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Faculty of Biosciences
Department of Animal and Aquacultural Sciences

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A new approach for predicting milk production responses and feed utilization in dairy cows

En ny tilnærming for å forutsi melke-produksjonsrespons og fôrutnyttelse hos melkekyr

Clementina Álvarez

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“All models are wrong, but some are useful”

George E. Box

About the Author

María Clementina Álvarez graduated in 2013 as an Agronomist from Universidad de la República in Uruguay. In 2016, she obtained her Master of Science degree (MSc) from Aarhus University and Debrecen University under the Erasmus Mundus joint master programme: Sustainable Animal Nutrition and Feeding (EMSANF). She started her PhD in March 2018.

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Contents

List of papers

The thesis is based on the following papers and are referred to in the text by their roman numerals:

I. **Álvarez, C.**, E. Prestløkken, N. I. Nielsen, H. Volden, G. Klemetsdal and M. R. Weisbjerg. 2020. *Precision and additivity of organic matter digestibility obtained via in vitro multi-enzymatic method.* Journal of Dairy Science. 103 (5): 4880-4891. DOI: https://doi.org/10.3168/jds.2019-17778 **Published**.

II. **Álvarez, C.**, N. I. Nielsen, M. R. Weisbjerg, H. Volden and E. Prestløkken. 2021. *A static model for estimating energy content of compound feeds in a dynamic feed evaluation system.* Journal of Dairy Science. 104(8): 9362-9375. DOI: https://doi.org/10.3168/jds.2020-19816 **Published.**

III. **Álvarez, C.**, M.R. Weisbjerg, N.I. Nielsen, E. Prestløkken and H. Volden. 2020. *Effect of digestibility of silage and concentrate intake on milk yield: a meta-analysis.* In: Proceedings of the 28th General Meeting of the European Grassland Federation: Meeting the future demands for grassland production. Pages 179-181. Helsinki, Finland. **Published.**

IV. **Álvarez, C.**, N. I. Nielsen, M. R. Weisbjerg, H. Volden and E. Prestløkken. *High-digestible silages allow low concentrate levels without affecting milk production*. **Submitted.**

V. **Álvarez, C.**, C. Wiik, T. Aschjem and H. Volden. *Machine learning is a useful tool to determine milk yield responses to diet changes from real-time farm data.* **Submitted.**

Summary

Feed evaluation systems (FESs), such as the Nordic Feed Evaluation System (NorFor), aim to help dairy producers compare the nutritive value of various types of feed, formulate of balanced diets, and predict animal performance given a certain diet. To cover the changing demands of the dairy industry, FESs are in continuous need of improvement and updating. Therefore, this thesis contributes to improving NorFor by developing a model that can estimate the energy content of compound feeds and the development of a model describing milk production responses to the changes in silage digestibility and concentrate intake. The practical use of this knowledge is central because the common goal is to implement them into NorFor for use in farms.

For the development of the energy estimation model of compound feeds, the activities are described in two scientific articles (Papers I and II). Paper I show that the enzymatic digestibility of the organic matter method (EDOM) is precise for the determination of organic matter digestibility (OMD) of compound feeds, here with a high correlation between measures of a sample within and between labs. This is vital for use in practice because a high correlation between the measurements of a sample means that values can be compared between countries and through time. Thus, is a viable method if OMD is to be included in the energy estimation equation developed in Paper II. The best fit model (2.08% RMSE) for estimating the net energy of lactation at 20 kg DM (NEL_{20}) of compound feeds include OMD measured by EDOM and the chemical components crude fat, NDF, and crude protein (urea corrected) as the explanatory variables. The model can be used as an alternative when information about ingredients for the calculation of NEL_{20} in the mechanistic model is lacking.

For the development of the responses in milk production regarding changes in diet, a meta-analysis was developed in Paper III. The meta-analysis showed a curvilinear response of ECM to concentrate intake. Hence, ECM showed a decreasing increment to concentrate intake. This marginal response was also affected by silage digestibility, showing lower responses with increasing silage digestibility. Paper IV is the result of an animal experiment involving 60 Norwegian Red cows. The objective was to test the meta-analysis by feeding two silages with different digestibility and concentrate levels. Milk production, intake, and methane emissions were measured throughout the experiment. The

silage with a higher digestibility was able to maintain high milk yields with low concentrate supply levels, but this could not be achieved with silage with lower digestibility. When the concentrate supply was increased, low-digestible silages showed a higher response in milk production. Cows in the high-digestible silage treatment were offered a lower concentrate supply, but the methane production did not differ between the silage treatments. Methane per unit of feed and milk decreased with increasing total intake, regardless of the type of silage or level of concentrate.

Paper V produced, based on the meta-analysis, a developed response approach that could be adapted to farm data, here by transforming the responses variable—ECM—into concentrate efficiency responses. According to an algorithm developed using machine learning, silage is classified as high or low digestibility. A silage is classified as highly-digestible because of the steeper decrease in concentrate efficiency with the increase in concentrate supply because of lower marginal milk responses. The algorithm also allows the evaluation of the concentrate efficiency for a specific silage use in farms at both, group and individual cow level. An economic response approach was also developed, showing that for highly-digestible silages, the optimal concentrate supply level is sensitive to changes in concentrate prices.

Sammendrag

Fôrevalueringssystemer (FESs), slik som the Nordic Feed Evaluation System (NorFor), har som formål å hjelpe melkeprodusenter til å sammenlikne næringsverdi av ulike typer fôr, formulere balanserte rasjoner og å predikere ytelse gitt en bestemt rasjon. For å møte endrede krav i melkeindustrien, er fôrevalueringssystemer under stadig oppdatering og forbedring. Denne avhandlingen bidrar til forbedring av NorFor gjennom utvikling av en modell som kan estimere energiinnhold av kraftfôr og gjennom utvikling av en modell som beskriver respons i melkeproduksjon ved endringer i fordøyelighet av surfôr og kraftfôrinntak. Den praktiske bruken av denne kunnskapen er sentral da det overordnede målet er å implementere den i NorFor.

Aktivitetene brukt for utviklingen av modellen for estimering av energi i kraftfôrblandinger er beskrevet i to vitenskapelige artikler (Artikkel I og Artikkel II). Artikkel I viser at metoden for enzymatisk fordøyelighet av organisk stoff (EFOS) er presis for evalueringen av fordøyelighet av organisk stoff (OMD) i kraftfôrblandinger, med høy korrelasjon både innen og mellom laboratorium. Dette er viktig for praktisk bruk da en høy korrelasjon mellom målinger av en prøve betyr at verdiene kan sammenliknes mellom land og over tid. Likningen utviklet for estimering av energi i Artikkel II er derfor en anvendelig metode dersom OMD skal benyttes. Den beste modellen (2.08% RMSE) for estimering av nettoenergi i kraftfôr ved 20 kg tørrstoffinntak (NEL20) inkluderer OMD målt med EFOS og de analytiske komponentene råfett, nøytral løselig fiber og ureakorrigert råprotein som forklarende variabler. Modellen kan brukes som et alternativ når informasjonen om ingrediensene for utregningen av NEL20 i en mekanistisk modell mangler.

For å undersøke respons i melkeproduksjon som et resultat av endring i rasjonen, ble en metaanalyse gjennomført (Artikkel III). Metaanalysen viste en krumlinjet respons av kraftfôrinntak på energikorrigert melk (EKM). Følgelig viste EKM en avtagende marginalrespons for kraftfôrinntak. Den marginale responsen ble påvirket av fordøyeligheten av surfôr, og viste lavere respons med økende kraftfôrinntak og med økende surfôrfordøyelighet.

Artikkel IV beskriver resultatene fra et produksjonsforsøk med 60 kyr av rasen norsk rødt fe (NRF). Formålet var å teste metaanalysen ved å fôre dyrene surfôr med ulik fordøyelighet kombinert med ulike kraftfôrnivå. Melkeproduksjon, fôrinntak og metanutslipp ble målt gjennom hele forsøket. Surfôr med høy

fordøyelighet klarte å opprettholde høy melkeproduksjon med lav tildeling av kraftfôr, men dette var ikke mulig for surfôr med lav fordøyelighet. Ved økende kraftfôrtildeling viste surfôr med lav fordøyelighet høyere respons i produksjon av melk. Kyr i gruppen som ble gitt surfôr med høy fordøyelighet ble tildelt mindre kraftfôr, men metanproduksjonen var ikke signifikant forskjellig mellom gruppene. Produksjonen av metan per enhet fôr og melk ble redusert med økt totalt fôrinntak, uavhengig av type surfôr og kraftfôrnivå.

Artikkel V presenterer en responstilnærming som kan tilpasses gårdsdata ved å transformere responsvariabelen– EKM –til kraftfôreffektivitetsrespons. I en algoritme utviklet ved hjelp av maskinlæring, er surfôr klassifisert som høyt fordøyelig på grunn av brattere nedgang i kraftfôreffektivitet med økende kraftfôrtildeling på grunn av lavere marginalrespons i melkeproduksjonen. Algoritmen tillater i tillegg evaluering av kraftfôreffektiviteten for en spesifikk bruk av surfôr på gårdsnivå for kyr både på gruppe- og individnivå. En økonomisk responstilnærming ble også utviklet. Den viste at for surfôr med høy fordøyelighet, er den optimale kraftfôrforsyningen sensitiv for endringer i prisen på kraftfôr.

List of abbreviations

AAT: amino acids absorbed in the small intestine (g/day)
ADL: acid detergent lignin (g/kg DM)
AIC: Akaike information criterion
AICc: Akaike information criterion corrected
AMS: Automated milking system
BIC: Bayesian information criterion
C.F ratio: concentrate ratio in the diet
CH_4: methane
Concentrate efficiency: ECM/kg concentrate intake
CNCPS: The Cornell Net Carbohydrate and Protein system
CFat: crude fat (g/kg DM)
CF: crude fiber (g/kg DM or % DM)
CP: crude protein (g/kg DM or % DM)
CPcorr: crude protein urea corrected (g/kg DM or % DM)
DE: digestible energy (MJ/day)
DIM: days in milk
DMIc: concentrate intake in dry matter (kg DM/day)
DMIs: silage intake in dry matter (kg DM/day)
DMI: total intake in dry matter (kg DM/day)
DOM: digestible organic matter (% DM)
DVE-OEB system: Dutch protein system
ECM: energy corrected milk (kg)
EDOM: enzymatic digestibility of OM method
FES: feed evaluation system
FST: NorFor's feedstuff table
GP: gas production method
HCl: hydrochloric acid
HDS: High-digestible silage
ICC: intra-class correlation
iNDF: indigestible nutrient detergent fiber (g/kg DM or % DM)
ILC: intra-laboratory correlation
LDS: low-digestible silage
ME: metabolizable energy (MJ/day)
ML: machine learning
MP: metabolizable protein (g/day or kg/day)

NCDX: Nordic Cattle Data eXchange
NDF: neutral detergent fiber (g/kg DM or % DM)
NE: net energy (MJ)
NEL: net energy of lactation (MJ)
NELc: net energy of lactation intake from concentrate (MJ/day)
NELs: net energy of lactation intake from silage (MJ/day)
NEL_8: standard feed value for NEL at 8 kg DMI (MJ/kg DM)
NEL_{20}: standard feed value for NEL at 20 kg DMI (MJ/kg DM)
NFE: Nitrogen free extract
NHR: Norwegian Herd Recording system
NIR: near-infrared spectroscopy analysis
NorFor: Nordic Feed Evaluation system
OMD: organic matter digestibility (%)
OMDs: organic matter digestibility of the silage (%)
PMR: partial mixed ration
PreP: pre-experimental period
PRESS: predicted residual error sum of squares
R^2: coefficient of determination
rcoef: repeatability coefficient
RestCHO;: rest carbohydrate (g/kg DM or % DM)
RMSE: root mean square error
RMSEP: root mean square error of prediction
RP1: response period 1
RP2: response period 2
SEP: separate feeding strategy
SR: substitution rate (kg DM silage/kg DM concentrate)
ST: starch (g/kg DM or % DM)
TT: Tilley and Terry method
TMR: total mixed ration
UFL: Unité Fourragère Lait (in French), unit for net energy lactation (kcal/kg/1760)

1. Introduction

The dairy industry faces new challenges and opportunities. The volatile prices of inputs needed for production (such as feeds, fertilizers, and electricity, among others) and of the produced goods in the international market (Alqaisi and Schlecht, 2021) are not the only challenges. For example, climate change has put the dairy sector in the spotlight, with some claiming a need to reduce its environmental impact. Reducing the greenhouse gas emissions (Paris Agreement, 2015) and nutrient losses of the system, such as N and P (Kanter and Brownlie, 2019), are a priority. These economic and environmental concerns need to be combined with a high level of productivity on the farm because a rising global population translates into higher demands for dairy products (FAO and GDP, 2018). Therefore, there has been a paradigm shift in dairy production, from a focus on the maximization of production into including the efficient use of inputs, hence reducing the environmental impact and improving sustainability. Feed accounts for more than half of the cost of milk production, and a cow's diet has a direct impact on yields, efficiency, and the environment. Therefore, improvement of feed knowledge and its relationship with milk production as a way to improve feed efficiency and reduce environmental losses is needed.

On the other hand, we have seen a significant increase in the available data because of new technologies used in farms, such as milking robots, scales, and other sensors. This large data flux is known as "big data", which is seen as the new technological revolution in agriculture (Mark, 2019). Big data opens up new opportunities for knowledge to be developed and used in commercial farms.

