Lin (1989). The evaluation was done while accounting for a random study effect. Next to calculating goodness of fit parameters, the resulting average Kp-D-ST- and Kd-D-ST-values of the roughages and concentrates in the dataset were also calculated.

The same goodness-of-fit parameters were calculated to evaluate the current estimation of RFST in the DVE/OEB-2007 system and to evaluate the best models on North American studies/diets. A summary of the NA dataset is given in Appendix 1.

3 Results and discussion

3.1 Evaluation DVE/OEB-2007 calculation rules

Table 2 shows the goodness-of-fit parameters of the prediction of RFST in the current DVE/OEB-2007 system. The current DVE/OEB-2007 model performed well in predicting Kp-D-ST and Kd-D-ST as assessed by calculating the RFST and comparing the calculated values with the observed data in the EU dataset. While most of the prediction error was due to random error (ED=94.60%), current DVE/OEB-2007 model predictions resulted in high R² =0.95 and CCC=0.97 and relatively low RMSPE=9.05%. On the other hand, the current DVE/OEB-2007 model did not perform well in predicting RFST in the NA dataset with a considerably lower R²=0.43 and CCC=0.61 and a higher RMSPE of 31.41% and a considerable portion of the prediction error). Figure 1 gives the observed versus calculated/predicted RFST values of the DVE/OEB-2007 system for EU (on the left) and NA (on the right) dataset. For the EU dataset the slope is very close to 1 and the intercept very close to 0. For the NA dataset, the current DVE/OEB-2007 system calculation rules results in a moderate underprediction of RFST.

Table 2	Overall performance of the current DVE/OEB-2007 system for predicting rumen degraded
	starch (RFST, kg/d) using the EU and NA datasets.

	Data	aset
Model performance parameters ¹	EU	NA
RMSPE (% Mean observed)	9.05	31.41
ECT (% MSPE)	4.91	9.20
ER (% MSPE)	0.49	1.81
ED (% MSPE)	94.60	89.00
R ²	0.95	0.43
CCC	0.97	0.61

Overview parameters (%/h)²

Mean Kp-D-ST	6.00	6.00
Mean Kd-D-ST	28.01	9.05
Mean Kp-W-ST	8.00	8.00
Mean Kd-W-ST	93.51	55.61

¹RMSPE is the root mean square prediction error expressed as a percentage of the observed mean; ECT is the error due to bias, as a percentage of total MSPE; ER is the error due to deviation of the regression slope from unity, as a percentage of total MSPE; ED is the error due to disturbance (or random error), as a percentage of total MSPE; R² is the coefficient of determination; CCC is the Lin's Concordance correlation coefficient; AIC is the Akaike information criterion.

²Average weighted model values for roughages and concentrates with respect to Kp-D-ST, for Kd-W-ST, Kd-D-ST and Kp-W-ST.



Figure 1 Observed versus predicted or calculated values for rumen fermented starch (RFST; kg/cow/d) with the current DVE/OEB-2007 system for EU (left) and NA (right) dataset.

3.2 New models

Parameter estimates and goodness-of-fit characteristics for models 1–6 for the three approaches of the EU dataset are presented in Tables 3 (approach 1; separate Kp-D-ST for roughages and concentrates), 4 (approach 2; a single Kp-D-ST for both roughage and concentrate starch) and 5 (approach 3; separate Kp-D-ST for coarse and finely ground starch). In Table 6 the alternative calculation of Kp-W-ST is used for models 1a, 4a and 6a as the average between Kp-fluid and the predicted Kp-D-ST. This resulted in separate Kp-W-ST-values for roughages and concentrates.

Table 3Parameter estimates (±SE) and overall model performance for new equations (models 1a-6a; separate Kp-
D-ST for roughage and concentrate feedstuffs) for predicting rumen fermented starch (RFST, kg/cow/d)
using the EU dataset.

