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Predicting fibre digestibility in Holstein dairy cows fed dry-hay-based rations through machine learning

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ABSTRACT

Calculating the requirements and predicting the feed digestibility are essential to building robust dairy cattle rationing programmes. In the field, a huge number of *in vivo* observations are needed to develop accurate equations and reliable predictions. The aim of this study was to develop an equation to estimate total-tract potentially digestible NDF digestibility (**TTpdNDFD**) for lactating cows fed hay-based rations. Individual data from 11 studies, 69 cows, 35 different treatments, and 1 614 observations were included in this study. To develop the prediction equation, the following traits, descriptors of the total mixed ration, were used: ash, starch, CP, NDF, acid detergent fibre, acid detergent lignin, undegradable NDF and potential degradable NDF. Before building the equation with bidirectional stepwise selection in the JMP software, outliers were removed and multicollinearity was checked for all the predictors of fibre digestibility. The model was trained with 10-folds cross-validation. Results showed an R^2 of 0.91 and 0.90, and a RMSE of 2.99 and 3.26 in the model for training and validation, respectively. The promising performance of the model suggested that, the fibre digestibility in lactating dairy cows fed dry-hay-based rations can be accurately predicted in advance just by using the diet characteristics. From the obtained equation, we predicted the weight and slope of the included covariates, and outcomes confirm that in general the TTpdNDFD is reduced as dietary starch and fast-fermentable fibre increase. This study found that the equation extracted from a neural network, when combined with precision farming techniques, can improve the management of lactating cows and optimise feed planning, monitoring, and cost. It can be used in areas where silages are not used in rations. This provides evidence that accurate equations can be developed from historical data for precision feeding implementation. Further research is needed to expand the dataset and develop equations that can be applied on a large scale. Improving accuracy would involve incorporating representative data from other areas with similar diets into the training set.

Implications

Feeding cows with an adequate diet is pivotal to guarantee optimal animal welfare conditions, cover the requirements, and sustain the performances. The importance of providing a fast and reliable tool to predict in the field ration digestibility in lactating dairy cows fed hay-based rations is well-known. Throughout the present study, we propose a preliminary equation for the prediction of cows' digestibility to support nutritionists and rationing programmers with this effort. Maximising ruminants' diet digestibility is recommended to avoid inefficient carbon use and will allow for a smarter allocation the feedstuffs and nutrients, reducing the feed costs and pollutant excretion.

Introduction

Meeting the nutrient requirements of dairy cows is the key goal of a feeding programme. To build a rationing programme, data on fibre digestibility are needed, as the main aspect influencing ration utilisation in lactating dairy cattle is the relationship between nutrient content and digestibility (Nicholson et al., 2019; Cavallini et al., 2023a; 2023b).

In lactating dairy cows, the digestibility of fibre is a complex process that is influenced by various factors. One of the most significant determinants of fibre digestibility is the feed lignin

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content. Lignin is a complex and indigestible molecule that is present in plant cell walls and can limit the availability of other nutrients to the cow's digestive system (Raffrenato et al., 2017). Therefore, high lignin content in the feed can reduce the fibre digestibility in lactating dairy cows. However, it is important to note that unbalanced nutrient proportions can also limit fibre digestibility in lactating dairy cows (Miller et al., 2021). For example, if the diet is deficient in protein or minerals, the cow's ability to digest fibre may be reduced, as these nutrients are essential for the proper functioning of the rumen microorganisms that break down fibre (Palmonari et al., 2023). On the other hand, excesses of certain nutrients, such as carbohydrates and fats, can also negatively impact fibre digestibility (Weimer et al., 2010; Calsamiglia et al., 2012). Therefore, it is crucial to ensure that the nutrient composition of the feed is balanced and adequate for the animal's needs to maximise fibre digestibility and overall health and productivity of the lactating dairy cow (Raffrenato et al., 2019; NASEM, 2021).

Kendall et al. (2009) considered the outcome of NDF digestibility on intake and milk production and reported an enhancement in DM intake (**DMI**) and milk yield when *in vitro* NDF digestibility of the rations augmented (complete abbreviation list is reported in Table 1). Other studies (Oba and Allen, 1999; Fustini et al., 2017; Miller et al., 2021) assessed the association of NDF digestibility and cow intake and performance with rations based on different sources of forages.

Further, intake level effects on diet digestibility are not constant but depend on diet composition and particle size of forages (Allen, 2000; Fustini et al., 2011; Bonfante et al., 2016). Higher intake increases the passage rate of feed and, thus, depresses ruminal fibre degradation (Raffrenato et al., 2019). Consequently, associative effects between dietary components combined with feeding level responses lead to a significant overprediction of energy intake, when the digestibility values determined at a maintenance level of feeding are applied to dairy cows fed mixed diets at high levels of intake (de Souza et al., 2018). However, a clear understanding of dietary factors associated to fibre digestibility, and their interactions, are still lacking in hay-based rations. The equations used by the National Research Council - Nutrient Requirements of Dairy Cattle (**NRC**) (NRC, 2001), NASEM (2021), Cornell Net Carbohydrate and Protein System (**CNCPS**) (Higgs et al., 2015; Raffrenato et al., 2019), and NorFor (Huhtanen et al., 2009) to estimate fibre digestibility are based on silage-based rations or pasture-based rations (Dineen et al., 2021c, 2021b; 2021a).