The current work was carried out within the Industrial PhD Scheme and was funded by the Research Council of Norway (project number 284008), TINE SA, and Nordic Feed Evaluation System (NorFor), while the Norwegian University of Life Sciences (NMBU) acted as the degree-conferring institution. The overall objective of the present thesis was to provide new feed knowledge that could contribute to solving the new paradigm the dairy industry is facing. This new knowledge is to be incorporated into NorFor and is intended to be used in farms to improve their performance. Therefore, the practicality of the current work was fundamental. This work has a strong practical approach, and I hypothesized that these results would be relevant for implementation in

NorFor and use in farms. Based on this objective and hypothesis, the current work developed a model describing the response in milk production to changes in diet components as a way to improve the understanding of the relationship between silage and concentrate. This response approach allows for the evaluation of multiple scenarios, such as different production goals that include economic and environmental perspectives. Finally, the use of machine learning allows the model to be adapted to big data coming from commercial farms. Additionally, a model to evaluate the energy content of compound feeds was created, as an alternative when information for the original mechanistic model is not available. This model compensates for the lack of information about the selected feeds, which can translate into under- or overfeeding, hence affecting production goals and/or leading to environmental losses. This model is intended to be to be commonly used in practice by incorporating it into NorFor.

2. Background

In the next pages of this chapter, a detailed background of this work is described by outlining the gaps in knowledge that precede my research. At the end of each section, questions that arise from the text will be elaborated on to help in better understanding the "whys" of this work.

2.1. Feed evaluation systems

The concept of a feed evaluation system (**FES**) is far from new and was already proposed in 1810 by Thaer. However, there are assumptions that there may have been even earlier attempts at FESs (Tyler, 1956). The aims of a FES are the accurate comparison of the nutritive values of feeds, formulation of balanced rations to reach a production goal, and prediction of performance when the feed characteristics are known (Martin-Rosset et al., 1994). The selection of a FES depends on what the user needs (Oddy, 1989). Normally, a FES developed in the same region or country will adapt better to the user's requirements. This is because FES and the regions' dairy production are developed in parallel, and FESs developed with local data to cover the needs of those production systems.

Earlier, FESs were developed on empirical data usually showing a linear relationship between the feed values and animal requirements. A deeper understanding of nutrient metabolism and rumen functioning has made the development of new FESs possible, including mechanistic models in their system. The main difference between empirical and mechanistic models is that empirical models are created to fit the data the best, while mechanistic models incorporate nutrient digestion, metabolism, and interactions under specific physiological conditions of the animals from lower levels of aggregation (Tedeschi et al., 2005b). The Cornell Net Carbohydrate and Protein System (**CNCPS**; Fox et al., 2004), INRA Feeding System for Ruminants (INRA, 2018), Nordic Feed Evaluation System (**NorFor**; Volden, 2011c), Dutch Protein System (**DVE-OEB system**; Tamminga et al., 1994), and Scandinavian Protein System (Madsen et al., 1995) are all examples of FESs that include mechanistic models. Despite being cataloged as "modern," continuous updates and improvements of these systems are essential to keep them relevant. This is because the user experience after several years of use, together with new feed knowledge, increase of the animals' genetic merits, new technologies and changes in consumer demands will need the continuous adaptations of FESs. There are several examples of FES updates. CNCPS was first published in 1992 but has had continuous updates since then, with the most recent ones being by Higgs et al. (2015) and Van Amburgh et al. (2015). More than 40 years ago, the first version of INRA was published, with three major updates in 1988, 2007, and 2018 (INRA, 2018). NorFor was launched in 2011, and several equations and feed table updates have been included on the system's webpage. The Scandinavian protein model (AAT/PBV model) was developed in 1985 (Madsen, 1985) and updated in 1995 by Madsen et al. (1995). Finally, the Dutch Protein System was first developed in 1991 and updated by Van Duinkerken et al. (2011).

2.1.1. Feeding standards

The development of FESs has gone hand in hand with the development of feeding standards. Feeding standards refer to matching animal nutritional needs with feed supply through a common unit of expression (Tyler, 1956). Hence, the concept of a feeding standard is key and has been the basis of FESs, allowing for the effective nutritional management of animals in varying conditions (Corbett, 1983). Classical FESs have used tabulated feed values to compare feed composition with animal requirements. The "Hay equivalent" from Thaer compared different feeds using meadow hay as a reference value. The possibility of chemical fractioning, which is known as the proximate system or Weende analysis, allowed for the use of broad chemical fractions, such as moisture, crude ash, crude protein (**CP**), crude fat (**CFat**), crude fiber (**CF**), and nitrogen free extracts (**NFE**), as feeding standards. The concept of digestibility, that refers to the balance between the diet and the feed residues that escaped digestion and are found in the feces (Van Soest, 1994), was proposed later by Henneberg and Stohmann in 1860, allowing for the inclusion of nutrient availability of feeds in the feed standards (Tyler, 1956; Weisbjerg et al., 2010b). Kellner's work introduced the starch equivalent and described the energy necessary for fat deposition in adult steers and dairy cows. From his and other authors' work, the use of digestible energy (**DE**), metabolizable energy (**ME**), and net energy (**NE**) has been the energy units used as feeding standards in FESs until current times (Weisbjerg et al., 2010b).

2.1.1.1. Feed standards today

Modern mechanistic models can incorporate time as an independent variable, together with the interaction of feeds during digestion and metabolization

(Åkerlind and Volden, 2011). Feed standards values now depend on which components are included in the diet and on the animal's characteristics. This means that for diet formulation, the concept of fixed and tabulated feed standard values is outdated. However, tabulated feed standards are still relevant because a comparison of feeds is important for ranking, trading, and the final selection of feedstuff (INRA, 2018). Moreover, tabulated values will give information about the impact of a certain feed compared with other feeds. For this, default feed values have been created. For example, the new INRA system, which was updated in 2018, still uses the unit for net energy lactation (**UFL**) but shifted away from a fixed energy value. However, to provide table values for feed comparison, the tabulated UFL is calculated based on a common feeding level (2% of body weight), with a rumen protein balance and concentrate level equal to 0. In NorFor (Åkerlind and Volden, 2011), feed standards are provided using the fixed feed intake level, body weight, concentrate proportion in the diet and diet CP, neutral detergent fiber (**NDF**), starch (**ST**), and rest fraction (**RestCHO**) content (see Table 1). Based on these, other parameters such as passage rates, rumen load index, and the efficiency of microbial synthesis can be calculated. To calculate the standard feed value for a specific feedstuff, parameters such as nutrient composition, degradation rates, and indigestible fractions of that specific feedstuff are used. For energy content, two standard values are calculated: net energy of lactation at 8 or 20 kg of dry matter intake (**NEL_8** and **NEL_{20}**). The NEL_{20} is the most popular one for lactating cows because 20 kg DM is comparable to the intake level of a cow producing 30 kg energy corrected milk (**ECM**) daily.

For NEL_{20} calculation, chemical composition is obtained through chemical analyses, while degradation rates and indigestible fractions of CP, NDF, and ST are measured by the in sacco technique, as described by Madsen et al. (1995). This technique takes a large number of resources, such as the need for cannulated animals, research facilities, and time. Therefore, NorFor collected results from previous research in sacco studies, including the rates and fractions for numerous types of forages and concentrate feedstuffs, to generate feed tables.

Table 1: Values for the fixed parameters used for determining standard feed values (Åkerlind and Volden, 2011).

Parameter	Equation	8 kg DMI	20 kg DMI
Current body weight, kg		600	600
Dry matter intake, kg/d		8	20
Concentrate proportion of the ration, %		50	50
CP, g/kg DM		160	160
NDF, g/kg DM		370	370
ST+RestCHO, g/kg DM		310	310
rdST+rdRestCHO, g/kg DM		280	280
Correction factor for NDF degradation rate	13.2	0.89857	0.89857
Passage rate for liquid, %/h	13.3	7.07943	12.5236
Passage rate for CP and ST in concentrate, %/h	13.4	3.33733	6.08733
Passage rate for NDF in concentrate, %/h	13.5	1.43505	2.61755
Passage rate for CP and ST in roughage, %/h	13.6	2.30177	4.47943
Passage rate for NDF in roughage, %/h	13.7	0.998465	1.64242
Efficiency of microbial CP synthesis, g/kg rd_OM	13.8	133.383	184.329

Compound feeds refer to a uniform mix of different ingredients and additives that are offered mainly in pellet form and produced commercially to fulfill specific animal requirements. Regarding these mixes, the rates and fractions are not available in the literature, so NEL_{20} is calculated based on the ingredient composition. The NEL_{20} of compound feeds is calculated based on the weighted sum of ingredient composition, according to Equation 1

$$NEL_{20}compound_i = \sum\left(NEL_{20}\ ingredient_j \times \%\ inclusion\ of\ j\ in\ compound_i\right) \qquad \textit{Equation 1}$$

The problem is that the ingredient's proportions in compound feeds do not need to be made public by the formulation companies. Hence, NEL_{20} values can only be obtained from their producers. In addition, compound feeds have become an important part of ruminant diets. In 2019, the average concentrate intake of a cow in Norway was 7.8 kg (Tine Rågiving, 2020), of which most was compound feeds, making up 45% of cows' diets (Animalia, 2020). For these reasons, NorFor needs an alternative model that can measure the NEL_{20} of compound feeds, independent of the ingredient composition, degradation rates, and indigestible fractions.

Previous studies showed that equations to predict feed energy by involving only chemical components was not as accurate as when digestibility is also included in the equations (De Boever et al., 1986b; Aufrère and Michalet-Doreau, 1988). Moreover, experience from other systems (INRA, German system, Feed into Milk, Scandinavian Feed Unit) shows the use of organic matter digestibility (**OMD**) as an important parameter, in addition to chemical components.

2.1.1.2. Determination of OMD of feedstuffs

As mentioned, OMD is used together with chemical components in other FESs for estimating the energy of concentrate feeds. The "gold standard," or reference method, for determining OMD is in vivo with sheep that are fed to the maintenance level (EAAP, 1969). Digestibility is calculated by the input–output method using the total collection of feces. However, as with the in sacco method mentioned before, this method is expensive and time-consuming for everyday use. Therefore, laboratory methods are popular alternatives; these can be classified as rumen fluid and enzymatic methods.

The Tilley and Terry method (**TT**), which was published in 1963, is probably the most well-known rumen fluid method. It is a popular method even nowadays because it measures digestibility with a high level of accuracy compared with the "gold standard" (Tilley and Terry, 1963). The method consists of a "two-stage technique" (as described by the authors) involving the incubation of the sample in rumen fluid from sheep for 48 hours and then another 48-hour incubation with pepsin-HCl. The gas production method (**GP**) also involves rumen fluid, but digestibility determination is performed by collecting the total gas produced (Menke and Steingass, 1988). The disadvantages of these methods are that they still need cannulated animals to obtain the fluid and animal facilities, so they are still expensive and laborious. Methods using commercially available cellulase have been developed with the objective of replacing rumen fluid.

An early example of an enzymatic method is the proposed by Kellner and Kirchgessner (1977) for forages, which could be summarized as a forage sample being incubated on hydrochloric acid (**HCl**), cellulase, and a pepsin solution. Many adaptations of this method, which is also known as the "HCl-pepsin-cellulase" method, have been published. Some popular examples are Dowman and Collins (1982) and Aufrère and Michalet-Doreau (1983) in their works to adapt the method to concentrate feedstuffs. The method by Aufrère and Michalet-Doreau (1983) was further modified by De Boever et al. (1986a). The goal of these modifications was to better mimic animal digestion, improve its accuracy and simplicity, and adapt the methods to new feeds. An example is the further modifications of the method developed by De Boever et al. (1986a), as described by De Boever et al. (1994). The method includes gammanase to improve the accuracy of palm kernel cake and a mixture of cellulase enzymes to improve the accuracy of fiber-rich feeds. Weisbjerg and Hvelplund (1993) also included gammanase and a mix of cellulases in the method (**EDOM**, or EFOS in Scandinavian languages), making these two methods similar. These last modifications can be categorized as “multi-enzymatic” methods. The use of NDF digestion with cellulase and amylase can also be used as another enzymatic method (Dowman and Collins, 1982).

In commercial laboratories, the use of near infra-red (**NIR**) analysis is widely used for digestibility determination in forage samples because it allows for the rapid estimation of many samples. However, a parallel biological reference method is needed for accuracy control. The control is common for forages because of the amount of analyzed samples, but it is difficult in rarely analyzed samples, such as concentrates and compound feeds (Weisbjerg and Hvelplund, 2005).

Within FESs, the INRA system proposes an estimation of OMD in compound feeds by using prediction equations and an enzymatic method. OMD measured by the pepsin-cellulase method proposed by Aufrère and Michalet-Doreau (1988) and the equation proposed by Giger-Reverdin et al. (1990) are the endorsed methods for compound feeds when the feed ingredients are unknown. The Feed into Milk System (Thomas, 2004) recommends an equation based on neutral detergent cellulase and gammanase digestibility (Thomas, 1988). CNCPS has two levels for energy determination: one mechanistic and one empirical (Tylutki et al., 2008). If information for calculating the mechanistic level (digestion and passage rates) is unknown, the empirical equation is used. In the empirical method, DE is calculated through an equation where the heat combustion coefficients are used instead of digestion fractions. The calculated DE could be further adjusted by intake level (Tedeschi et al., 2005a).

Regarding these systems, two main ideas can be considered: First, OMD is used as a parameter for energy estimation, though by different determination methods. Also, empirical equations can still be valuable in mechanistic models because data are unavailable. Therefore, two questions arise: 1) Is it possible to develop an empirical model to measure NEL_{20} in compound feeds within NorFor? 2) Can OMD, together with chemical components, be used as proxies to substitute for the need for degradation rates and indigestible?

For developing milk responses to changes in diet, knowledge of the evaluated components of the diet is essential. Any errors in the diet formulation will translate into errors in the energy estimation for the final diet (Dijkstra et al., 2005). Lower-than-targeted animal responses or excess nutrients will lead to environmental losses and unnecessary costs. These are some of the obvious consequences of errors.