Coefficients ¹						
	1a	2a	3a	4a	5a	6a
INTr	0.36 ± 0.10	22.54±7.78	12.79±9.45	31.96±9.37	307.43±6e+05	3.62±1.00
INT _c	1.45 ± 0.10	-0.17±3.70	32.52±2.75	31.74±3.57	2.84 ± 0.35	2.95±0.49
B ₀ (FL _r)		-9.18±3.02		-18.09 ± 9.14		
B1 (FLc)		0.53±1.42		-0.03±1.05		
B ₂ (CL _r)			-52.35±46.98	60.06±70.20		
B ₃ (CL _c)			-50.71±4.85	-49.25±4.84		
B ₄ (COR _r)					-311±8e+04	
B ₅ (COR _c)					-31.21±4.53	
INT _w	31.34±1.32	30.13±8.14	47.36±6.21	47.83± 7.18	53.97±13.02	163±25
B ₆ (Kd-D-ST)						-2.92±0.61

Overview estimated parameters (%/h)²

Mean Kp-D-STr	0.36	-2.09	-10.95	10.66	223.43	3.62
Mean Kp-D-STc	1.45	1.25	9.51	9.32	-5.57	2.95
Mean Kd-W-ST	31.34	30.13	47.36	47.83	53.97	81.08
Mean Kd-D-ST	28.01	28.01	28.01	28.01	28.01	28.01
Mean Kp-W-ST	7.33	7.33	7.33	7.33	7.33	7.33

Model performance parameters³

RMSPE (% Mean observed)	9.94	14.57	10.69	12.17	17.49	10.74
ECT (% MSPE)	0.13	6.84	10.29	1.75	12.34	0.00
ER (% MSPE)	0.60	0.01	4.15	2.03	1.23	0.26
ED (% MSPE)	99.27	93.16	95.56	96.22	86.43	99.73
R ²	0.94	0.87	0.93	0.91	0.83	0.93
ССС	0.97	0.93	0.96	0.95	0.90	0.96
AIC	10	39	11	29	27	10

¹INT_r is the intercept value for the Kp-D-ST equation for roughages; INT_c is the intercept value for the Kp-D-ST equation for concentrates; FL (% of BW) is the effect of feeding level on Kp-D-ST calculated as a percentage of the body weight (BW; kg) for roughages (FL_r) and for concentrates (FL_c); CL (ratio) is the effect of concentrate level on Kp-D-ST for roughages (CL_r) and concentrates (CL_c) in the diet; COR (ratio) is the effect of the ratio coarse starch to the total amount of starch in the diet on Kp-D-ST for roughages (COR_r) and for concentrates (COR_c); INT_w is the intercept value for the Kp-W-ST value; Kd-D-ST is the estimated effect of current CVB Kd-D-ST values of feedstuffs (%/h) on Kp-W-ST.

²Average estimated and weighted model values for Kp-D-ST for roughages (Kp-D-ST_r) and concentrates (KP-D-ST_c) and for Kd-W-ST, Kd-D-ST and Kp-W-ST. ³RMSPE is the root mean square prediction error expressed as a percentage of the observed mean; ECT is the error due to bias, as a percentage of total MSPE; ER is the error due to deviation of the regression slope from unity, as a percentage of total MSPE; ED is the error due to disturbance (or random error), as a percentage of total MSPE; R² is the coefficient of determination; CCC is the Lin's Concordance correlation coefficient; AIC is the Akaike information criterion. All model performance parameters are based on cross-validation results.

Table 4Parameter estimates (±SE) and overall model performance for new equations (models 1b-6b;
no differentiation in Kp-D-ST between roughage and concentrate feedstuffs) for predicting
rumen fermented starch (RFST, kg/cow/d) using the EU dataset.

Coefficients ¹	Model						
	1b	2b	Зb	4b	5b	6b	
INT	0.61±0.54	-0.20±1.14	0.32±1.12	-0.08±1.93	1.60 ± 0.42	3.40±0.49	
B7 (FL)		0.37±0.40		0.23±0.85			
B ₈ (CL)			0.82±1.30	0.53±2.28			
B ₉ (COR)					-1.31±0.34		
INTw	29.34±7.25	30.90 ± 6.51	29.52±7.32	30.40±8.18	31.98±6.17	177±25	
B10 (Kd-D-ST)						-3.18±0.54	

Overview parameters²

Mean Kp-D-ST	0.61	0.80	0.69	0.78	1.25	3.40
Mean Kd-W-ST	29.34	30.90	29.52	30.40	31.98	87.98
Mean Kd-D-ST	28.01	28.01	28.01	28.01	28.01	28.01
Mean Kp-W-ST	7.33	7.33	7.33	7.33	7.33	7.33