These equations may not adequately predict the digestibility of dry-hay diets that are finely chopped, such as those adopted in the Parmigiano Reggiano (**PR**) consortium area, and in other parts of the world where silages are sporadically included or not allowed in dairy cows' nutrition. The PR product specification indicates that dietary inclusion of silages is not permitted, and forages must be included at least 40–50% of dietary DM (Righi et al., 2016a; Buonaiuto et al., 2021b). Alfalfa hay represents one of the main forage sources in the rations, and it is well-known that the forage maturity and quality can impact digestibility and cow performance (Palmonari et al., 2014, 2016; Fustini et al., 2017).

Table 1
Abbreviations list used in the Italian Holstein dairy cow study.

Abbreviation	Full name
OM	Organic matter
aNDFom	Amylase NDF organic matter corrected
uNDF24	Undegradable NDF after 24 h *in vitro* fermentation
uNDF240	Undegradable NDF after 240 h *in vitro* fermentation
pdNDF24	Potentially degradable NDF after 24 h *in vitro* fermentation
pdNDF240	Potentially degradable NDF after 240 h *in vitro* fermentation
peNDF	Physically effective NDF
TTpdNDFD	Total-tract potentially digestible NDF digestibility

The objective of this study was to develop an equation to estimate total-tract potentially digestible NDF digestibility of DM (**TTpdNDFD**) using recent data derived from individual observations of high-producing lactating cows fed hay-based rations. The final scope is to provide practicing nutritionists with an easy and fast tool to estimate the TTpdNDFD on the field and using the common rationing programmes.

Preliminary results from our study have been previously published in abstract form (Cavallini et al., 2022b).

Material and methods

The data employed in the current research were obtained from individual observations from 11 studies conducted at the University of Bologna Dairy Research Unit. The entire database contained 1 614 observations from 69 cows on 35 different treatments obtained from 11 experimental trials whose details are reported in Table 2.

In this database, eight cows participated to more than one experimental trial, but at different lactations and years. This means that, both within and across experiments, repeated measurements were present. Additionally, the large selection of experimental traits, and therefore dietary treatments, allows providing enough variability and representativeness in the dataset. This can help to ensure that the results are robust and not overly influenced by any one particular treatment or study design. By including a diverse range of studies in the analysis, the authors are able to capture a broader range of data and potentially improve the generalisability of the results. This can help to increase confidence in the conclusions drawn from the analysis and provide more insights into the overall relationship between the different covariates and the outcomes of interest.

Descriptive statistics of the data used in the present study are depicted in Table 3. On average cows had 630 kg of BW, daily produced 37 kg of milk with ~140 days in milk, and the DMI was 26 kg.

The forage sources in all cases were mainly alfalfa hay, meadow grass hay (Table 3), wheat hay, and wheat/barley straw. The diets contained different amounts of each forage source in various combinations, ensuring enough variability/representativeness for the purpose of this study. Total Mixed Ration (**TMR**) preparation was accurate, and the hay was finely chopped to avoid selection by the cows.

The variables included in the database were the TMRs' nutrients compositions: ash, organic matter (**OM**), starch, CP, amylase NDF corrected for the OM (**aNDFom**), NDF from forage (**fNDF**), ADF, ADL, potentially digestible NDF after 24 and 240 hours of *in vitro* fermentation (**pdNDF** 24 and 240), undegradable NDF after 24 and 240 hours of *in vitro* fermentation (**uNDF** 24 and 240), physically effective fibre (**peNDF**). More details regarding the chemical analyses are reported in previous studies (Buonaiuto et al., 2021a; Mammi et al., 2022). Among the 11 studies, the methods for total-tract digestibility determination followed the same protocol and methodology and used the uNDF240 as an internal marker and sampling at the minimum and maximum excretion rate in order to have representative values (Cavallini et al., 2023a).

The average observed total-tract digestibility was 76.38% pdNDF240 (1 614 records). The TMRs included in the dataset were characterised by 44% DM of forages on average, the main forage was alfalfa (20% DM), followed by meadow grass hay (16% DM), wheat hay and straw (~3% DM). Included cereals were mainly corn (10–12%) and sorghum (10–12% DM) and fibrous by-products were low represented (<15% DM). TMRs had an average starch of 23%

Table 2
Overview of the 11 experimental designs whose data were merged and used for the present Italian Holstein dairy cow research.

Study	No. of cows[1]		No. dietary treatments	Experimental design	Season
	Primiparous	Multiparous			
Fustini et al. (2016)	0	8	2	Cross Over	Spring
Fustini et al. (2017)	0	8	4	Latin Square	Winter-Spring
Cavallini et al. (2018)	0	8	4	Latin Square	Fall-Winter
Palmonari et al. (2018)	4	3	2	Cross Over	Winter-Spring
Cavallini et al. (2019)	0	8	4	Latin Square	Fall-Winter
Cavallini et al. (2020a)	4	4	4	Latin Square	Winter-Spring
Cavallini et al. (2020b)	3	5	4	Latin Square	Fall-Winter
Cavallini et al. (2021a)	0	8	4	Latin Square	Spring
Mammi et al. (2022)	4	4	4	Latin Square	Fall-Winter
Cavallini et al. (2023a)	0	4	1	Longitudinal	Winter
Unpublished data	7	1	4	Latin Square	Winter-Spring

[1] Only Holstein cows were present.

Table 3
Mean, SD, and 95% CI of animal characteristics, total-tract digestibility, and diet ingredients and chemical composition calculated on the whole Italian Holstein dairy cow dataset.