2.1.2. Response approach

A desired update of the modern FESs is the inclusion of a milk response to feed approach. Currently, the formulation of diets is based on matching animal requirements with the nutrient supply of feeds, which means giving the necessary nutrients for a predefined production goal. However, a predefined diet optimization does not describe how performance will change with changes in the diet, which is a big limitation (Dijkstra et al., 2007). The predefined production approach cannot adapt to the evaluation of the different objectives that characterize the dairy industry today. A response approach will allow for the assessment of different diets for the same production goal and for an evaluation of different production goals in different circumstances, by, for example, evaluating the economic return of an improved diet (Brun-Lafleur et al., 2010).

Over the last decade, several attempts have been made to create empirical models of milk responses to changes in diet. Huhtanen and Nousiainen (2012) developed a model for ECM for silage-based diets, including a quadratic effect of ME, CFat, and non-fiber carbohydrates intake and a linear effect for metabolizable protein (**MP**). Jensen et al. (2015) described ECM responses to diet composition for specific breeds, parity, and stages of lactation. The explanatory dietary variables included a logarithmic response to the NEL supply for multiparous cows, including the linear effects of diet NDF, amino acids absorbed in the small intestine (**AAT**), and CFat. Moreover, from the models with the best fit, marginal ECM responses have been developed, which the authors suggested as the best approach for economical optimization. Daniel et al. (2016) also developed an empirical equation of milk responses to changes in the NE and MP supply; the results showed a curvilinear response for both variables and a positive interaction between them. From these results, Daniel et al. (2017) proposed an adaptation to cow's milk yield potential. Of all these models, only Daniel et al.'s (2017) was adapted into a FES when it was included in the INRA system (Faverdin et al., 2018).

All these efforts include changes in nutrient supply, such as energy, MP, fat, and fiber, but they do not consider the relationship between forage and concentrate in the diet. This relationship is important because forage and concentrate are the main feed components in ruminant diets (Van Soest, 1994), providing different nutrients into the rations. Forages are characterized by high content of the plant's cell wall, consisting of cellulose, hemicellulose, and lignin, also referred to as structural carbohydrates or NDF content (Van Soest, 1994). However, the group has a very variable composition and nutritive value (Wilkins, 2000). Forages comprise plant parts other than the grain, such as leaves, stems, and flowers, that are produced for grazing or harvesting for animal feed (Allen et al., 2011). Sown and permanent grasslands, straw, crops and their residues, and browse that can be grazed or harvested can be considered types of forages (Barnes and Baylor, 1995). On the other hand, concentrates can be defined as the group characterized by high energy or protein content, such as grains and by-products like oil-seed meals, bran, and vegetable and fruit waste (Wilkins, 2000). Concentrates are also referred to as supplements because forages usually cannot sustain high production levels on their own.

2.1.2.1. Forage quality

In northern European countries, the use of grass forage conserved as silage ensures the possibility of feeding cows during the winter (Bernardes et al., 2018), with minor energy and nutrient losses (Rinne et al., 1997; Mahanna and Chase, 2003). However, silage quality varies greatly because it is influenced by numerous factors during sowing, growing, harvesting, and ensiling. Solar radiation, temperature, water deficits, botanical composition, fertilization, and management are some of the factors influencing silage quality (Buxton, 1996). Although the concept of silage quality can be ambiguous, it is related to voluntary feed intake and digestibility (Buxton et al., 1995). Maturity is the parameter influencing quality the most (Rinne et al., 1997; Harrison et al., 2003). Maturity should be clearly separated from growth. Although growth is linked to mass and directly impacts yield, maturity refers to physiological events in the plant cycle, such as the leaf production, boot, heading, and bloom (Ritchie and Nesmith, 1991). As a plant matures (Figure 2.1), the proportion of stems increases and at the expense of leaves proportion decreasing and stems also thicken for support and protection. This results in an increased proportion of cell walls and NDF concentration. The increased NDF also has lower digestibility because cell wall thickening mainly occurs because of lignification

(Harrison et al., 1994), which makes NDF less digestible (Van Soest, 1994). The total protein production still increases, but the proportion of leaves decreases, thus decreasing the CP proportion in the plant (White and Wolf, 2009). Sugar content will increase with plant maturity (Weisbjerg et al., 2010a), with a switch in the sugar location from leaf to stems to grain.

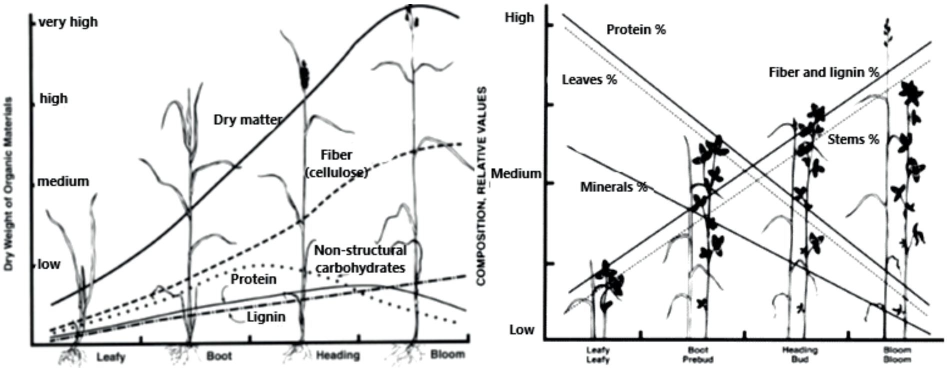


Figure 2.1: Changes in plant components' dry weight (left) and relative composition in the plant (right) as the plant matures (adapted from White and Wolf, 2009).

From a management viewpoint, the date of harvest is the most important tool for manipulating maturity at harvest (Table 2). Later harvest of a forage translates into lower OMD and CP content. Also, as mentioned, the higher NDF contents of later-harvested silages are characterized by lower digestibility. This is reflected by the higher content of indigestible NDF (**iNDF**) or acid detergent lignin (**ADL**) fractions. Although iNDF and ADL are not the same, they are highly correlated (Jančík et al., 2008), representing the indigestible fraction of the NDF.

The rate of maturity of a plant is ruled by temperature, so for a given age, plant maturity can vary (Ritchie and Nesmith, 1991; Hatfield and Prueger, 2015). Hence, at similar harvest dates, latitude, altitude, and yearly variation can affect plant composition. Differences in species (legumes vs. grasses and annual vs. perennial) and varieties (short vs. long season) included in the forage mix will also affect maturity because they present different temperature requirements for development (Hatfield et al., 2011; Hatfield and Prueger, 2015) and composition (Buxton, 1996). Moreover, the harvest season also impacts forage quality, which means that forage harvested from the primary growth will show a different composition from subsequent regrowth (Kuoppala et al., 2008). The number of factors involved explains the high variability of forages as a feed group.

Table 2: Effect of harvest date (early or late) on the chemical components of the silage.

Author	OMDs (%)		NDF g/kg DM		iNDF g/kg DM		ADL g/kg DM		CP g/kg DM	
	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late
Randby et al. (2012)[1]	80.6	70.8	477	601			46.4	64.7	166	113
Kuoppala et al. (2008)[2]	76.7	69.2	498	594	50	97	24	31	160	127
Prestløkken et al. (2008)[3]	81.4	74.7	450	545	58.5	111			202	138
Aston et al. (1994)[4]	82.8	68.8	479	647					191	135
Dønnem et al. (2011)[5]	83.2	66.3	433	584	-	-	46.8	75.9	156	105
Rinne et al (1997)[6]	79.5	62.3	409	623			18	23	187	109
Rinne et al. (1999)[7]	79.5	68.4	486	645			28	52	172	113

1 Early harvest: 30th May, Late harvest: 14th June
2 Early harvest: 5th June, Late harvest: 17th June
3 Early harvest: 1st June, Late harvest: 15th June
4 Early harvest: 19th May, Late harvest: 12th June
5 Early harvest: 19th May, Late harvest: 12th June
6 Early harvest: 29th May, Late harvest: 25th June
7 Early harvest: 13th June, Late harvest: 4th July

Digestibility determines the energy values of feeds (Allen, 1996) by defining the amount of nutrients that are available for potential use for different body functions. In silages, together with fermentation quality, digestibility has a large impact on intake (Rinne et al., 1997; Huhtanen et al., 2002; Huhtanen et al., 2008), directly affecting milk production (Martin and Sauvant, 2002; Hristov et al., 2005). Because of the factors discussed above, silage digestibility presents large variations, and this is reflected in Table 3. In this table, samples from silages from commercial farms shows large ranges of OMD of the silages (**OMDs**), from very low to high, with variations between location and year. Therefore, the importance of digestibility on milk production, together with this variability, makes studying how milk responds to different silage digestibility essential.

Table 3: Grass silage OMD (%) averages (minimum–maximum) for three years and three locations (NorFor, 2021a).

	OMDs (%)[1]		
	Denmark	Norway	Sweden
2018	74.0 (46.5–84.3)	71.9 (45.9–83.8)	73.6 (48.0–84.9)
2019	75.8 (44.2–87.5)	71.5 (48.8–83.3)	72.6 (48.0–83.9)
2020	74.7 (56.2–86.6)	72.7 (53.6–83.1)	74.2 (54.9–87.6)

[1] OMDs: organic matter digestibility of the silage

Additionally, the supplemented concentrate also has an impact on silage and total intake, and this impact could be further influenced by the quality of the silage that is fed to the animals (Huhtanen et al., 2008). Therefore, the relationship of concentrate level and silage digestibility could have a direct impact on how milk responds to the changes of this components in the diet.

Hence, there are some questions derived from the discussion above: How does milk production respond to different silage digestibility? How does the concentrate level and silage digestibility relate to and impact milk production? Is it possible to develop milk responses to changes in the concentrate supply level and silage digestibility to account for their effect? Is it possible to adapt the response functions to NorFor?

2.1.2.2. Factors affecting milk responses

The responses of milk to diet changes could be influenced by animal characteristics such as parity (primiparous vs. multiparous), stage of lactation (or days in milk), breed, milk yield potential, and management practices such as feeding strategy.

Because of the climate, Nordic dairy production is considered an indoor production system because most of the year, animals are housed, so their diets are based on conserved grass, mainly in the form of silage. Indoor feeding strategies can be divided into total mixed rations (**TMR**) and separate feeding (**SEP**) as the two extremes, with “in-between” strategies, which are referred to as partial mixed rations (**PMR**). TMR consists of the total mix of concentrate and forage prior to feeding. This strategy is based on group feeding and assumes that the herd average milk yield and animal characteristics balance the risks of over- or underfeeding. This feeding strategy is commonly used in Denmark

(Alstrup, 2015), which is characterized by big herds with more than 200 cows (Statistics Denmark, 2020). In Norway, the average herd size is 28 cows (SSB, 2020), whereas in Sweden, it is 98 cows (Jordbruksverket statistik, 2021), making SEP or PMR more popular strategies. Although SEP has a total separation between concentrate allocation and forage, PMR mixes a proportion of the concentrate with the forage. These strategies are usually based on feeding cows silage ad libitum but still optimize the concentrate individually. The benefits and drawbacks of feeding strategies have been widely discussed (Holter et al., 1977; Gordon et al., 1995a; Schingoethe, 2017). Little et al. (2016), Purcell et al. (2016), and Henriksen et al. (2019) showed that milk and total intake did not differ between strategies, but the intake pattern differed. Little et al. (2016) showed that for the TMR rations, increased milk because of higher intake could be explained by an increase of both concentrate and silage. However, the increase of milk production in SEP intake was because of a concentrate increase, with a decrease in silage intake. The authors showed a lower response of milk yield per kg of dry matter intake (**DMI**) in the TMR group because cows with high yields should eat more silage to obtain more concentrate. Therefore, when evaluating milk responses to the changes in any of these components, the differences in feeding strategies could be important.

Within animal characteristics, the lactation stage can also have an impact on the milk response because the physiological state of the animal could affect nutrient partitioning into milk production. This can be reflected in the negative effect of days in milk (**DIM**) in the response model developed by Huhtanen and Nousiainen (2012) and the higher marginal response in the early lactation stages reported in Jensen et al.'s (2015) response function and by Kirkland and Gordon (2001). The early lactation stage partitions more nutrients into milk, resulting in higher milk responses to dietary changes. Coulon and Rémond (1991) reviewed the linear response of milk to energy supply in early lactation, showing the response was curvilinear in mid-lactation and stating that this linear behavior in early lactation could be attributed to the energy requirements never being met during this lactation stage. Lactation number was shown to also affect milk response because primiparous cows will divide the nutrients between milk and growth, which could result in a lower response of milk to changes in the diet (Coulon and Rémond, 1991; Jensen et al., 2015). Finally, breed and potential milk yield has shown that although the total milk yield could be different for the same diet intake, similar milk marginal responses were shown for different breeds (Jensen et al., 2015) and milk potential (Daniel et al., 2017).

2.1.2.3. Environmental impact

Dairy production account for 20% of the greenhouse gas emissions of the livestock sector, of which 46% is represented methane emissions (**CH_4**, Gerber et al., 2013). CH_4 from ruminants is a by-product of ruminal and hindgut microbial fermentation, which comes from a process called as methanogenesis. When feed is fermented by the microbial community, nutrients become available for cow utilization, while H^+ and CO_2 are also produced, where the methanogen community can convert them into CH_4 (McAllister and Newbold, 2008; Martin et al., 2010), as shown in Equation 2.

$$CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O \qquad \textit{Equation 2}$$

Several strategies, such as feed additives like tannins (Duval et al., 2016), 3-nitrooypropanol (Haisan et al., 2014), garlic (Olijhoek et al., 2016), red algae (Roque et al., 2019), and fat supplementation (Alvarez-Hess et al., 2019), have been studied for CH_4 mitigation. Silage digestibility and concentrate level can also impact CH_4 emissions because of their effect on total feed intake and diet composition (concentrate:forage ratio), which are known to impact CH_4 emissions.