Model performance parameters³

RMSPE (% Mean observed)	9.54	10.25	10.57	12.33	9.81	9.53
ECT (% MSPE)	0.09	0.10	0.004	0.04	0.29	0.004
ER (% MSPE)	0.22	1.32	0.11	0.88	0.85	0.68
ED (% MSPE)	99.69	98.58	99.89	99.08	98.86	99.32
R ²	0.94	0.93	0.93	0.90	0.94	0.94
ССС	0.97	0.96	0.96	0.95	0.97	0.97
AIC	8	12	12	20	9	6

¹INT is the intercept value for the Kp-D-ST equation for all the feedstuffs (%/h); FL (% of BW) is the feeding level calculated as a percentage of the body weight (BW; kg); CL (ratio) is the effect of concentrate level in the diet on Kp-D-ST; COR (ratio) is the effect of the ratio of coarse starch to total amount of starch in the diet on Kp-D-ST; INT_w is the intercept value for the Kp-W-ST value; Kd-D-ST is the estimated effect of current CVB Kd-D-ST values of feedstuffs (%/h) on Kp-W-ST.

²Average weighted estimated model values for Kp-D-ST, Kd-W-ST, Kd-D-ST and Kp-W-ST (estimates valid for both concentrate and roughage feedstuffs).

³RMSPE is the root mean square prediction error expressed as a percentage of the observed mean; ECT is the error due to bias, as a percentage of total MSPE; ER is the error due to deviation of the regression slope from unity, as a percentage of total MSPE; ED is the error due to disturbance (or random error), as a percentage of total MSPE; R² is the coefficient of determination; CCC is the Lin's Concordance correlation coefficient; AIC is the Akaike information criterion. All model performance parameters are based on cross-validation results.

Table 5Parameter estimates (±SE) and overall model performance for new equations (models 1c-
6c; separate Kp-D-ST for coarse (cor) and fine ground (grd) starch) for predicting rumen
degraded starch using the EU dataset.

Coefficients ¹	Model					
	1c	4c	6c			
INT _{cor}	0.37±0.53	39.11±18.77	3.62±1.00			
INT _{grd}	1.54±0.42	29.21±4.52	2.96±0.49			
B ₁₁ (FL _{cor})		-14.14±8.64				
B ₁₂ (FL _{grd})		0.15±1.41				
B ₁₃ (CL _{cor})		-13.32±12.63				
B ₁₄ (CL _{grd})		-45.13±4.45				
INTw	31.78±6.30	57.51±17.56	163.39 ± 25.30			
B ₁₅ (Kd-D-ST)			-2.93± 0.61			

Overview parameters²

Mean Kp-D-ST _{cor}	0.37	-4.87	3.62
Mean Kp-D-ST _{grd}	1.54	9.14	2.96
Mean Kd-W-ST	31.78	57.51	81.23
Mean Kd-D-ST	28.01	28.01	28.01
Mean Kp-W-ST	7.33	7.33	7.33

Model performance parameters³

RMSPE (% Mean observed)	9.89	49.38	10.73
ECT (% MSPE)	0.12	1.38	0.01
ER (% MSPE)	0.65	54.71	0.28
ED (% MSPE)	99.22	43.91	99.72
R ²	0.94	0.32	0.93
ссс	0.97	0.52	0.96
AIC	9	86	10

¹INT_{cor} is the intercept value for the Kp-D-ST equation for feedstuffs with coarse starch; INT_{grd} is the intercept value for the Kp-D-ST equation for feedstuffs with finely ground starch; FL (% of BW) is the effect of feeding level calculated as a percentage of the body weight (BW; kg) on Kp-D-ST of for feedstuffs with course starch and feedstuffs with finely ground starch; CL (ratio) is the effect of concentrate level in the diet on Kp-D-ST for feedstuffs with course starch and feedstuffs with finely ground starch; INT_w is the intercept value for the Kp-W-ST value; Kd-D-ST is the estimated effect of current CVB Kd-D-ST values of feedstuffs (%/h) on Kp-W-ST.

²Average weighted estimated model values for Kp-D-ST of course starch (Kp-D-ST_{cor}) and finely ground starch (Kp-D-ST_{grd}), for Kd-W-ST, Kd-D-ST and Kp-W-ST.