Variable	Mean	SD	95% CI	
			Upper	Lower
Animal characteristics				
BW, kg	629.9	65.82	633.1	626.7
Milk yield, kg/d	36.92	6.750	37.25	36.60
DIM	139.0	48.05	83.02	199.3
DMI, kg/d	25.94	4.002	26.13	25.13
DMI, %BW	4.140	0.610	4.172	4.110
TTpdNDFD				
%pdNDF240	76.38	8.870	76.81	75.95
TMR ingredients, %DM				
F:C[1]	43.71	5.640	43.98	43.44
Alfalfa	20.26	15.01	20.98	19.53
Meadow grass hay[2]	16.42	16.14	17.20	15.64
Wheat hay	3.250	8.710	3.670	2.830
Straw	3.304	3.760	3.480	3.120
TMR nutrients, %DM				
OM	92.79	0.942	92.85	92.74
Starch	23.12	2.650	23.26	22.99
CP	14.41	0.893	14.46	14.37
NDF	35.34	2.970	35.49	35.19
peNDF	16.95	3.271	14.88	19.86
ADF	25.51	2.671	25.65	25.38
ADL	5.160	0.980	5.210	5.110
uNDF24	20.44	3.390	20.62	20.27
pdNDF24	14.81	2.503	14.94	14.68
uNDF240	12.12	2.372	12.24	12.00
pdNDF240	23.32	1.781	23.41	23.23

Abbreviations: CI = Confidence interval; DIM = Days in milk; DMI = DM intake; TTpdNDFD = Total-tract potentially digestible NDF digestibility, expressed as percentage of total potentially digestible NDF; TMR = Total Mixed Ration; F:C = Forage and concentrate ratio; OM = Organic matter; aNDFom = Amylase NDF organic matter corrected; peNDF = physically effective NDF; uNDF24 = undegradable NDF after 24 h *in vitro* fermentation; pdNDF24 = potentially degradable NDF after 24 h *in vitro* fermentation; uNDF240 = undegradable NDF after 240 h *in vitro* fermentation; pdNDF240 = potentially degradable NDF after 240 h *in vitro* fermentation.

[1] Used concentrates are fibrous feeds (beet pulp, wheat bran, soybean hulls, <15% DM), starchy concentrates (corn, sorghum, 20–25% DM, below the EU maximum tolerable level (Girolami et al., 2022)), proteic concentrates (heat-treated soybean meal, rapeseed meal and sunflower meal, 5–10% DM), liquid feeds (cane and beet molasses, 1–2% DM), minerals & vitamins (1% DM).

[2] Quality and composition checked according to (Cavallini et al., 2022c) and resulted as a mixture of grasses: Italian ryegrass (*Lolium multiflorum*), perennial ryegrass (*Lolium perenne*), wild oats (*Avena fatua*); with a small presence of legumes species: alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), white clover (*Trifolium repens*) (Giorgino et al., 2023).

DM, CP of 14%DM, NDF of 35% DM, and peNDF of 17% DM. The degradability of the fibre was 15 and 23% DM for pdNDF24 and pdNDF240, respectively.

All statistical analyses were performed using JMP pro v. 16 (SAS Institute Inc., Cary, NC). Before developing the prediction model, TTpdNDFD phenotypes were adjusted through a general linear model (**GLM**) to correct for the following fixed effects: cow within lactation, DIM, and BW.

The model was expressed as

$$Y_{gjkn} = \mu + C_g \times L_j + D_k + B_n + \varepsilon_{gjkn}$$

where Y is the vector of observations for TTpdNDFD; μ is the overall intercept of the model; C is the cow of the gth level; L is the lactation of the j^{th} level; D is the average DMI of the k^{th} level; B is the BW of the n^{th} level; and $\mathcal{E}$ is the random residual term.

Subsequentially, observations with studentised residuals greater than ±3.5 were considered as outliers and removed (<1% of data). All the TMR characteristics (i.e., TMR nutrients composition) included in the equation as predictors of TTpdNDFD were jointly checked for multicollinearity; in particular, variance inflation factors (**VIF**) and Pearson r were calculated to identify the predictors that inflate the variances of the parameter estimates due to collinearity. The VIF is calculated for each trait using regression analysis as follows:

$$VIF_j = \frac{1}{(1 - R_j^2)}$$

where R_j^2 obtained when the predictor j is regressed on all the other predictors. When traits were highly correlated ($r \geq 36\%$ (Raspa et al., 2020)) or their VIF was $\geq$10, the covariate with lower interest was removed from the analysis. From a practical perspective, covariates that are more challenging to determine, costly to measure, or less accurate in their measurement may be considered of lower interest in the analysis. This process was iterated until all covariates in the model presented a VIF < 10.

Subsequently, the model containing the selected predictors (F:C, OM, CP, starch, NDF, fNDF, NDF/ADF ratio, pdNDF24, uNDF240 and peNDF) was trained with a bidirectional stepwise that combines forward and backward variables selection, tests interactions, and chooses the most significant predictors and combinations of predictors according to the significance in the model ($P < 0.05$). Predictors that satisfy the *P*-value threshold within the loop were allowed to enter, or otherwise removed from the model. The decision to use a supervised approach was undertaken in order to create a model based on easily measurable nutritional variables, and that can be of practical use for both nutritionists and farmers. Variables resulting from the bidirectional stepwise procedure were: F:C, OM, CP, starch, NDF, pdNDF24, and uNDF240.