As feed intake increases, the digestibility of the total diet decreases because of an increased passage rate in the rumen. As a result, microbes will have less fermentable matter per kg available but more total fermentable matter (Mathison et al., 1998), increasing total H^+ production. In contrast, a higher passage rate promotes propionate production, which is a sink source of H^+ (Boadi et al., 2004). All of this translates into increased total CH_4 production but in a decrease of CH_4 per kg feed (Johnson and Johnson, 1995). Moreover, as previously mentioned, intake has a positive effect on milk production; thus, CH_4 per unit of milk could also decrease.

Altering the concentrate level—thus the concentrate and forage proportion—also affects CH_4 production because it affects the acetate:propionate ratio and pH in the rumen. Forage promotes acetate production, which results in more H^+ available in the rumen for methanogenesis. Concentrates will promote propionate production, which is a sink source of H^+, resulting in less substrate for methanogenesis. In addition, high rates of concentrate fermentation will reduce ruminal pH, inhibiting the growth of methanogens. A review by Van Gastelen et al. (2019) showed that for dairy cattle, an increase of the concentrate ratio in the diet increases CH_4 production due to an increase of intake, but this leads to a decrease of CH_4 per unit of feed and milk by 14% and 12%, respectively. However, total CH_4 production was shown to decrease with increase concentrate ratio by some studies (Ferris et al., 1999; Aguerre et al., 2011), while others showed no differences (Jiao et al., 2014; Patel et al., 2011).

Finally, more mature forages can increase CH_4 production because they present more structural carbohydrates and, therefore, a higher retention time in the rumen (Moe and Tyrrell, 1979), with slower fermentation rates. This will lead to increased acetate production, hence promoting methanogenesis (Johnson and Johnson, 1995). The results collected by Van Gastelen et al. (2019) showed that the higher digestibility of silage promotes total intake, increasing total CH_4 production by 14% but reducing CH_4 per unit of feed and milk by 19% and 10%, respectively.

Changes in silage digestibility and concentrate level can affect the proportions of crude protein and phosphorus offer and its utilization, hence affecting N and P excretions to the environment. The availability of P is affected by the total P intake, protein content, ST degradability, and grain proportion in the diet (Braithwaite, 1976; Klop et al., 2013). P excretion influences the level of P in the water, which causes rapid algae growth, which will increase oxygen consumption, harming aquatic animal life (Knowlton et al., 2001). N excretion via urine can undergo ammonification of urea, causing ammonia volatilization into the air, which can be deposited in solid form and converted to NO_3^-, leaking into subsoils. Also, denitrification can escape as nitrous oxides, which is a well-known greenhouse gas (Tamminga, 1996).

2.2. Big data in agriculture

Adopted technologies that collect data systematically and solutions that store these data have resulted in a tremendous growth of data in agriculture. Within animal production, technologies for data gathering include historical data of animal characteristics, ground sensors (milking robots, automatic feeders, ear tags, weight scales, sound detectors, eating sensors), and camera sensors (body condition score, movement, etc.; Kamilaris et al., 2017). This large influx of data has been given the name 'big data.' Morota et al. (2018) defined big data as "data often so large that limits visual inspection, most of the time the number of columns is higher than rows. This data is 'messy' because it misses some observations and contains noise." The characteristics of this data makes it out of scope for the manipulation by traditional statistics used by scientists. Instead, big data analytics has appeared as an alternative, and several authors have highlighted machine learning (**ML**) as a viable and the most commonly used tool for analysis (Kamilaris et al., 2017; Neethirajan and Kemp, 2021).

ML is a branch of artificial intelligence, which has the main goal of learning from example data (i.e., learning from training data) to predict and infer from new incoming data (test data). As defined by Ayodele (2010), the concept of "learning" does not involve consciousness but finding patterns and relationships in the data in difficult learning environments, such as big data. The learning process can be supervised when a clear task is commanded or unsupervised, which is defined as the discovery of hidden patterns in the data. The created algorithms can include regression, classification, clustering, decision trees, and others.

2.2.1. Big data in livestock production

Although ML is used frequently in crop agriculture, Ellis (2021) and Liakos et al. (2018) clearly demonstrate the slower development of big data analytics in livestock production by comparing the few studies found in this sector. This could be attributed to the slower adoption of technologies and digitalization, unclear view users have of its worth, and the high efforts required for creating a common dataset because it involves the fusion of several data sources (Liakos et al., 2018). Examples of ML use in the dairy sector are even scarcer. The identification of important events such as eating, laying, and walking using neck sensors (Dutta et al., 2015; Vázquez Diosdado et al., 2015), inertial measurement units attached to the back of the cow (Achour et al., 2019), GPS data (Williams et al., 2019), accelerometers (Arcidiacono et al., 2017), and the identification of chewing patterns (Pegorini et al., 2015) are the most popular use cases of ML. Within production, the use of milk fatty acid composition in milk to detect the molar proportions of individual fatty acids has shown promising results (Craninx et al., 2008) and would allow for the evaluation of different diets for milk production. Finally, the prediction of weight trajectories from a few early measurements could allow for the accurate estimation of weight evolution of individual animals (Alonso et al., 2015).

The Norwegian dairy sector is an excellent case for using big data analytics in dairy production. First, it has a high level of digitalization, with more than 45% of farms including automatic milking systems (**AMS**, Vik et al., 2019). From this system, information about each milking, milk composition, feed intake, and animal characteristics, such as weight and body condition score, can be obtained. Additional data about the animal, such as breed, lactation stage and parity, and milk analysis, can also be obtained from the Norwegian Dairy Herd Recording System (**NHR**, TINE SA, Ås, Norway). Moreover, the problem of data storage from several sources is being successfully solved by using a cloud-based system called Nordic Cattle Data eXchange (**NCDX**; Kyntäjä et al., 2018). However, because of the early stages of NCDX, few attempts have been made to use it in productivity tools and solutions. Big data becomes an opportunity for FESs because data are crucial for further development. Also, it allows for gathering many diets and milk production scenarios with real-time changes. The availability of different diets, with different proportions of concentrate level and silage, is a great opportunity for the adaptation of the response functions to a farm level. Hence, is it possible to use big data obtained from Norwegian dairy farms in a milk response approach to changes in diet? Is it possible to adapt the milk response approach to specific farms and use them as a decision tool? Is it possible to use a large influx of data as an input and to use ML within FESs?

3. Aims of the thesis

The purpose of the current project was to create new knowledge to be included in the NorFor system and to be used in practice. Based on the questions that originated from Chapter 2, I found two main areas for research and adaptation in NorFor: the development of an alternative model to estimate the net energy of compound feeds and the development of a model describing milk production responses to changes in diet components supply.

For the energy estimation of compound feeds, a series of activities were conducted, leading to two scientific papers (Papers I and II). Concerning the response function's development, the activities performed in this project translated into three scientific papers (Papers III, IV, and V). In this chapter, each paper's specific objectives and hypotheses are explained. These specific objectives tend to be small steps that work toward achieving the overall goal.

3.1. Paper I

The objective of Paper I, titled "Precision and additivity of organic matter digestibility obtained via in vitro multi-enzymatic method," was to evaluate the precision of EDOM (Weisbjerg and Hvelplund, 1993) as a way to measure the OMD of compound feeds. This paper evaluated the repeatability and reproducibility of EDOM and established a relationship between the OMD of individual feed ingredients and the compound feed produced thereof. We hypothesized that the OMD measured by EDOM would be precise.

3.2. Paper II

The objective of Paper II, titled "A static model for estimating energy content of compound feeds in a dynamic feed evaluation system," was to develop an empirical model for the prediction of NEL_{20} of compound feeds. The model should be independent of ingredient composition, degradation rates, and indigestible fractions. Our hypothesis was that OMD as measured by EDOM and the chemical components measured in compound feeds are good predictors of NEL_{20} of compound feeds.

3.3. Paper III

The goal of Paper III, titled “Effect of digestibility of silage and concentrate intake on milk yield: a meta-analysis,” was to develop a model describing the responses in milk production to the changes in grass silage digestibility and concentrate supply, here based on previous experiments. We hypothesized that lower concentrate levels could be used with highly digestible silages when compared with low-digestible silages, without compromising milk production.

3.4. Paper IV

The aim of Paper IV, titled “High-digestible silages allow low concentrate levels without affecting milk production,” was to test the model developed in Paper III by challenging silage digestibility against different concentrate levels and evaluating their interactions. Also, the study evaluated these scenarios from a climate perspective, analyzing CH_4 emissions for different silage digestibility and concentrate levels. The hypothesis was that the model developed in Paper III would be accurate, so high-digestible silage (**HDS**) with low concentrate levels would have milk production similar to low-digestible silages (**LDS**) with higher concentrate levels.

3.5. Paper V

The intention of Paper V, titled “Machine learning is a useful tool to determine milk yield responses to diet changes from real-time farm data,” was to adapt the developed response function from Paper III and have it work using real-time data from farms. Our hypothesis was that the use of ML would allow for the visualization and analysis of data coming from farms (and within each farm, each cow, and each milking) and that these data could be adapted using theoretical functions to specific farms. The developed specific functions can serve as a decision-making tool within farms regarding feed efficiency and economy.

4. Methods and results

This chapter summarizes the methods and main findings of the five papers included in the current thesis. The goal of this chapter is to briefly explain the experimental procedures, that is, sample collection, chemical analysis, and statistical analysis, along with the key results to provide a better understanding of the discussion that follows. A more detailed description of the methods and results can be found in each paper, which can be found at the end of this thesis.

4.1. Paper I

Precision and additivity of organic matter digestibility obtained via in vitro multi-enzymatic method

This study was designed to evaluate whether EDOM was a good method to predict OMD of compound feeds that could be used in the estimation of NEL_{20} (Paper II). To meet the objectives, 70 compound feed samples were collected from Denmark, Sweden, and Norway from six feed companies. The ingredients used for the formulation of these compound feeds were also collected, resulting in 79 ingredients (149 samples total). The inclusion proportion of these ingredients into the collected compound feeds was known because the recipes were collected for the 70 compound feed samples. The 149 samples were analyzed for OMD by EDOM, DM, and ash at lab1, with two repetitions per sample that were separated in space and time. A subset of 49 compound feed samples were sent to lab2 for OMD measurement by EDOM.

EDOM precision was evaluated by repeatability (agreement of measurements within the lab of the same sample) and reproducibility (agreement of the measurement of same sample between laboratories). Repeatability was evaluated for the 149 samples (70 compound feeds and 79 ingredients) for the repeated measurements performed in lab1 by intraclass correlations (**ICC**, using rptR package and linear mixed model) and repeatability coefficient (**rcoef** = $\sqrt{2} \times 1.96 \times SD_{within\_sample}$). Reproducibility was evaluated for the 49 compound feeds analyzed in both laboratories by a Bland-Altman plot and interlaboratory correlation (**ILC**, using rptR package and linear mixed model).

Repeatability of EDOM was high, showing an ICC of 0.989, meaning that only 1.1% of the variation was because of errors in the measurements. The estimated rcoef was 2.2%, which can be described as the maximum future differences of OMD measured by EDOM, in 95% of the cases, between the measurements (in the same lab). The reproducibility results showed an ILC of 0.926, meaning that 7.4% of the differences were because of measurement errors between laboratories. Moreover, the estimated OMD mean difference between laboratories was 0.4% OM, suggesting that lab2 showed higher OMD results than lab1, even though the differences were not significant.

4.2. Paper II

A static model for estimating energy content of compound feeds in a dynamic feed evaluation system

This paper aimed to create a model to estimate the energy content of compound feed samples out of OMD (measured by EDOM) and chemical components as measured directly in compound feeds. For this, 75 compound feed samples, together with their ingredients (108 samples), were collected from six feed companies in Denmark, Sweden, and Norway over a period of two years. Forty-nine of these compound feed samples corresponded to the compound feed samples evaluated in Paper I.

For model development, reference values of NEL_{20} for the 75 compound feeds were calculated according to NorFor by adding the NEL_{20} values of the ingredients according to their proportion in the mixture (see Equation 1). Calculation of the ingredients' NEL_{20} was done according to NorFor by using degradation rates and indigestible fractions from NorFor Feed Table.

An empirical model was developed, here with NEL_{20} of compound feeds as the dependent variable. Independent variables for potential inclusion in the model were digestible organic matter (**DOM** = OMD % x OM % DM), CP urea corrected (**CP_{corr}**), CFat, NDF, ST, and ash. All of these variables were analyzed in the compound feed samples. The order of inclusion of the independent variables was determined by Akaike information criterion (**AIC**) stepwise forward selection. Year and company effects were included as random effects and compared with models without their inclusion. The model was validated by cross-validation using predicted residual error sum of squares (**PRESS**) and root mean square error of prediction (**RMSEP**). The additive property of the independent variables was evaluated to analyze its effect on the prediction of NEL_{20}.

The best model, NEL_{20} = 3.69 -0.0435NDF +0.0997CFat +0.0393DOM +0.0234CP_{corr}, presented the lowest AIC corrected (**AICc**) and RMSE (0.149 MJ/kg DM). Models including company and year of production as random effects showed higher AICc, so they were not included in the final model. Urea correction from total CP showed a better fit than models with uncorrected CP.