³RMSPE is the root mean square prediction error expressed as a percentage of the observed mean; ECT is the error due to bias, as a percentage of total MSPE; ER is the error due to deviation of the regression slope from unity, as a percentage of total MSPE; ED is the error due to disturbance (or random error), as a percentage of total MSPE; R² is the coefficient of determination; CCC is the Lin's Concordance correlation coefficient; AIC is the Akaike information criterion. All model performance parameters are based on cross-validation results.

Table 6Parameter estimates (±SE) and overall model performance for new equations (models 1a,
4a and 6a) for predicting rumen degraded starch (RFST, kg/cow/d) using the EU dataset and
with Kp-W-ST dependent on Kp-D-ST (calculated as the average of Kp-fluid and estimated
Kp-D-ST).

	Model			
	1a′	4a′	6a′	
INT _r	0.35±0.51	26.89±8.70	3.53±0.91	
INT _c	1.15±0.42	22.77±2.81	2.87±0.52	
B ₀ (FL _r)		-14.34±7.09		
B ₁ (FL _c)		0.42±1.05		
B ₂ (CL _r)		42.08±50.37		
B ₃ (CL _c)		-36.24±3.68		
INT _w	22.62±5.87	42.05±7.51	142.73±25.66	
B₅ (Kd-D-ST)			-2.57±0.59	
Overview parameters (%/h) ²				
Mean Kp-D-STr	0.35	7.50	3.53	
Mean Kp-D-ST _c	1.15	7.45	2.87	
Mean Kd-W-ST	22.62	42.05	70.78	
Mean Kd-D-ST	28.01	28.01	28.01	
Mean Kp-W-STr	5.34	8.92	6.93	
Mean Kp-W-STc	5.74	8.89	6.60	
Model performance parameters ³				
RMSPE (% Mean observed)	10.01	13.07	10.84	
ECT (% MSPE)	0.08	2.77	0.0003	
ER (% MSPE)	0.41	4.17	0.19	
ED (% MSPE)	99.51	93.06	99.81	
R ²	0.94	0.90	0.93	
ССС	0.97	0.95	0.96	
AIC	10	33	10	

¹INT_r is the intercept value for the Kp-D-ST equation for roughage feedstuffs; INT_c is the intercept value for the Kp-D-ST equation for concentrate feedstuffs; FL (% of BW) is the effect of feeding level on Kp-D-ST calculated as a percentage of the body weight (BW; kg) for roughages (FL_r) and for concentrates (FL_c); CL (ratio) is the effect of concentrate level on Kp-D-ST for roughages (CL_r) and concentrates (CL_c) in the diet; COR (ratio) is the effect of the ratio coarse starch to the total amount of starch in the diet on Kp-D-ST for roughages (COR_r) and for concentrates (COR_c); INT_w is the intercept value for the Kp-W-ST value; Kd-D-ST is the estimated effect of current CVB Kd-D-ST values of feedstuffs (%/h) on Kp-W-ST. ²Average estimated and weighted model values for Kp-D-ST for roughages (Kp-D-ST_r) and concentrates (KP-D-ST_c) and for Kd-W-ST, Kd-D-ST and Kp-W-ST.

²Average weighted estimated model values for Kp-D-ST for roughages (Kp-D-ST_r) and concentrates (KP-D-ST_c), for Kd-W-ST, Kd-D-ST and for Kp-W-ST for roughages (Kp-W-ST_r) and concentrates (Kp-W-ST_c).

³RMSPE is the root mean square prediction error expressed as a percentage of the observed mean; ECT is the error due to bias, as a percentage of total MSPE; ER is the error due to deviation of the regression slope from unity, as a percentage of total MSPE; ED is the error due to disturbance (or random error), as a percentage of total MSPE; R² is the coefficient of determination; CCC is the Lin's Concordance correlation coefficient; AIC is the Akaike information criterion. All model performance parameters are based on cross-validation results.



Figure 2 Observed versus predicted values of RFST (kg/cow/d) for model 1a (left) and model 6a (right) for the EU dataset.

The best goodness of fit parameters were obtained for models 1 and 6 for all 3 approaches. Comparing results from model 1 (for all three approaches) with results from model 6 (for all three approaches) shows that average estimated Kp-D-ST values are substantially lower for model 1 compared to model 6). Figure 2 gives the observed versus predicted values for models 1a and 6a.