The obtained predictors and adjusted TTpdNDFD have been analysed with two approaches: a traditional meta-analytic approach, the GLM, and a machine learning-based procedure, the neural network (**NN**). The NN are popular machine learning algorithms that recognise patterns and hidden relationships in the data, based on the structure and function of biological neurons. The NN has layers of interconnected nodes that process data using an activation function and produce output; in this study, three activation functions were tested in JMP software, namely Hyperbolic tangent, Linear and Gaussian. During the model training, the NN adjusts the weights and biases of each node to minimise error using backpropagation and an optimisation algorithm. Once trained, NN can make predictions on external data by processing it through the network (Fernández et al., 2006).

In this study, in fact, the entire data set was repeatedly partitioned into a training (90%) and a testing set (10%). Parameters estimated from the training data set were used for prediction in the test data set. In NN, rows with missing values are ignored. The key performance metric used to evaluate the model(s) was the correlation between predicted and actual values in the test data set. Data were divided into 10-folds so that, in turn, each fold was used for validating the model developed using the rest of the data, fitting a total of 10 models.

The final model chosen by the NN was the one showing the best average performance in validation. For the chosen model, the following fit statistics metrics in both training and validation were evaluated: R^2, RMSE, mean absolute deviation (**MAD**), Log Likelihood (−**LogL**), and the sum of square due to the error. Visually, we plotted the prediction patterns and the diagram equation (Fernández et al., 2006).

As regards the GLM, the model performance consisted in the overall R^2 and RMSE; records were processed altogether without splitting for the testing step.

Results

TTpdNDFD values were adjusted for the effects of cow within lactation, DIM, and BW to account for individual variability. All of the fixed effects were found to be highly significant and were useful to account for the variance of the trait.

In the first step of the statistical analysis, we assessed multicollinearity and correlation between the TMR traits used as predictors. The variables ADF, ADL, pdNDF240 and uDNF24 were removed because they were strongly significantly correlated ($P < 0.05$) to other variables of greater interest. For example, ADL and pdNDF240 were removed to keep uNDF240 in the model and uNDF24 was removed to keep pdNDF24. For the TTpdNDFD equation, the bidirectional stepwise procedure resulted in the following list of interesting covariates: F:C, OM, CP, starch, NDF, pdNDF24, and uNDF240.

The conventional approach, GLM, resulted in a moderate R^2 (0.55) and in a RMSE of 5.97, with normally distributed residuals. Table 4 presents the obtained summary statics of the NN for TTpdNDFD prediction. The best model in training resulted in R^2 of 0.91 and in RMSE of 2.99 (% pdNDF), respectively, values close to the ones obtained in validation: 0.90 and 3.26 for R^2 and RMSE (% pdNDF), respectively.

The final prediction equation obtained for TTpdNDFD resulted as:

$$TTpdNDFD = 74.45 + 4.72 \ast Eq1 \pm 5.23 \ast Eq2 \pm 9.09 \ast Eq3$$

where three sub-equations, or sub-models, were included. In particular, each sub-model was characterised by different intercepts and slopes for the selected covariate, as follows:

$$\begin{aligned} Eq1 = TanH(0.5 \ast (&-243.41 - 0.86 \ast OM - 7.08 \ast uNDF240 \\ &+ 1.69 \ast F:C + 5.23 \ast starch + 2.82 \ast aNDFom \\ &+ 10.68 \ast CP - 2.36 \ast pdNDF24)) \end{aligned}$$

$$\begin{aligned} Eq2 = TanH(0.5 \ast (&1347.48 - 14.79 \ast OM - 1.15 \\ &\ast uNDF240 - F:C + 1.24 \ast starch - 0.42 \\ &\ast aNDFom + 3.48 \ast CP - 1.37 \ast pdNDF24)) \end{aligned}$$

$$\begin{aligned} Eq3 = TanH(0.5 \ast (&358.66 - 4.24 \ast OM + 0.43 \ast uNDF240 \\ &+ 0.26 \ast F:C + 0.57 \ast starch + 0.36 \ast aNDFom \\ &-0.78 \ast CP + 0.19 \ast pdNDF24)) \end{aligned}$$

where TanH is Hyperbolic tangent function, OM is the TMR organic matter (% of DM), uNDF240 is the TMR undegradable NDF after 240 h of *in vitro* fermentation (% of DM), F:C is the TMR forage concentrate ratio (% of DM), starch is the TMR starch (% of DM), NDF is the TMR amylase treated NDF corrected for the organic matter (% of DM), CP is the TMR CP (% of DM), and pdNDF24 is the TMR potentially digestible NDF after 24 h of *in vitro* fermentation (% of DM).

The model is graphically showed as diagram in Fig. 1; profiler curves are reported in Fig. 2 where red crosses stand for the overall average TTpdNDFD value (i.e. 85.16% pdNDF240) on the curve.

We report the predictive performance of the model in Table 4. Overall, the model was promising, and resulted with better fit than the GLM, with R^2 of 0.89 and RMSE at 2.73; the residuals were normally distributed. Final model slope SE was 0.76 and intercept SE was 0.01. Plotting measured *vs* predicted values mean bias and slope bias resulted 2.01 and 0.76, respectively. The visual represen-

Table 4
Summary statistics of the neural web model in training and validation of the equation for predicting the TTpdNDFD (Total-Tract potentially digestible NDF Digestiblity) in Italian Holstein Dairy Cows.