The predicted NEL_{20} values from the model with the best fit model were compared against the reference NEL_{20} values and against the European Union's tolerance limit for error in the declaration of energy values (± 5%). No predicted values lay outside these limits. Differences between values of the explanatory variables (NDF, CFat, DOM, and CP_{corr}) calculated by the weighted sum of ingredients and measured directly on compound feeds of was the main source of model error.

4.3. Paper III

Effect of digestibility of silage and concentrate intake on milk yield: a meta-analysis

Paper III's objective was to develop responses of milk production to changes in concentrate intake based on silage digestibility. For this, the literature was reviewed. To be included, studies should have included grass as the predominant silage species, with at least two levels of silage digestibility and two levels of concentrate within digestibility tested. This resulted in 80 group means from nine published studies. A summary of the included studies can be seen in Table 4 below.

The evaluated dependent variable was ECM (3.14 MJ/kg). The evaluated independent variables included OMDs, silage intake as net energy (**NELs**), dry matter intake of the concentrate (**DMIc**), concentrate intake as net energy (**NELc**), concentrate to forage ratio in the diet (**C:F ratio**), and chemical components of the ration (NDF, AAT, CFat). Data were analyzed using mixed models with study as the random effect. The level of significance for the inclusion of an effect in the model was 0.05. Model fit comparison was done using the Bayesian information criterion (**BIC**) statistic, coefficient of determination (**R^2**) for mixed models, and RMSE.

Table 4 : Studies included in Paper III's meta-analysis

References	Cows	Breed[2]	OMDs (%) [3]	Concentrate level[4]
Aston et al. (1994)	40	H	68.8, 82.5	3, 6, 9, 12 kg
Rinne *et al.* (1999) [1]	32	FA	68.2, 74.9, 77.9, 79.6	7, 10 kg
Ferris et al. (2001)	48	H	71.2, 81.4	Hi: 10%, 30%, 50%, 70% Me: 32%, 48%, 64%, 80%
Kuoppala *et al.* (2008)	24	FA	67.3, 68.9, 69.2, 72.7, 73.4, 76.7	8, 12 kg
Prestløkken *et al.* (2008)	32	NR	74.1, 74.7, 81.4, 81.6	4, 10 kg
Randby *et al.* (2012)	66	NR	69.3, 76.3, 80.6	0, 4, 8, 12, 16 kg
Sairanen and Juutinen (2013) exp 1	36	FA	72.7, 74.2, 77.3	9, 11, 13 kg
Sairanen and Juutinen (2013) exp 2	40	FA	69.2, 76.3	4, 6.5, 8, 11.5 kg
Alstrup et al. (2016)	24	H	73.9, 74.2, 76.2, 79.1	20%, 50%

[1] The treatments without a rapeseed effect were taken into consideration. [2] H: Holstein, FA: Finnish Ayrshire, NR: Norwegian Red. [3] Range of in vivo organic matter digestibility tested in each study. [4] Concentrate levels presents as percentage correspond to the total mixed ration feeding systems. Hi: High-digestible silage. Me: medium digestibility silage.

The best model for ECM responses presented a curvilinear effect of the DMIc, linear effect of OMDs, and an interaction between DMIc and OMDs:

$$ECM = -16.1 + 4.2\ DMIc - 0.07\ DMIc^2 + 0.50\ OMD - 0.03\ DMIc * OMDs.$$

Higher digestible silages achieved higher yields with lower concentrate intakes. Moreover, the interaction between DMIc and OMDs translates into lower marginal yields obtained with high-digestible silages when the concentrate intake increases. The substitution rate (**SR**), meaning the change dry matter intake of the silage(**DMIs**) when DMIc is change by 1 kg DM was 0.53 and was not affected by silage digestibility or concentrate level.

4.4. Paper IV

High-digestible silages allow low concentrate levels without affecting milk production

A representation of the experimental design can be seen in Figure 4.1. The study was divided in three periods with a completely randomized block (parity) design. For the pre-experimental period (**PreP**), 60 Norwegian Red cows, 26 primiparous and 34 multiparous, were fed a common silage (half early and half late cut) and concentrate level according to yield, as optimized by NorFor. For response period 1 (**RP1**), after PreP, the cows were randomly allocated to two groups (30 cows per group), where each group was offered an LDS, or a HDS and a common concentrate optimized according to milk yield from PreP according to NorFor. For response period 2 (**RP2**), each silage group was further divided into three subgroups (10 cows each), increasing 2 kg DM concentrate (Plus2), decreasing 2 kg DM concentrate level (Minus2), or not changing concentrate level form RP1 (Standard).

<table>
<tr><th>PreP</th><th>RP1</th><th>RP2</th></tr>
<tr><td>20 days</td><td>17 days</td><td>20 days</td></tr>
<tr><td rowspan="6">60 cows
Mix silage</td><td rowspan="3">High-digestible silage
group (HDS)
30 cows</td><td>HDS-Plus2 group (10 cows)
Increase 2 kg DM/day</td></tr>
<tr><td>HDS-Standard group (10 cows)
Same concentrate as RP1</td></tr>
<tr><td>HDS-Minus2 group (10 cows)
Decrease concentrate by 2 kg DM/day</td></tr>
<tr><td rowspan="3">Low-digestible silage
group (LDS)
30 cows</td><td>LDS-Plus2 group (10 cows)
Increase concentrate by 2 kg DM/day</td></tr>
<tr><td>LDS-Standard group (10 cows)
Same concentrate as RP1</td></tr>
<tr><td>LDS-Minus2 group (10 cows)
Decrease concentrate by 2 kg DM/day</td></tr>
</table>

Figure 4.1: Schematic representation of the experimental design of Paper IV

Silage and concentrate intake were recorded daily. Silage and concentrate were fed separately, and their samples were chemically analyzed for chemical composition and OMD. Milk production was recorded in the AMS for each individual cow at each milking. Milk samples were taken at the end of each experimental period for each milking for a 48-h period. The samples were analyzed for fat, protein, and lactose. CH_4 daily emissions were recorded with two GreenFeed units throughout the experiment.

For RP1, the effect of silage digestibility (HDS vs. LDS), change of concentrate (from PreP), and their interactions were evaluated for milk yield, ECM, milk components, DMIs, DMIc, DMI, weight, CH_4, and CH_4 per unit of DMI and ECM. For each dependent variable, a covariate of the same parameter from PreP was included in the model. For RP2, the effect of concentrate level (Minus2, Standard, Plus2) was evaluated for the same dependent variables; silage digestibility was only included in the interaction term. For this period, results from RP1 were included as covariate in the model . Models were analyzed using mixed models, where cow was included as random effect.

Effect of silage digestibility: the DMI was 3.0 kg DM higher for cows in HDS than for LDS. Although DMIc was higher for LDS because of a higher offer (because of NorFor optimization), LDS presented 3.8 kg DM lower DMIs, explaining the lower total DMI. The higher yields for HDS were not expected because the treatments in RP1 were optimized to achieve similar yields. Higher DMI for HDS could explain the higher milk and ECM yields (3.0 kg and 3.5 kg higher, respectively) for this treatment because the feed efficiency (milk/kg DMI and ECM/kg DMI) was similar between silage treatments. Regarding milk composition, only lactose content had a trend as being higher for HDS, so milk fat, protein, and lactose yield were higher for HDS because of a higher milk yield.

Daily CH_4 emissions showed no differences between silages, but because HDS presented higher DMI, the CH_4/DMI was lower for HDS. Both CH_4 per DMI and ECM showed a significant decrease with an increase in DMI, regardless of silage digestibility.

Effect of concentrate level: For HDS, DMI was not affected by increase or decrease of the concentrate level because of the decrease or increased of silage intake, respectively. However, DMI had a different pattern for LDS, with a lower intake for the Minus2 group compared with the other concentrate levels, because DMIs did not increase when the concentrate level was reduced. On average, the substitution rate was 0.59 but was higher for HDS than for LDS (0.78 vs. 0.42).

Milk yield was affected by concentrate level in the same way for both silages, with higher yields for Plus2 and lower for Minus2. However, ECM was affected differently, with no differences when concentrate level was reduced for HDS, while LDS showed low ECM yields for the Minus2 group. Feed efficiency was similar between concentrate levels, regardless of silage digestibility.

Daily CH_4 emissions were higher with concentrate increase, though this did not translate to differences in methane per DMI nor ECM.

4.5. Paper V

Machine learning is a useful tool to determine milk yield responses to diet changes from real-time farm data

To develop a model to adapt the results from Paper III to handle real farm data, two datasets were created: a training dataset included studies with known OMDs, DMIc, and ECM yield, resulting in three data sources: the research studies used in Paper III, Paper IV's results from RP2, and data from two commercial Norwegian farms where Paper III was tested. The model developed from the training data was subsequently applied to the "Live farm dataset." This dataset included data from two sources: NCDX and NHR.

Prediction of silage digestibility was performed by a logistic regression of kg ECM per kg of concentrate (**concentrate efficiency**) to changes in the concentrate. The evaluation of individual cow responses in the algorithm was done by comparing the algorithm results using the data of Paper IV with the changes in concentrate efficiency from RP1 to RP2 of individual cows.

Prices of milk, silage, and two prices for concentrate were considered for the economic calculation of profits per cow, here reflecting Norwegian prices. A substitution rate of 0.53 was considered in the calculation.

The training dataset resulted in 19 data points, of which each data point was a group of at least three cows. The live farm data resulted in 1,290 animals from 157 farms.

Only a binomial classification of silage digestibility—low and high digestibility—was possible because of the size of the training dataset. The gradient of the linear regression, meaning how concentrate efficiency is reduced with increasing concentrate, was the most important feature for labeling silage digestibility. High-digestible silages showed a steeper reduction of concentrate efficiency with increased concentrate intake when compared with LDS. Individual cow's concentrate efficiency to changes in concentrate intake above 4 kg DM showed comparable results with the algorithm, allowing the use of this algorithm for the individual level.

The inclusion of prices for an economic evaluation showed that HDS would be more sensible to concentrate price changes than LDS. Although HDS optimum could be affected by concentrate prices, LDS profit increases with increasing concentrate because of the lower impact that concentrate increases have on concentrate efficiency. However, similar profits for LDS can be obtained with HDS and lower concentrate levels. However, this economic evaluation tool is incomplete because silage intake is a missing parameter from the live farm data and could not be included in the costs.

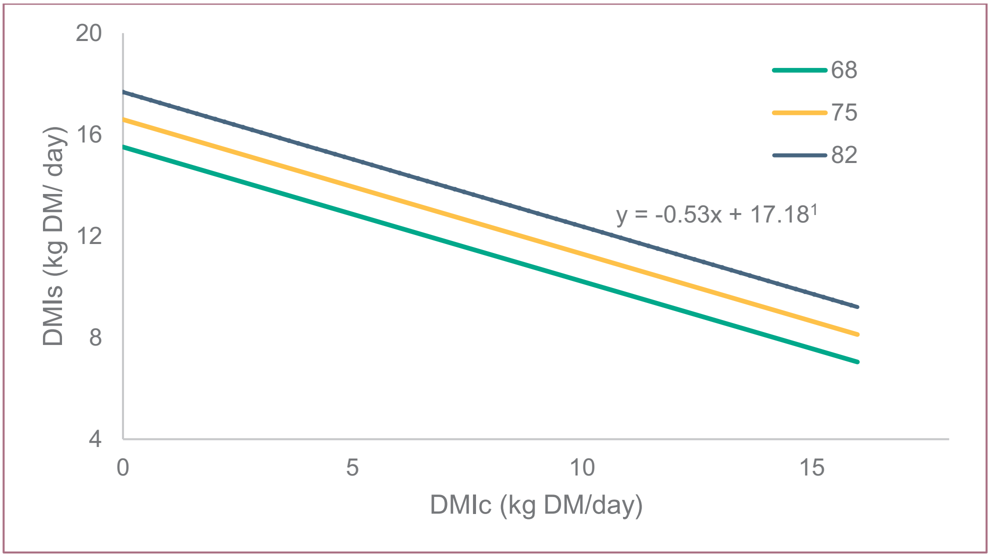
5. Supplementary results

In this chapter, the results that are not contained in the papers but are relevant for the discussion are included.

5.1. Paper III

This meta-analysis was published as a conference paper, which has strict length rules. Therefore, many interesting results were omitted or reduced. Hence, below, some additional results for Paper III are shown.

As shown in Figure 5.1, the predicted DMIs showed a linear relationship with DMIc, with higher intakes for higher OMDs (0.15 kg increase per percentage of OMDs increase). The reduction of DMIs with increasing DMIc was 0.53, which was reported as the SR. No interaction was found between DMIc and OMDs. The model resulted in DMIs = 4.88 – 0.53 DMIc + 0.15 OMDs.



[1]Regression equation in the figure is OMDs = 82% and was set as example for the visualization of the SR (0.53). DMIs: dry matter intake of silage. DMIc: dry matter intake of concentrate.

Figure 5.1: Dry matter intake of silage according to concentrate level for three silage digestibility profiles used (68%, 75%, and 82% OMDs).

Feed efficiency (ECM/kg DMI, DMI calculated as DMIc + DMIs) and ECM responses to an increase in DMIc are shown in Figure 5.2. Comparing the optimums, the feed efficiency optimums are achieved with a lower concentrate intake compared with maximum ECM. Higher OMDs show higher efficiencies at low concentrate levels. In this example, DMIc higher than 8 kg DM shows higher efficiencies for lower OMDs.