Model 1 is the simplest model with for model 1a separate fixed Kp-D-ST values for roughages and concentrates; model 1b has a fixed Kp-D-ST for all feedstuffs, and model 1c has separate fixed Kp-D-ST for coarse and fine ground starch.

Model 2 has the intercept and FL (the feeding level calculated as a percentage of the body weight) as the predictor. In the first approach (a; separate Kp-D-ST for roughages and concentrates), model 2a resulted in a negative mean Kp-D-ST for roughages that is not biologically logical. Model 2b resulted in a very low value for Kp-D-ST. The DM intake effect is likely the outcome of its influence on the particulate outflow from the rumen, which competes with starch digestion. Offner and Sauvant (2004) calculated a theoretical value of Kp to achieve the best fit of the observed digestibility data in the rumen, and there was no distinction between roughage and concentrate. In this case, the effect of DM intake on calculated Kp was significant (regression slope = 0.030 ± 0.006). The model 2b approach in the present study is similar to the latter approach and still, the outcome is drastically different. Differences in obtained values are possibly because of the differences in methodology and the presence of the coefficient of Kd-W-ST (INT_w) in the models used in the present study. Anyhow, the negative sign of FL coefficients contradicts the general perception of a positive correlation between DMI and passage rate of digesta in the rumen.

Model 3 has an intercept and CL (the concentrate level in the diet) as explanatory variable for Kp-D-ST. In the first approach (a; separate Kp-D-ST for roughages and concentrates), the model resulted in a negative mean Kp-D-ST for roughages that is not biologically logical. Also, model 3b was not superior in predicting RFST, based on the overall model performance.

Model 4 has both CL and FL as predictors besides the intercept. This model has a higher RMSPE and lower R² value compared to models 1 and 6 in approach 1 (a models) and 2 (b models), but the resulting values for Kp-D-ST are higher in model 4a, even higher than the value for Kp-W-ST, which is not logical. The resulting value for Kp-D-ST in model 4b is almost as low as the value in model 1b.

When model 4a is modelled with the Kp-W-ST value dependent on the Kp-D-ST value (Table 6, model 4a') this results in average Kp-values which are very close to the values of the current DVE/OEB-2007 system. Figure 3 shows the observed and predicted values for RFST (kg/d) with models 4a and 4a'.



Figure 3 Observed versus predicted values for RFST (kg/cow/d) with model 4a (left) and model 4a' (right) for the EU dataset.

Model 5 has an intercept and the percentage of coarse starch as predictor. In the first approach this results in a very high value for Kp-D-ST for roughages and a negative value for Kp-D-ST for concentrates (model 5a, Table 1), which is not logical. In the second approach (Table 4) the value for Kp-D-ST is more logical and in between the value that is the result of model 1b and model 6b. In model 6, with all three approaches, there was an intercept and Kd-D-ST (CVB values) as explanatory variables for Kd-W-ST.

The relatively low RMSPE and AIC values and high R² and CCC values for models 1 and 6 for all three approached suggest that these models are potential candidates for predicting Kp-D-ST and RFST in an updated DVE/OEB system.

The current DVE/OEB-2007 calculation rules performed well in predicting Kp-D-ST as assessed by calculating the RFST and evaluating the calculated values against the observed data in the EU dataset. Also none of the tested models performed better than the current DVE/OEB-2007 system based on the overall model performance parameters. Neither the DVE/OEB-2007 system (Table 2) nor the new equations (Table 8) could properly predict RFST in the NA dataset.

It is remarkable that both the DVE/OEB-2007 calculation rules for Kp-D-ST and Kd-W-ST and the estimated Kp-D-ST and Kd-W-ST values in this study (for models 1 and 6) result in similar predicted RFST values and similar goodness-of-fit characteristics as values for Kd-W-ST and Kp-D-ST differ substantially. For example, the mean calculated Kd-W-ST for the EU dataset is 93.5 %/h when using DVE/OEB-2007 calculation rules (Table 2) whereas Kd-W-ST ranges from 29.3 to 31.8 %/h when using the results of models 1a, 1b and 1c in this study. Furthermore, the Kp-W-ST is 6.00 %/h for both roughages and concentrates when using DVE/OEB-2007 calculation rules (Table 2) and is estimated to be 0.36 and 1.45 %/h for roughages and concentrate feedstuffs, respectively in model 1a or 0.61%/h for both roughages and concentrate feedstuffs in model 1b.