Measures	Training	Validation
No. records	90%	10%
R^2	0.91	0.90
RMSE	2.99	3.26
Relative RMSE	4.3%	4.7%
MAD	2.28	2.55
−LogL	2 370.86	270.52
SSE	8 429.85	1 106.31

Abbreviations: Relative RSME = as percentage of observed mean; MAD = Mean absolute deviation; −LogL = Negative Log Likelihood; SSE = Sum of squared estimate of errors.

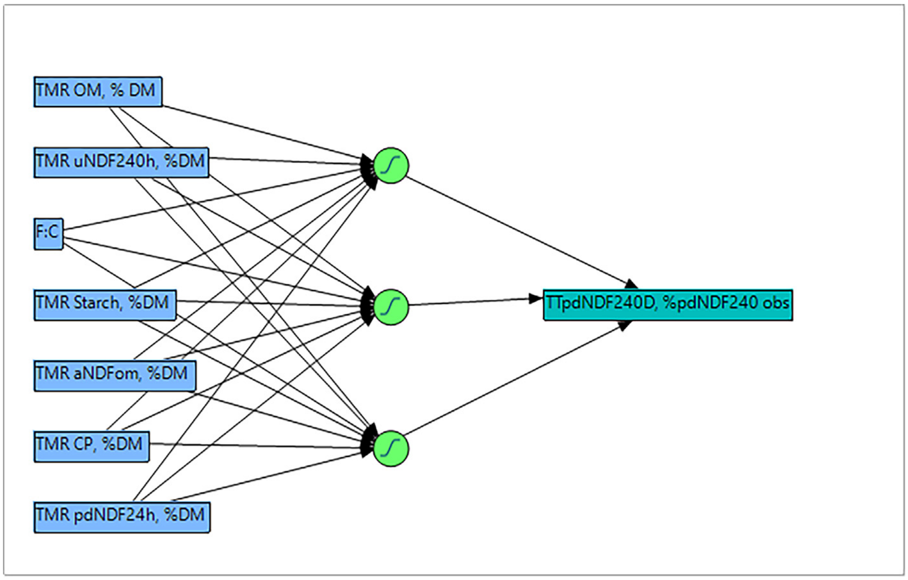

Fig. 1. The TTpdNDFD (Total-Tract potentially digestible NDF Digestibility) in Italian Holstein dairy cow equation shown as a neural network model, with interconnections between covariates. The model comprises sub-models linked by nodes, and each covariate is repeated in each equation to predict different parts of the slope. TMR = Total Mixed Ration; F:C = Forage and concentrate ratio; Obs. = Observed; see Table 1 for other abbreviations.

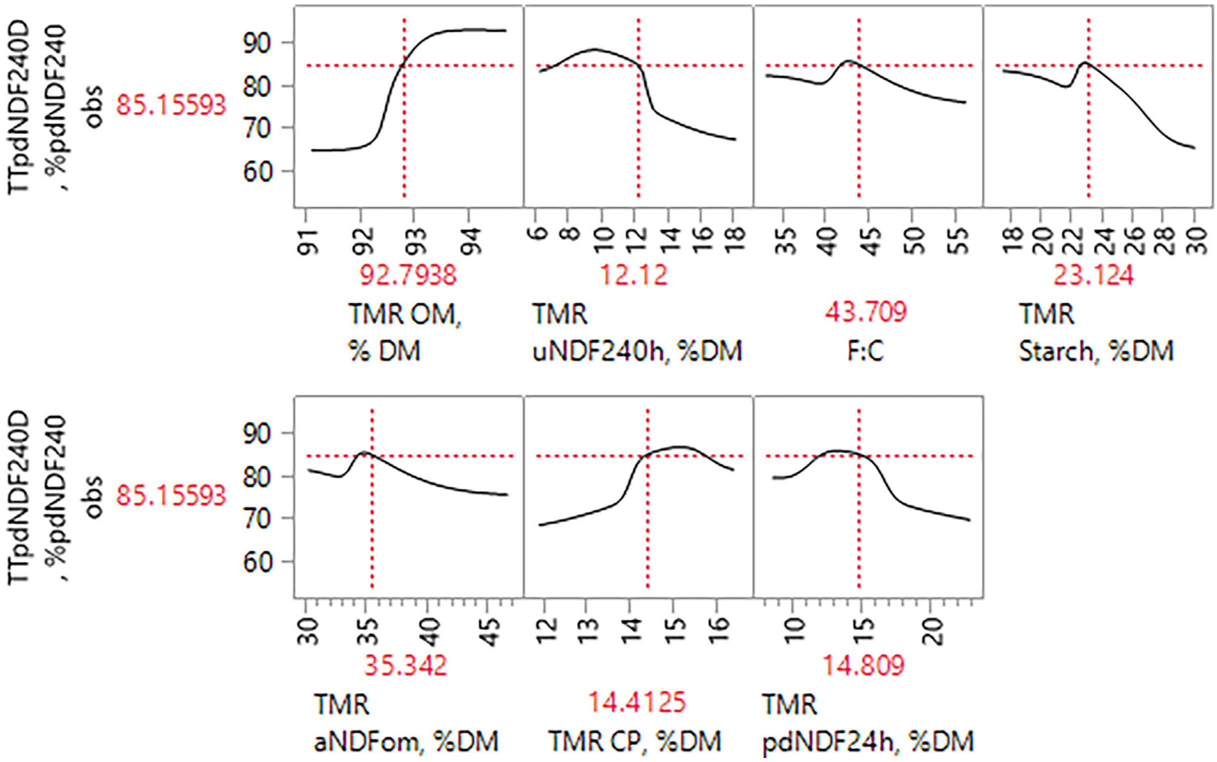

Fig. 2. Pattern of prediction slopes for covariates and their relationship with estimated effect on TTpdNDFD (Total-Tract potentially digestible NDF Digestibility) in Italian Holstein Dairy Cows. The red values on the graph represent the predicted average TTpdNDFD for each combination of covariates, and the red cross indicates the location of each value on the curve. TMR = Total Mixed Ration; F:C = Forage and concentrate ratio; Obs. = Observed; see Table 1 for other abbreviations.

tation of the pattern of covariates in Fig. 2 helps to illustrate the relationship between the covariates and the predicted TTpdNDFD values.