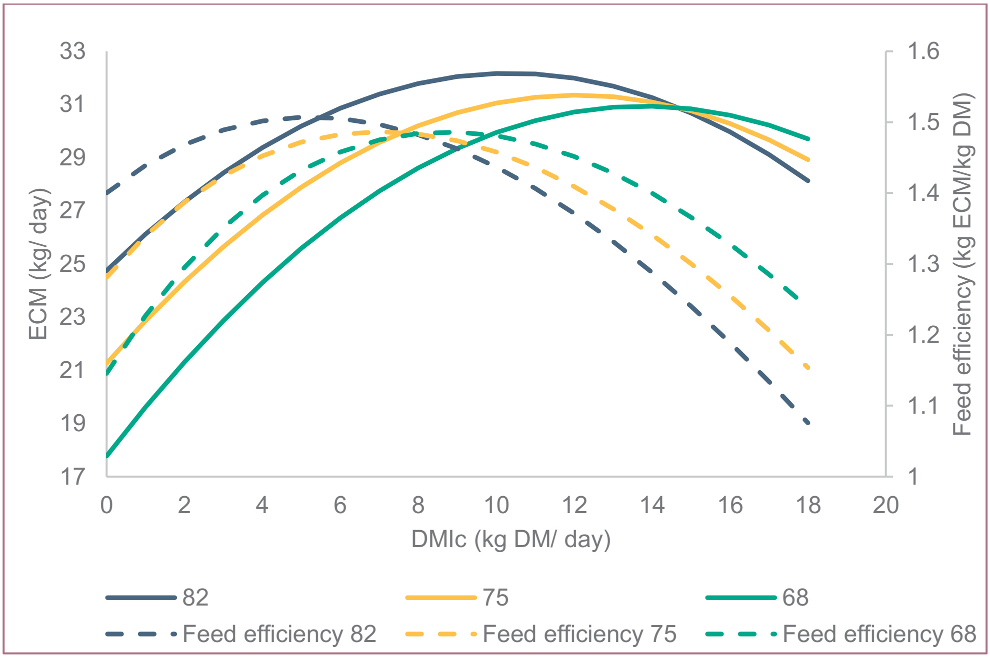
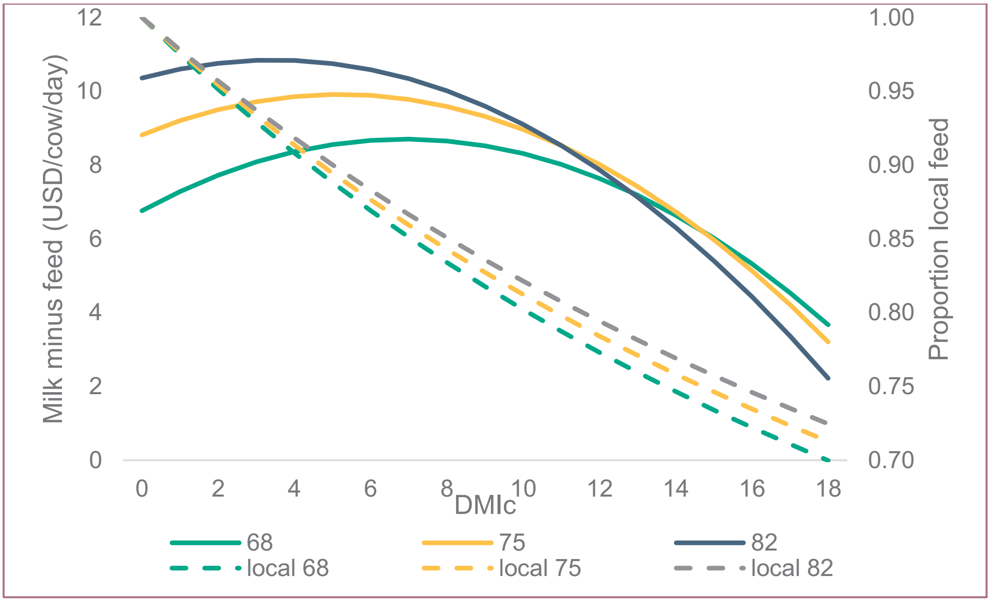


Figure 5.2: ECM response and feed efficiency according to concentrate intake level for the three silage digestibility profiles used.

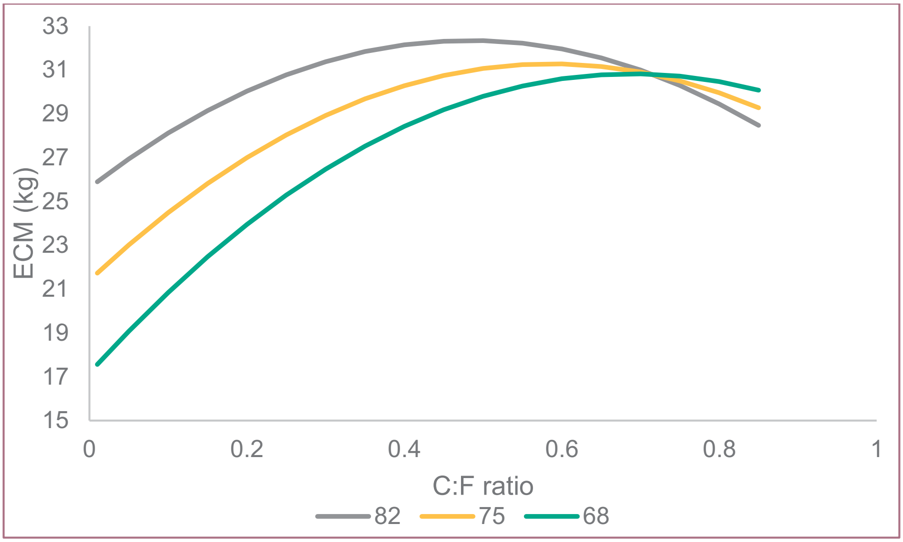
Figure 5.3 shows the responses of profit per cow and percentages of local feed to increase DMIc for different silage digestibility levels. The economic response shows higher profits for higher OMDs to increase DMIc for low concentrate levels, with the differences disappearing as DMIc increases. Higher OMDs showed a higher percentage of local feed in the diet, regardless of concentrate level.



[1]Milk minus feed = ECM * Milk price – DMIs * Silage price – DMIc * Concentrate price. Milk price 0.59 UDS/ECM 0.69 UDS/kg DM concentrate 0.24 UDS/kg DM silage (prices used in Paper V).
[2] Proportion of local feed: Proportion of feed in the diet with Norwegian origin. Silage assumed to be 100% Norwegian origin; concentrate assumed to be 60% Norwegian origin (Animalia, 2020).

Figure 5.3: Economic response (milk minus feed)[1] and proportion of local feed[2] in the diet according to concentrate intake level for the three silage digestibility profiles used.

Although DMIc was the parameter that showed lower RMSE in the selected model (1.29 kg ECM), the use of concentrate intake as a proportion of inclusion in the diet is important for production systems that TMR as a feeding strategy. Therefore, Figure 5.4 shows the model with the best fit, including the concentrate ratio as a variable describing the concentrate intake (RMSE = 1.39 kg ECM).



ECM= -30.0 + 115.9 C:F ratio – 35.0 C:F ratio^2 + 0.667 OMDs – 1.02 OMDs * C:Fratio.
C:F ratio: concentrate to forage ratio in the diet

Figure 5.4: ECM response with a developed model including concentrate:forage ratio instead of DMIc as an explanatory variable level for the three silage digestibility profiles used.

6. General discussion

6.1. NEL_{20} model

In most cases, mechanistic models require a high level of information, which is not easily obtained. The challenge of FESs is to develop systems that can reflect our understanding of the biology that governs the process but that will still be accessible for use (Tedeschi et al., 2005b). Paper II demonstrates that the use of empirical models as supplements in mechanistic feed systems is still valuable.

6.1.1. Parameters in the model

6.1.1.1. Organic matter digestibility

The selection of OMD as a potential proxy to be included in the NEL_{20} model was based on several motives. First, digestibility is the basis for energy evaluation systems, and table values rely on standard digestibility rather than on a variable digestibility that is dependent on the actual production situation (Weisbjerg and Hvelplund, 2005). Second, as mentioned in Section 2.1.1.2, experience from other equations includes OMD as explanatory variable, and studies have demonstrated an increase of accuracy when OMD is included in the prediction equation rather than only in chemical components (Cottyn et al., 1984). Finally, as it will be discussed next, the study of methods for estimating OMD is vast, allowing the selected method for OMD determination to be supported by scientific evidence.

The selection of EDOM as the method for OMD determination of compound feeds was a decision based on scientific facts, but also was made from a practical perspective because the end goal was to include this method in NorFor and for it to be useful in practice.

From a scientific standpoint, the accuracy of EDOM has been tested against other methods for estimating OMD. Here, accuracy is defined as the closeness of a measure to its real value. As mentioned in Section 2.1.1.2, in vivo values are considered the reference values for OMD; therefore, a comparison with them is considered a measurement of accuracy. Palić and Muller (2006) evaluated EDOM's accuracy for a mix of forage and concentrate samples, showing lower errors for EDOM than for GP but higher than TT (RMSE = 3.6%, 5.5%, and 3.0%

OMD, respectively). Palić and Leeuw (2009) compared the same methods for complete diets and showed the lowest error for EDOM (RMSE = 2.7% and 6.6% and 3.8% OMD, respectively). The results for forages are contradictory, with Søegaard et al. (2001) showing a lower error for EDOM against a rumen fluid method (RMSE = 3.1% vs. 3.5% OMD, respectively) and Weisbjerg et al. (2007) showing better results for the latter (RMSE = 3.7% vs. 2.0% OMD). Weisbjerg and Hvelplund (1996) showed that EDOM is accurate for compound feeds when compared with digestibility results from in vivo in sheep (2.3% RMSE with regression coefficient of 0.87 and R2 = 0.90). No accuracy comparison against other methods has been performed for EDOM in compound feed samples. However, De Boever et al. (1986b) showed that for compound feeds and concentrate ingredients, there was a lower error for the enzymatic method developed by De Boever et al. (1986a) against TT (RSD = 1.7% vs 2.1 % OMD). De Boever et al. (1994), which added gammanase and more cellulases to the previously mentioned method, showed better results than TT for compound feeds rich in protein and fat (RSD = 1.84% vs. 2.97%). Although these are not the same methods, EDOM was based on De Boever et al. (1986a) for its development (see Section 2.1.1.2) by adding similar enzymes as De Boever et al. (1994). Therefore, this could suggest that similar accuracy results could be achieved with EDOM for compound feeds.

Precision studies of EDOM for compound feed samples were lacking in the literature, and this was the main objective of Paper I (see Section 3.1). Precision is a synonym of consistency, so it refers to the closeness between measurements of the same sample. In this work, precision was evaluated through repeatability (closeness between measures within the same lab) and reproducibility (closeness between measures of different labs). High repeatability and reproducibility ensure that EDOM can be applied as a common method, and values can be compared over time and regions. Thus, if EDOM was to be selected for an energy estimation equation to be used in practice, the study of EDOM's precision in compound feeds would be central.

In Paper I, we evaluated EDOM's repeatability by analyzing two repetitions per sample in batches separated by time. Only the variability between batches was evaluated because a higher variability between than within batches was shown by De Boever et al. (1986b). This is reasonable because analyzing samples in different batches will increase the error sources, such as the handling processes and separate dates. The results showed that EDOM's error of measurements between batches in the same lab (rcoef of 2.2% OM) is similar to that reported by De Boever et al. (1986b) for an enzymatic method testing two cellulase types (2.5% and 2.3% for two cellulase types). In line with this, the reproducibility of the method was expected to show higher variation than the measurements between batches because even more sources of variations are involved, such as different technicians, instruments, and locations. This was confirmed in Paper I because the correlation between batches was ICC = 0.989, while that of between labs was ILC = 0.926.

A critique of Paper I could be that the reproducibility results were only performed by comparing two laboratories (see Section 4.1). However, Søegaard et al. (2001) and Weisbjerg et al. (2007) showed, for example, the repeatability and reproducibility of EDOM compared with methods using rumen fluid. The higher reproducibility of other enzymatic methods compared with rumen fluid methods has also been shown in other studies (De Boever et al. 1986b). The lower reproducibility of the rumen fluid method could be attributed to the use of different donor animals (Holden, 1999), which the enzymatic method would be able to standardize because of the use of commercially available enzymes. The selection of a method reliant on commercially produced enzymes could also be a critique of Paper I because a cease in production could mean a threat to the whole methodology. However, the possibilities for replacing lacking enzymes with new enzymes had been registered before for EDOM, and the possibilities for future substitution have been demonstrated by Kokić et al. (2013).

From a practical point of view, the choice of EDOM was an advantage for numerous reasons. EDOM has been used since 1994 in Denmark as the official method for declaring energy values of compound feeds in the Scandinavian Feed Unit System. Moreover, it is the reference method in NorFor to determine OMD for some forage types (Åkerlind et al., 2011). This means that laboratories across Scandinavia already use this method. Moreover, the use of commercial enzymes—not rumen fluid—makes the method easy to implement. Also, the instruments required for this procedure are few and are commonly available in any laboratory (pH indicator, ash determination oven, crucibles, vacuum wash, and bath incubator). Thus, the accuracy demonstrated when it comes to EDOM by other authors, the precision demonstrated in Paper I, and the convenience of its use support the selection of EDOM as the method for OMD estimation as a potential variable for NEL_{20} estimation.

6.1.1.2. Chemical components

The chemical components included as the other potential variables for the energy estimation model are in accordance with the feed fractioning suggested by NorFor (Volden, 2011b). It was considered important to include easily obtained variables. Therefore, only the analytical feed fractions of CP, NDF, CFat, ST, and ash were included, avoiding subfractions and degradation rates. Moreover, the RestCHO was not included either because it is calculated and highly correlated with the other fractions (RestCHO = 100 - the analyzed fractions). Likewise, sugar was not included because it is not a routine analysis.