The fact that both the DVE/OEB-2007 calculation rules and the model results in this study result in similar predicted RFST values and goodness-of-fit characteristics while having substantial differences in Kd-W-ST and Kp-D-ST values shows that the higher calculated rumen fermentation of W-ST using the DVE/OEB-2007 calculation rule is offset by a reduced rumen fermentation of D-ST due to a higher rumen passage rate as compared to using model outcomes in this study. This is an indication that the dataset used in the present study to estimate Kp-D-ST and Kd-W-ST values for roughages and concentrate feedstuffs lacks the necessary contrasts in dietary W-ST and Kd-D-ST levels.

When comparing the differences between Kd-D-ST and predicted Kd-W-ST for both the DVE/OEB-2007 system and the results from models tested in this study it appears that for the DVE/OEB-2007 system and using the EU dataset the average Kd-W-ST : Kd-D-ST ratio is (93.51 : 28.01) = 3.3 (this ratio of 3.3 is the consequence of applying the calculation rule: Kd-W-ST (%/h) = $37.5 + 2 \times \text{Kd-D-ST}$ (%/h)) whereas for model 1a the average Kd-W-ST : Kd-D-ST ratio is (31.34 : 28.01) = 1.1. In a study by de Jonge et al. (2015) the in vitro starch degradation rates of 6 concentrate feedstuffs (barley, faba beans, maize, oats, peas and wheat) were determined for the total feed ingredients and for the D-ST fraction of the ingredients (residue after using the wool wash procedure to remove the W-ST fraction). Based on the difference between in vitro degradation of the total ingredient and the D-ST fraction the Kd-W-ST was calculated. It appeared that Kd-W-ST : kd-D-ST ratios ranged from 0.96 for oats to 2.39 for maize with an average ratio of the 6 ingredients of 1.59. The average Kd-W-ST : kd-D-ST ratio for model 1a of 1.1 (a ratio value which is similar to ratio values for models 1b and 1c) seems more in line with ratio's found by de Jong et al. (2015) than the average ratio value of 3.3 when using the DVE/OEB-2007 calculation rules.

With respect to scientific literature on Kp of starch there are some studies, employing rumen evacuation techniques (studies not included in the dataset), reporting a broad range of starch Kp values in the rumen. For instance, Oba and Allen (2003) utilized the rumen evacuation technique in a duplicated 4×4 Latin square design with a 2 \times 2 factorial arrangement of treatments, involving either high moisture maize or dried ground maize at two dietary starch concentrations. They reported starch Kp values ranging from 13.9 to 21.2 %/h and starch Kd values ranging from 12.2 to 28.2 %/h resulting in 45.9 – 71.1% of starch digested in the rumen. Voelker and Allen (2003) gradually replaced high moisture maize with sugar beet pulp in a duplicated 4×4 Latin square design. They reported starch Kp values ranging from 15.9 to 23.5 %/h and starch Kd values ranging from 1.92 to 11.3 %/h resulting in 16.9 – 46.5% of starch digested in the rumen. Most of these reported Kp values are substantially higher than expected Kp-fluid rates of around 11%/h and it seems unlikely for Kp-W-ST to be higher than Kp-fluid.

It is concluded that current DVE/OEB-2007 calculation rules perform well in predicting RFST for the EU data. It is furthermore concluded that models 1 and 6 (for all three approaches) may be considered as potential candidates for predicting Kp-D-ST and Kd-W-ST in an updated DVE/OEB system. It is furthermore concluded that both the current DVE/OEB-2007 calculation rules and models 1 – 6 evaluated in this study could explain substantially more variation in RFST for the EU dataset compared to the NA dataset.