Discussion

To our knowledge, this is the first analysis that used individual cow's observations (instead of treatment means) from several studies to jointly evaluate the effect of hay-based TMR diet characteristics on total-tract fibre digestibility. Unlike treatment means, the use of individual observations increases the variability, and therefore, the representativeness of the training, which is pivotal to guarantee robust and applicable prediction models. Moreover, it is fundamental avoiding model overfitting and thus better accounts for the variability among the different studies, animals, diets, etc. (de Souza et al., 2018).

The fact that dietary ADF and ADL content were removed because statistically irrelevant was expected as they were in a strong relationship with the uNDF240.

The NN approaches are generally considered advantageous for their ability to model complex relationships between variables, handle large datasets, and provide robust predictions. Traditional meta-analytic approaches, such as mixed models and random-effects models, have been widely used and well-established in the field. In accordance with our results, these methods are generally simpler to implement and interpret, and can provide valuable insights into the overall effect size and heterogeneity in the data.

Whether a NN approach is better than a traditional approach depends on the specific research question and the nature of the data. In fact, NN may be more appropriate when dealing with complex relationships, multiple and correlated predictors, or large datasets, while traditional approaches may be more appropriate for simpler relationships and more straightforward data (Fernández et al., 2006).

The resulting equation in the paper is constructed by three sub-equations that are connected by nodes, as shown in Fig. 1. These sub-equations are interconnected to represent the relationships between the different covariates and the outcomes of interest. Fig. 2 illustrates the slopes of the three sub-models used in the equation. Each sub-model represents a different aspect of the data, and the combination of these sub-models allows for a more comprehensive understanding of the relationships between the covariates and the outcomes. By combining these three sub-models in the equation, the authors are able to account for the complex relationships between these different levels of analysis and provide a more accurate representation of the data. The resulting equation can then be used to make predictions about the outcomes of interest based on the different covariates included in the model.

Furthermore, the NN analysis in this study offers valuable insights into the pattern of prediction slopes for covariates and their impact on TTpdNDFD (Fig. 2). This innovative tool provides nutritionists with practical guidance by indicating the optimal range for each covariate that can be incorporated into the TMR diet. It is important to note that this tool surpasses the capabilities of more traditional evaluation methods, making it a powerful asset for nutritionists in their decision-making process.

Overall, the evaluation of model performance and the visual representation of the covariate patterns for TTpdNDFD prediction provide valuable insights into the accuracy and robustness of the model, and can help to guide future research or interventions aimed at improving TTpdNDFD equations.

One strength for the TTpdNDFD equation developed in the current research was the inclusion of indigestible and potential digestible NDF (uNDF 24 and 240 and pdNDF 24 and 240, respectively) information during the modelling process. In fact, this information was not previously available in most of the published experiments by other authors (de Souza et al., 2018). The inclusion of uNDF and pdNDF was reported to enhance the prediction of TTpdNDFD at production level because they are important NDF fractions related to digestibility (Righi et al., 2016b; de Souza et al., 2018). An important aspect of our analysis was that the database was composed only of experiments involving lactating cows, with most cows producing over 35 kg of milk per day. This implies the model proposed in our study may have limited value for heifers, dry cows, animals not belonging to Holstein breed, or cows producing outside of this range.

Furthermore, studies investigating the feeding level effect on diet digestibility are difficult to conduct in dairy cows (de Souza et al., 2018), requiring strict protocols, careful planning, specialised equipment, and expertise. Researchers must also consider the ethical implications of conducting such trials and take steps to ensure that the well-being of the cows is protected. Moreover, in general, there are several confounding effects (stage of lactation, changes in environment, feed quality, etc. (Heinrichs et al., 2021)).

Our database was composed of animals with moderate to high DMI level (25.4 ± 3.2 kg DMI/d), which precludes conclusions about digestibility at lower intakes. Finally, all the forages used in the rations included in the analyses were of medium to high quality (Brogna et al., 2009; Palmonari et al., 2014; 2016). If low-quality forages are used instead, the model may not be able to achieve its maximum potential in terms of optimising the nutrient content and balance of the ration.

It is important to highlight that the 11 studies whose data were merged for the NN model development differed in terms of diet but were characterised by the same location (experimental farm), identical environmental, herd genetic level, and same management practices. Therefore, since the data are from locations in the northern Italy, our overall equations should be most valuable in similar climates and be used with caution in colder or warmer climates.

A strength of our study is that the laboratory methods were consistent, in fact, published research comparing the digestibility of the same hay indicated considerable variation in digestion coefficients among laboratories (Cochran et al., 1986). The results from our study could be used to improve ration balancing, where the coefficients estimated in our equation can be used to adjust a basal digestibility for hay-based TMR diets.