Table 5 summarizes examples of the equations predicting the energy of compound feeds that include OMD as a variable measured by enzymatic methods. In addition to OMD, all equations include CFat, while structural carbohydrates (expressed as crude fiber or NDF) are shown in most, followed by CP. The inclusion of nonstructural carbohydrates was shown by including ST in the German equation and as sugar in the Scandinavian Feed Unit system for high sugar feed samples. The negative coefficient of NDF in our model agrees with the other equations in the table and could be explained by the negative correlation between NDF and energy density because structural carbohydrates, in general, are less digestible than other fractions (Weiss, 1993). Therefore, the NDF content in concentrates will reduce the average energy value. In contrast, CFat and CP will increase it.

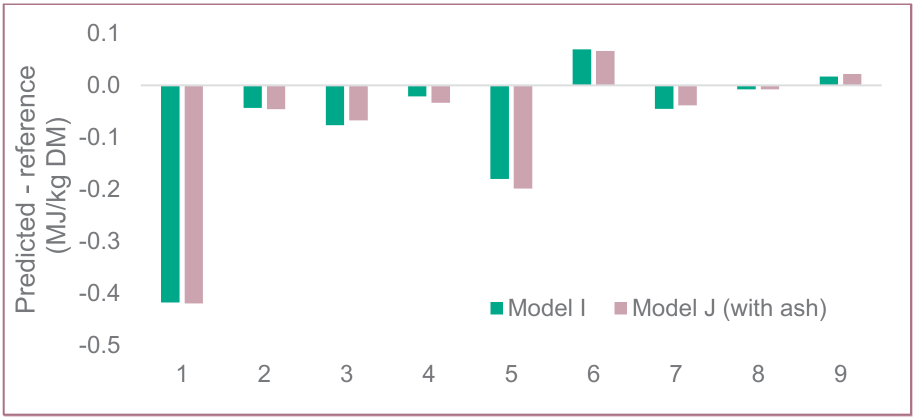
Most examples in Table 5 also include ash in their equations, which differs from the equation developed in Paper II. The inclusion of ash as a parameter in Paper II had a worse fit (higher AICc) and increased the error of prediction (RMSE from 2.08% to 2.11%). De Boever et al. (1994) found that the chemical parameters included depended on the digestibility method used, type of concentrate, and type of energy expression (DE, ME, NE). In their study, ash was included in the equation for fiber-rich samples, which were characterized by including more than 29% NDF. Nine samples presented this NDF content in Paper II's dataset, but no differences in the prediction error were found for these samples when compared with the model including ash (model J in Paper II), as shown in Figure 6.1a. When the residuals were regressed against the ash content for all samples, no differences in the predictive error were found (Figure 6.1b). Weiss (1993) attributed the low variability of ash in concentrates as being the reason for inadequate energy estimation, which, in our study, is also the chemical parameter with the lowest SD.

Table 5: Summary of the equations in the literature for predicting energy of compound feeds using enzymatic methods.

Author	Country/Region (FES)	Energy unit measured	Parameters* in model	OMD method	RSD	RMSE
Paper II	Scandinavia (NorFor)	NEL (NEL_{20})	OMD[1] CFat NDF CP_{corr}	EDOM: Multienzyme[2]	2.0%	0.15 MJ 2.08%
Sauvant et al. (2002)	France (INRA, 2018)	Ed	OMD CFat NDF CP Ash	HCl-pepsin-cellulase[3]		1.5% Ed
GfE (2009)	Germany	ME	OMD CFat NDF CP Ash ST	HCl-pepsin-cellulase[4]		2.04%
Cottyn et al. (1984)	Belgium	NEL (VEM)	OMD CFat NDF	HCl-pepsin-cellulase[5]	3.0%	
Thomas et al. (1988)	UK (Feed into Milk)	ME	OMD CFat	NDGD[6]		
Weisbjerg and Hvelplund (1993)	Denmark (SFU system)	NEL (SFU)	OMD CFat CP C Ash Sugar[8]	EDOM: Multienzyme[2]	0.062 SFU	
DeBoever (1994)	Belgium	NEL	OMD CFat CFiber[9] Ash	Multienzyme[10]	2.8% (5.4%[11])	
Aiple et al. (1996)	Germany	NEL	OMD CFat CP Ash	HCl-pepsin-cellulase[7]		2.6%

*Abbreviations of the parameters explained in the List of Abbreviations.
[1]OMD in the equation as DOM_{EDOM}
[2] Weisbjerg and Hvelplund (1993)
[3]Aufrere and Mechalet-Doreau (1988)
[4]VDLUFA (1993) from De Boever et al. (1986b)
[5]Kellner and Kirchgessner (1976)
[6]NCDG: Neutral detergent cellulase plus gammanase digestibility (MAFF, 1993)
[7]DeBoever et al. (1986)
[8]Sugar correction if sugar content is higher than 20% DM.
[9]Crude fiber and ash for fiber-rich concentrates
[10]De Boever (1994)
[11]RSD for fiber-rich concentrates

a.



b.

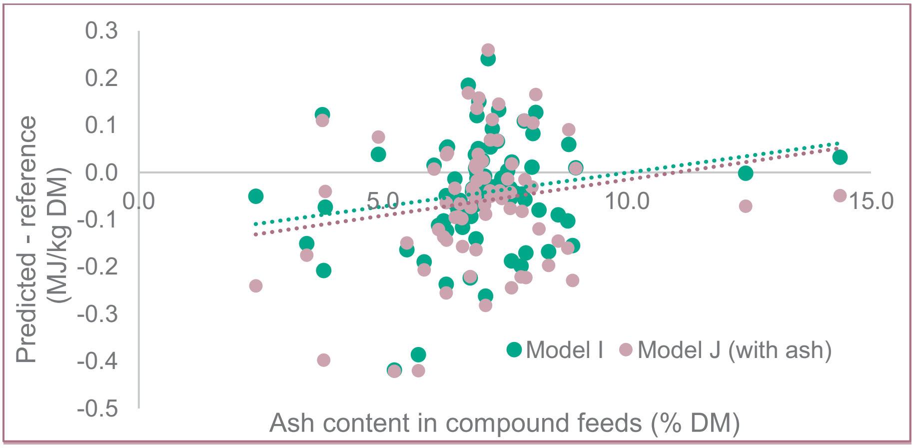


Figure 6.1. a. Comparison of residuals (NEL_{20} predicted – NEL_{20} reference) when comparing the selected model (model I) with the model including ash as a variable (model J). b. Comparison of errors (NEL_{20} predicted – NEL_{20} reference) when comparing the selected model (model I) with the model including ash as a variable (model J).

6.1.2. NEL_{20} and other energy estimation models

The NEL_{20} equation error is comparable—or in some cases—lower than others (Table 5). This is an advantage of accepting the new model. However, because the equations were developed with different datasets, a strict comparison cannot be made. A comparison of the equations could be done by evaluating the predictive ability of the same dataset. However, the model developed in Paper II is in line with previously developed models in the literature, with the advantage of being adapted to predict NEL_{20} of Scandinavian compound feeds in NorFor. Thus, if the model depends on the dataset used for development, as De Boever (1994) suggested, it is adapted for the Scandinavian region. The calibration of NIR to measure NEL_{20} or the use of NIR for the determination of the chemical components of the model could make the developed method even cheaper and faster, while maintaining accuracy (De Boever et al., 1995; Aufrère et al., 1996).

6.2. Responses of milk to changes in the diet

6.2.1. Selection of response model's variables

The evaluation of concentrate intake in the meta-analysis was considered in mass (DMIc) and energy (NELc) terms. For an estimation of the concentrate intake in energy terms, using the equation developed in Paper II was not possible for the response function developed in Paper III because the main parameters, such as EDOM, were not reported in the studies included in the meta-analysis. Moreover, as discussed, NEL_{20} is an energy value calculated with fixed standardized parameters, but in this response approach, these conditions fluctuate, such as the proportions of diet components and intake level. Thus, the use of NEL_{20} was not appropriate. Therefore, to evaluate the intake of concentrate in energy terms, NELc was calculated as the proportion of total NEL intake (based on the C:F ratio in kg). However, because the total NEL depends on the intake levels and interactions between diet components, the calculated NELc is not completely independent from the total and the silage intake. Therefore, the best fit (lowest BIC) for DMIc as a description of concentrate intake was expected. The use of concentrate intake as DMIc is also convenient because it can be easily obtained from farms. As seen in Paper V, the use of DMIc allowed for the development of the algorithm to be used for real-time farm data. If NELc had been the selected variable, the adaptation would not have been possible because information on the total intake and energy concentration is not available from the NCDX platform.

In line with this, a better fit of the response model by including OMDs instead of NELs was expected because of the independence of OMDs with the total intake and concentrate intake. The selection of the concentration of DOM in silage DM (**D-value**), could have been used instead of OMDs as a descriptor of silage digestibility. However, the end goal was to develop a practical model, and OMDs are easier to obtain because no extra calculation from the analysis is needed. Also, OMD determination by NIR analysis is broadly used in practice, making it even easier and cheaper. If calibrated, portable NIR to determine OMD in farms would be even more useful, avoiding sampling and sample shipping to the laboratory.

6.2.2. Main outcomes

6.2.2.1. Use of meta-analysis

Individual animal studies are useful for establishing cause and effect and hypothesis testing, but general conclusions cannot be inferred because the conditions of a single study are very specific. Meta-analyses are useful because they summarize the results from several studies using objective statistical methods to test a global hypothesis (Sauvant et al., 2008). Therefore, the use of a meta-analysis in Paper III seemed appropriate to test the relationship between grass silage and concentrate level to create a general conclusion. Paper IV involved an animal trial to test this meta-analysis, so Paper IV's objective could be criticized: If the goal of a meta-analysis is to summarize studies, why perform another study? Paper IV was created from a different perspective than the studies gathered in the meta-analysis. Paper IV tested the meta-analysis by changing the concentrate supply from individually optimized diets, while the other studies compared similar concentrate supply levels with different silage digestibility profiles. Therefore, Paper IV tested Paper III by imitating today's practice in farms and simulating a possible use of this response function. This means optimizing diets according to yield but then integrating the new response approach to decide a level of concentrate to achieve a certain goal. As shown in Paper IV, the use of the response approach as a complementary tool for previously optimized diets could be a viable way to incorporate it into the current FES.

One of Paper III's main findings is that for a defined concentrate level, higher yields are achieved with high-digestible silages because of higher intakes. The effect of higher intake levels for high-digestible silages on milk production was also shown in Paper IV for both optimized diets (RP1). However, this intake comparison between silages is not fair because both silage treatments were optimized to have similar milk outputs, but HDS intake was higher than predicted. Nevertheless, at similar and not optimized concentrate levels (treatment HDS-Plus2 vs. LDS-Minus2), the intake and yield were numerically higher for HDS (because no statistical comparison between treatments could be made because of the experimental design). The effect of silage digestibility on the intake was shown by other authors. For example, a negative relation between OMDs and fill value was proposed for forages in the NorFor system (Volden, 2011a), with higher fill value translating into less intake potential of that forage. A higher fill value for low-digestible silages was also shown in Paper IV. The silage intake index proposed by Huhtanen et al. (2002) and Huhtanen et al. (2007) also showed higher intakes with increased digestibility because the D-value had a positive impact on silage and total intake (Huhtanen et al., 2008). Moreover, the meta-analysis of Weisbjerg and Johansen (2017) showed an increase of ECM and total intake with increased OMDs (up to 82% OMDs).

Another take from Paper IV is that high yields were maintained by feeding HDS when the concentrate level was reduced from the optimized level, but this could not be achieved when feeding LDS. This demonstrates the value of a response approach as a decision tool because different milk production responses are a result of changes in the diet.

6.2.2.2. Comparison with other response approach models

The response models in the literature and mentioned in Section 2.1.2 include different variables for the estimation of milk responses because they have different goals. Thus, a direct comparison of the model developed in this work with a previously developed model cannot be performed. However, the quadratic response of milk shown by Huhtanen and Nousiainen (2012) and Daniel et al. (2016) to energy intake agrees with the quadratic response to DMIc shown in Paper III. Jensen et al. (2015) showed a natural logarithmic relationship of milk to increased intake. Regardless of whether this response is quadratic or logarithmic, studies have agreed that increased intake led to diminishing increments of milk production. In Paper III, these decreasing increments with increasing DMIc were more pronounced for high-digestible silages, which are also referred to as lower marginal milk responses. The lower marginal responses explain the vanishing differences in milk yield for higher silage digestibility at high concentrate levels. Several explanations for the differences in milk responses can be found in the literature. Lower marginal milk responses for high-digestible silages could be driven by intake because the higher SR reported for high-digestible silages with increasing concentrate will translate into lower intake increases (Huhtanen et al., 2002; Kuoppala et al., 2008) and, therefore, a lower milk response. Faverdin et al. (1991) concluded that energy balance is the most important factor driving SR, with a higher energy balance showing higher SR. Therefore, at the same concentrate level, silages with higher digestibility and energy content will translate into cows with a higher energy balance and, thus, higher SR. However, the meta-analysis from Paper III showed no differences in SR between silages, nor were these differences found by the meta-analysis performed by Huhtanen et al. (2008). However, Paper IV showed higher SR for the high-digestible silage when concentrate offer was increased (0.78 for HDS-Plus2 vs. 0.42 for LDS-Plus2 treatments) and a lower marginal response of milk. Lower marginal milk responses could also be because of a shift of nutrient allocation toward body reserves. Randby et al. (2012) showed a linear increase of body weight with increased concentrate supply and silage digestibility, though this was not detected in Paper IV. Another explanation could be the negative effect of concentrate increases on NDF digestibility (Alstrup et al., 2016, Rinne et al., 1999) because of reduced pH in the rumen affecting cellulolytic bacteria. The decrease in NDF digestibility could be more accentuated in high-digestible silages because of the lower total pH in the rumen. To sum up, as explained by Ferris et al. (2001) and shown in Papers III and IV, higher milk yields can be achieved by improving silage digestibility or increasing the concentrate supply.