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Appendix 1 Summary North American (NA) dataset

An overview of the animal, diet and starch digestibility characteristics for the North American (NA) dataset is given in table 7. This dataset is very different from the EU dataset (Table 1), and diets in the NA dataset had markedly higher starch and lower NDF content. Both RFST and total tract digestible starch were lower in NA compared with the EU dataset. Overall model performance for models 1a, 1b and 6a for predicting rumen degraded starch (models fitted on the EU dataset) was evaluated against the NA dataset. These results are presented in table 8. Figure 4 shows the observed versus predicted values of NA dataset with model 1a (left) and model 6a (right). Overall performance of the models in predicting Kp-D-ST as assessed by calculating the RFST was poor, as indicated by relatively high RMSPE and AIC and low R² and CCC. Between the different models the change in RMSPE and R² seems not logical, as both RMSPE and R² increase from model 1a to model 1b. The probable explanation is a shift in error type from random error (ED) to an overall bias (ECT). In addition, none of the tested new equations (1a, 1b, and 6a) performed better than the current DVE model in predicting Kp-D-ST and RFST in the NA dataset (Table 1 vs. Table 8).

Table 7Overview of the animal, diet and starch digestibility characteristics for the North American
(NA) dataset.

Animal characteristics	n	Mean	Std Dev	Min	Max
BW (kg)	87	626	43.6	506	697
Milk (kg/d)	76	31.3	5.81	18.2	42.5
DMI (kg/d)	87	21.6	2.05	15.5	25.5
DMI (%BW)	87	3.47	0.35	2.49	4.24
Diet characteristics					
Concentrate (% in DM)	87	55.4	8.9	30.7	77.0
OM (g/kg DM)	87	913	10.8	884	931
CP (g/kg DM)	87	176	14.7	160	231
NDF (g/kg DM)	87	320	45.7	222	479
Starch (g/kg DM)	87	258	64.9	50	368
Digestibility characteristics					
Total tract digestible starch (%)	83	93.5	5.23	77.8	99.9
Ruminally degradable starch (%)	87	59.9	17.47	9.7	96.7
Ruminally degradable starch (g/kg DM)	87	154.8	57.74	15.1	263.0

Table 8Parameter estimates and overall model performance for new equations (models 1a, 1b and 6a)
(fitted on EU dataset) for predicting rumen fermented starch (RFST, kg/cow/d) using the North
American (NA) dataset.

	Model		
Coefficients ¹	1a	1b	6a
INTr	0.36		3.62
INT _c	1.45		2.95
INT		0.61	
INTw	31.34	29.34	163
B ₆ (Kd-D-ST)			-2.92
Overview parameters ²			
Mean Kd-D-ST	9.05	9.05	9.05
Mean Kp-W-ST	11.74	11.74	11.74

Model performance parameters³

RMSPE (% Mean observed)	40.48	49.11	36.06
ECT (% MSPE)	50.52	66.10	26.04
ER (% MSPE)	2.43	3.04	4.87
ED (% MSPE)	47.05	30.86	69.09
R ²	0.50	0.52	0.42
CCC	0.54	0.46	0.57
AIC	185	183	197

¹INT is the intercept value for the Kp-D-ST equation for all the feedstuffs (%/h); INT is the intercept value for the Kp-D-ST equation for roughages; INT_c is the intercept value for the Kp-D-ST equation for concentrates; INT_w is the intercept value for the Kp-W-ST value; Kd-D-ST is the estimated effect of current CVB Kd-D-ST values of feedstuffs (%/h) on Kp-W-ST. ²Average estimated and weighted model values for Kp-D-ST for both roughages and concentrate feedstuffs (Kp-D-ST), Kp-D-ST for roughages (Kp-D-ST) and concentrates (KP-D-ST) and for Kd-W-ST, Rd-D-ST and Kp-W-ST. ³RMSPE is the root mean square prediction error expressed as a percentage of the observed mean; ECT is the error due to

³RMSPE is the root mean square prediction error expressed as a percentage of the observed mean; ECT is the error due to bias, as a percentage of total MSPE; ER is the error due to deviation of the regression slope from unity, as a percentage of total MSPE; ED is the error due to disturbance (or random error), as a percentage of total MSPE; R² is the coefficient of determination; CCC is the Lin's Concordance correlation coefficient; AIC is the Akaike information criterion. All model performance parameters are based on cross-validation results.



Figure 4 Observed versus predicted values for RFST (kg/cow/d) with model 1a (left) and model 6a' (right) for the NA dataset.

To explore the potential of nature to improve the quality of life



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