According to the information presented in Fig. 2, there is a positive correlation between OM (%DM) and fibre digestibility. However, there are plateau situations where the correlation between the predictor and the response variable remains constant, i.e. between 92 and 93.5%.

The ash content of a diet is influenced by mineral supplementation and potential contamination of forages during processing (Brogna et al., 2009; Buxton et al., 2015; Palmonari et al., 2016). Nevertheless, in optimal situations, the range of ash content observed is quite narrow and has limited practical significance.

In the present study, the best TTpdNDFD is reported with a dietary forage content between 42 and 43%. Furthermore, regarding the forage quality, forage digestibility is positively related to DMI, because NDF that digests faster in the rumen contributes to a faster passage rate, thus, less filling (Allen, 2000; Fustini et al., 2017; Raffrenato et al., 2019). On the other hand, an excessive amount of forage in the diet has a filling effect, reducing the intake capacity of the cows (Grant, 2022) of both forage and concentrates. In fact, the fibre content of the diet, as well as its physical characteristics such as particle size, density, fragility, and digestibility, appear to be the main factors responsible for ruminal fill and daily DMI (Fustini et al., 2017; Miller et al., 2021; Grant, 2022). This physical fill control system is more evident when cows are fed for *ad libitum* intake and during the first phase of lactation, during which metabolic and endocrine signals are less effective to control appetite (Allen and Piantoni, 2014).

Ration concentrates included in the present study were characterised by the high content of cereals (corn and sorghum) and lower content of fibrous feeds (beet pulp, wheat bran and soyhulls).

The starch (%DM) inclusion pattern resulted optimal between 22 and 24% and higher levels of decreased fibre digestibility. This is consistent with a meta-analysis that also found decreased ruminal and total-tract NDF digestibility's as dietary starch is increased (Ferraretto et al., 2013). The depression in TTpdNDFD as the level of dietary starch increased is probably due to the greater dietary starch content decreases ruminal pH (Cavallini et al., 2021b and 2022a; Olijhoek et al., 2022) and thus creates an unfavourable environment for the cellulolytic bacteria (van Soest, 1994; Buxton et al., 2015). The starch sources used in the trials were mainly corn and sorghum flakes, characterised by high vitreous starch content and they probably did not result in lowering enough the rumen pH (Ferraretto et al., 2013 and 2015; Buonaiuto et al., 2021b). The pdNDF24 (%DM) content of the diet also exhibits an optimal range between 12 and 16%. The fast-fermentable fraction of the fibre behaves as non-structural carbohydrates in the rumen fermentation pattern (Calsamiglia et al., 2012; Raffrenato et al., 2019; Cavallini et al., 2021b), so, under overabundance of pdNDF24, it could lead to a ruminal volatile fatty acids overload, resulting in decreased pH and fibrolitics bacteria activity (van Soest, 1994; Weimer et al., 2010). On the other hand, the lack of fast-fermentable substrates in the rumen is associated with insufficient energy for the bacteria that are depressed in growth and degradation of the substrates (van Soest, 1994; Buxton et al., 2015; Palmonari et al., 2023), in this case the fibre. Moreover, when the dietary forage amount increases, the most indigestible fraction of the diet increases as well (de Souza et al., 2018; Raffrenato et al., 2019). The optimal range for fibre digestibility is around 34–35% when expressed relative to NDF (%DM), which supports the typical rations used in the Parmigiano Reggiano and hay-based TMR areas (Mordenti et al., 2015; Righi et al., 2016a). In the NASEM (2021), the typical NDF content of diets used in the model was around 31–32% for corn silage-based diet, which is less fragile than alfalfa-based diet, as used in the present study. It is important to highlight, that the dietary NDF in the present study was mainly driven by forages instead of fibrous feeds (Table 3). In the present study, the most abundant TMR forage was alfalfa (20% of the ration, Table 3). Our results are consistent with studies reporting that grass and corn silage have higher NDF digestibility than alfalfa (Miller et al., 2021). However, the filling effect of legumes is commonly less than grasses and independent of fibre digestibility (Kammes and Allen, 2012). This variance between legumes and grasses is associated to a quicker fibre digestibility and higher particle fragility for legumes, resulting in decreased retention time in the rumen, increased outcomes and enhanced intake (Kammes and Allen, 2012; Oba and Kammes-Main, 2022). Good quality hays (grass or alfalfa) are characterised by high native content of sugars and organic acids (Formigoni et al., 2003; Ferraretto et al., 2015; Fusaro et al., 2016) if compared with silages (corn silage mainly). Moreover, the pectin content of alfalfa is greater than corn silage (Formigoni et al., 2003; Palmonari et al., 2014; 2016). These two aspects enforced the previous statements and the differences showed in the present research.

All included rations had a moderately short particle size (peNDF = 16.95 [14.88–19.86] %DM, Table 3), as is common in the Parmigiano-Reggiano area and every ration without silages (Fustini et al., 2011, 2016; Righi et al., 2016a). These short dietary particle size permits for negligible feed sorting while using all dry components (Fustini et al., 2016). It is typical for these diets to have less than 1% particles larger than 19 mm and a high proportion on the inferior sieves (8–1.18 mm) and bottom pan. In a previous research, (Fustini et al., 2016) was reported that in typical Parmigiano-Reggiano rations, the presence of wheat straw may well maintain rumination time and ruminal pH above 5.5, even at low peNDF values.

Our model showed that when the undigestible portion of the fibre (uNDF240, %DM) exceeded 12%, there was a decrease in *in vivo* fibre digestibility. This was an expected result, as the additional amount of indigestible fraction in the faeces contributed to the drop in digestibility (Fustini et al., 2017). Other authors (Oba and Kammes-Main, 2022), have reported that uNDF240 may not be an effective indicator of the filling effects of roughage NDF. This is because uNDF240 is an end-point measurement that does not provide information on the digestion rate, fragility, or how NDF is digested. In addition, legume have higher uNDF240 content than grass, but they are more fragile and less filling in the rumen (Kammes and Allen, 2012). So, as reported by Oba and Kammes-Main (2022), it is not appropriate to use *in vitro* NDF digestibility to estimate *in vivo* NDF digestibility of forages. In fact, *in vivo* trials are required for precise prediction, as reported in the present research. It is reported that higher *in vitro* NDF digestibility is related to greater fragility, which reduces the ruminal pool of large NDF particles and decreases the retention time of ruminal digesta (Oba and Kammes-Main, 2022). Indeed, in vivo more fragile forages escape from the rumen earlier and result in lower total-tract digestibility (Raffrenato et al., 2019). Finally, literature also suggested a dairy cow requirement of uNDF240 for grass-based ration and legumes-bases ration, 0.28 and 0.48% of BW (Fustini et al., 2017), respectively, confirming our results.

Our equation also suggests that TMR's CP content has a positive effect on the fibre digestibility until the 15.5%, pointing out to the optimal range between 14 and 16% which is consistent with what reported in previous works (Daniel et al., 2022; Letelier et al., 2022). On the other hand, the diets included in the present analysis have a protein range quite small (Table 3), so that is a limitation for the study. As reported by Letelier et al. (2022) increasing the CP content of the diet has a positive effect on DMI and production until 17%; after this limit, no additional effects were found, except for fresh cows. Indeed, higher levels of dietary CP are related with higher rumen ammonia content available for bacterial growth, enhancing the fibre degradation (Fessenden et al., 2019).

Increased dietary CP intake can increase nitrogen retention in ruminants, as more nitrogen is available for absorption and incorporation into protein synthesis. However, this effect is limited by the animal's ability to use the nitrogen excess, and if the animal is unable to use it, it may be excreted in urine or faeces (Monteils et al., 2002). So, higher levels of dietary CP can increase nitrogen emissions through urine and faeces, as the excess nitrogen is excreted. This can contribute to environmental pollution, as it can be converted to nitrate and other forms that can leach into waterways or contribute to greenhouse gas emissions (Castillo et al., 2000; Kebreab et al., 2008).

However, the protein rumen degradation rate is an important parameter (Fessenden et al., 2020). In the present research, the main protein sources are alfalfa and heat-treated soybean meal. Alfalfa is characterised by high rumen degradable protein and heat-treated soybean meal by high by-pass protein (Fessenden et al., 2020).

The equation obtained from the NN can be a powerful tool for nutritionists and farmers to predict the fibre digestibility of hay-based TMR and manage nutrient requirements for lactating cows in the following ways:

I. Optimise feed planning: using the equation, nutritionists and farmers can predict the fibre digestibility of different formulations and select the optimal rations that meets the nutrient requirements of lactating cows. By selecting the option with the highest predicted fibre digestibility, they can improve the efficiency of feed utilisation and reduce feed costs.
II. Monitor feed quality: the equation can also be used to monitor the quality of the ration over time. By regularly measuring the TTpdNDFD on field and comparing it to the predicted values from the equation, nutritionists and farmers can identify changes in the feed quality and make adjustments to the TMR formulation as needed.
III. Manage nutrient intake: the equation can be used to predict the amount of fibre that will be digested by lactating cows and adjust the nutrient intake accordingly. By managing the nutrient intake, nutritionists and farmers can prevent under or overfeeding, which can lead to health problems and reduced milk production.

Future developments of this study will be useful to make the model more flexible allowing for evaluation of breeds different than Holstein, wider range (e.g., partial mixed ration) of diets, lactation stages and the possible effect of additional concentrates.

Conclusions

The study demonstrates that TTpdNDFD of lactating Holstein cows fed dry-hay-based rations can be predicted from the diet characteristics. This represents an important achievement in animal nutrition, opening the room for a fast and efficient optimisation of hay-based TMR formulations in the field.

Furthermore, our results confirm the negative relationship between dietary starch and fast-fermentable fibre and TTpdNDFD. Specifically, as the levels of dietary starch and fast-fermentable fibre increase, NDF digestibility decreases. We found that the minimum dietary CP should be 14% in order to maximise fibre digestiblity, while the maximum dietary uNDF240 level should be 12%. This highlights the importance of monitoring different types of fibre when the aim is to optimise nutrient utilisation and prevent digestive problems.

The obtained equation can be considered as a first attempt to aid nutritionists and farmers to implement precision feeding startegies, i.e. by making more accurate and informed decisions about feed planning, quality monitoring, and nutrient management for lactating cows. By using the equation and following the specific guidelines for optimising hay-based TMR formulations, farmers and nutritionists can maximise herd productivity at the same time minimising the feed costs and the environmental impact.

Ethics approval

Our study compiled data from studies previously carried out. Thus, no animal care and use protocol was needed.

Data and model availability statement

The data/models were not deposited in an official repository. The data/models that support the study findings are available from the authors upon request.

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.

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Declaration of interest

None.

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