6.2.2.3. Factors influencing the response approach model

As referred to in Section 2.1.2.2, milk production responses can be influenced by factors such as parity, breed, lactation stage, and feeding strategy. However, our meta-analysis results did not differentiate for these factors. Parity was not tested in the model because it was not included as a variable in the dataset. This was because some of the collected studies did not differentiate the results by parity. For breed effect, popular breeds such as Jersey were not included in the data because the collected studies fitting the criteria did not use the Jersey breed. The selected studies included Holstein, Norwegian Red, and Finnish Ayrshire, but no effect of breed was found ($P = 0.89$). This is different from the results shown by Jensen et al. (2015), who indicated higher yields for Holstein than for the Norwegian Red breeds included in our study. In that study, the differences between breeds in milk were absolute and expressed by models having different intercepts but showing similar marginal milk responses to the diet. Likewise, Huhtanen and Nousiainen (2012) found no interaction between the milk yield potential of the cow and ECM marginal responses. Therefore, transforming Paper III's response variable from absolute ECM to the marginal yield could be a way to analyze this, independent of the breed differences not detected in our study.

Days in milk were not significant in the meta-analysis. This could be explained by the characteristics of the studies involved, which reported results from calving up to mid lactation. Thus, it is not possible to identify studies with clear early, mid, or late lactation responses.

In the meta-analysis, two studies used TMR as a feeding strategy (Ferris et al., 2001; Alstrup et al., 2016), while the rest used an SEP strategy (see Table 4). When only studies with the SEP strategy were included as a dataset, the same type of ECM response was detected, curvilinear to DMIc and with an interaction between DMIc and OMDs. For the SEP dataset, a numerically higher response of ECM to linear DMIc was shown (5.4 vs. 4.2 kg ECM/ kg DMIc), along with a similar response to the quadratic term of DMIc, OMDs, and their interaction compared with the dataset including all strategies. The substitution rate was numerically lower for the SEP dataset compared with the dataset including all strategies (0.45 vs. 0.53). As discussed in Section 2.1.2.2, the higher response of ECM by more concentrate intake in the SEP agrees with the meta-analysis. However, the lower SR for the SEP dataset was a surprise because it was expected that silage intake would present a higher reduction when concentrate increases. The variability of SR shown by Thomas (1987) and the low number of TMR studies included in the meta-analysis could explain this. Despite the numerical differences, the type of response of ECM was similar for both strategies (quadratic to DMIc and linear to OMDs, with a significant interaction between the two). Therefore, it supports the inclusion of the two strategies in the dataset. The difference shown in SR could be a critique of the developed model and should be considered for future use.

6.2.3. Opportunities and challenges

The study of the relationship between silage digestibility and concentrate intake to milk responses is valuable from several perspectives. The possibility of evaluating multiple scenarios is the main advantage of the response approach compared with a predefined production level.

From a production perspective, we would be able to assess different production scenarios. Given silage digestibility, the model would allow for a determination of concentrate supply level for maximal or optimal production. It is clear that by maximum production, the highest milk production would be targeted; however, optimal production can have several connotations. If by optimal production we refer to the highest feed efficiency, the selected concentrate level would differ from the maximal production (Figure 5.2). On the other hand, if optimal refers to the highest profit, the optimal concentrate intake differs from the ones needed for maximal production and highest feed efficiency (Figure 5.3). It seems from these figures that the optimal concentrate level for the highest profit is closer to the concentrate level needed for achieving the highest feed efficiency than needed for maximum production. This is valuable when trying to understand that the maximum yields do not always translate into higher profit. However, it is important to emphasize that Figure 5.3 is an example based on Norwegian input variables. Regional differences and changes in the price scenarios make the economical optimal production fluctuate.

The developed response model can also be useful for prioritizing locally produced feeds over imported feeds, an issue that has gained importance in recent years. In the Norwegian ruminant market, on average 40% of the concentrate ingredients used in compound feeds are imported, represented mostly by soybean, rapeseed, beet pulp, molasses, and maize imports (Animalia, 2020). Maximizing silage intake by supplying high-digestible silages is a useful strategy to increase the proportion of locally produced feed in the diet, which is visualized in Figure 5.3. Favoring silage feeding is also an advantage for other reasons. Ruminants have the unique characteristic of digesting cell wall content because of microbial fermentation in the rumen, allowing for the utilization of nutrients from feeds not suited for other livestock (such as monogastric animals) or human consumption. Moreover, forages contain substantial amounts of protein, and high-digestible silages usually have more of this protein than low-digestible silages. As a result, high-digestible silage reduces the need for imported protein feeds, allowing for further increases in locally produced feeds, such as grains. In addition, silages in Nordic countries are based on perennial grass species, such as meadow fescue and timothy (Steinshamn et al., 2016). Perennial species prevent soil erosion because of a constant surface covering and deeper root system than the annual crops used for concentrate production. Also, higher carbon sequestration is achieved than with annual crops (Freibauer et al., 2004; Soussana et al., 2004). Finally, increasing the proportion of silages in the diet is also an advantage economically in Norway, where the price of concentrates is usually higher than that of forages.

Paper IV presented methane emissions as another layer of complexity in the relationship between silage digestibility and concentrate intake. The results from Paper IV showed that methane emissions per unit of milk and intake were driven more by the total intake than by diet composition. Lower emissions per unit of milk and intake were shown at higher intakes. Therefore, both feeding more digestible silages and increasing the concentrate intake in the case of low-digestible silages would be good alternatives for reducing methane intensity and yield. The relationship between silage digestibility and concentrate level from an environmental perspective should be further studied to create a response approach. A response model was not possible to develop because the studies included in the meta-analysis did not consider methane. In addition, several studies separately evaluated the effect of silage digestibility (Gordon et al., 1995b; Brask et al., 2013; Warner et al., 2017) and concentrate level (Moe and Tyrrell, 1979; Beauchemin and McGinn, 2005; Liu et al., 2012) on methane emissions, but studies exploring both were not found. Åby et al. (2019) used the production results from Randby et al. (2012), which were included in Paper III, for an evaluation of CO_2-equivalent emissions in farms through a farm-scale model (HolosNor). The results showed lower CO_2 equivalents per kg ECM for high-digestible silages, but the effect was not linear. Moreover, the concentrate level showed different results within silage digestibility, showing a possible interaction between the diet components. Considering methane in the response approach will add an extra level of complexity and probably have an impact on the selection of the concentrate level.

Finally, the response approach model could be useful for selecting OMDs to achieve a certain goal and optimize the harvest according to this target (Johansen, 2017). However, targeting a specific digestibility can be complex because of the curvilinear development of silage digestibility with increased maturity (Rinne et al., 1999). Also, the compromise between yield and digestibility should be considered because an earlier harvest translates into lower yields (White and Wolf, 2009). However, earlier harvests will allow for more cuts or more development time for the following cuts, reducing the effect of the total season biomass production (Weisbjerg et al., 2010a), but costs increase with an increasing number of cuts.

6.2.4. Response approach and big data

The opportunities of the response function that are described in Section 6.2.3 become even more useful when adapted to real-time data. Paper V could be described as an attempt to adapt the response function (Paper III) into real-time farm data. Currently, the developed algorithm can predict a silage digestibility class while regressing the concentrate efficiency to increase concentrate intake for individual farms. The results are useful to evaluate the quality of the fed silage, if not already analyzed, and to have an assessment of the farm's performance in efficiency terms. As discussed in Paper V, the training dataset was not large enough to allow for the division of more classes for silage digestibility (only two classes were possible) nor prediction as a continuous variable (as OMDs). A larger dataset will allow for more detailed prediction of silage digestibility. Moreover, the more detailed prediction would allow for using the algorithm backward. That is, selected OMDs could show a regression for concentrate efficiency. These selected OMDs could be the currently fed silage, a desired goal, or the OMDs that will be used for the next feeding period. The algorithm will allow the farmer and advisor to plan the concentrate levels.

The developed economic response is incomplete because the silage intake cannot be obtained from the AMS or NHR. The use of weighing troughs to measure the silage intake is discarded because of the infrastructure and investment involved; these are suitable for research facilities, not commercial farms (Pahl et al., 2016). A possibility will be the periodic evaluation of input–output forage and dividing this by the number of fed animals. Paper V showed that it is possible to consider individual cow responses in the algorithm. However, the forage intake calculation method described above is based on group averages. The use of intake prediction equations and sensors to predict forage intake could be an alternative to feeding bins with tag recordings (Beauchemin, 2018). For example, Øvreeide (2019) showed that chewing and ruminating activity, as measured by monitoring collars, together with body condition score, improved NorFor's intake prediction model by 38% (RMSE reduced from 1.6 to 0.99 kg). In addition, Halachmi et al. (2015) showed an error reduction of 29% (RMSE 4.1 vs. 2.9 kg/day) when feeding behavior (number and distribution of meals), as measured by a feed intake monitor system, was included in the NRC intake prediction equation. This type of monitor is available at the farm level, and the information from these monitors could be included in databases, like NCDX, for individual forage intake predictions.

6.3. Implementation in NorFor

6.3.1. Implementation of NEL_{20} empirical model

The model in Paper II was developed to estimate NEL_{20} of compound feeds when the ingredient composition is unknown. The model could also be used by farmers as a cost-efficient alternative to an ingredient analysis when creating farm mixtures. From the feed producers' outlook, the model could be used to verify that the final product matches the planned energy content. Feed producers already analyze for CP and CFat content, and only an NDF and EDOM analysis needs to be included. The use of NIR for analysis of the components included in the model could be beneficial because of the faster and cheaper determination when compared with a chemical analysis. In addition, the use of chemical reagents and the produced pollutants will be avoided (De Boever et al., 1995; Aufrère et al., 1996).

The model can also be used if disagreement about the energy content of a product arises between the producer and purchaser. The model could then be used to compare the energy value of the purchased product with the declared value in the label. Until now, this was not possible because a lack of ingredients composition information limited the original calculation model.

From the standpoint of NorFor, the NEL_{20} predicted value could be included in NorFor's Feedstuff Table (**FST**) as an additional parameter. Feed companies provide their declared NEL_{20} values to NorFor by uploading them into the FST, and NEL_{20} values obtained with the model could be compared with the declared values. To include the predicted value by the model in the FST, NorFor could collect yearly samples from feed producers for analysis.

In Paper II, the maintenance of the model was discussed. Maintenance is needed because the parameters used for the calculation of the reference NEL_{20} values could change, affecting model prediction. Also, new ingredients or by-products that were not considered when the model was developed could become popular in ruminant feeding. Moreover, changes, for example, digestion rates and indigestible fractions, could change the reference NEL_{20} and, thus, the dataset used for model development. To account for these changes, the model needs to be updated to keep it accurate and relevant.

6.3.2. Implementation of response approach model

The use of a response approach into a system using a predefined level of production for diet formulation is by no means antagonistic but instead complementary. Several ideas emerge when analyzing the ways of implementing the response function into NorFor. This is reasonable because of the flexibility of the response approach, which offers several opportunities, as discussed in Section 6.2.3.

The model developed in Paper III could be used during diet formulation. Diet formulation can be performed as usual, and the response functions can be offered to the user to evaluate the different needs and scenarios, such as feed efficiency and profit. In addition, it could also be implemented for the evaluation of the results of a given diet. Currently, NorFor offers the 'Feeding Control' tool within their optimizing software (NorFor, 2021b); this tool is used for an evaluation of the farm results (efficiency and economic return) for a given diet and to make decisions for future feeding. Thus, this is an opportunity to include the algorithm developed in Paper V. Feed efficiency can be assessed, as shown in Paper V, by evaluating the concentrate efficiency. From this, the decision to increase or decrease the concentrate supply can be made. Paper V also showed that an evaluation for individual cows is promising. However, this was only one example, and more tests on farms should be done to account for individual responses.

For the economical use of the algorithm, profit per kg of feed can be calculated as the absolute or relative profit. For the absolute profit, silage intake needs to be obtained to account for the total intake. Silage intake can be calculated as the average intake per cow through the input–output calculation or predicted by the NorFor intake. On the other hand, the relative profit can be performed by setting the current profit as a reference, showing marginal profit changes from this point. The advantage of the first option is that the absolute total profit is evaluated but to the detriment of using averaged or predicted silage intake values. As discussed in Section 6.2.4, this could have disadvantages, such as prediction errors. Alternatively, the relative profit will be independent of the calculation of silage intake. However, it will not deliver a total profit but will show marginal gains. Because the goal of the response function is to evaluate results because of changes, this option can be as useful as the total profit evaluation.

For both economic options, the inclusion of SR is important because the change in concentrate intake will impact the silage intake. The substitution rate is a variable that depends not only on silage quality (Paper IV), but also on concentrate type, concentrate level, and cow status. Inaccurate SR could impact the economic calculation and result in wrong decisions being made. Therefore, NorFor's efforts should be centered on an accurate prediction of SR. Finally, if individual silage intake is to be used, Paper IV showed the underprediction of the NorFor intake model for high-digestible silages. In the intake model, NorFor includes a metabolic regulation factor, which decreases intake for high quality silages, accounting for metabolic regulation. This factor could be influencing the underprediction of intake for these types of silages and further study of this problem should be considered.

The use of this algorithm, as with most opportunities, also comes with limitations. The first limitation is that its use is limited for AMS systems because it depends on the automatic outflow of data. For other systems, the use of the model developed in Paper III could be a good alternative. The second limitation appears between feeding strategies. The data for the developed algorithms in Paper V were mainly obtained from farms using SEP feeding. As discussed in Sections 2.1.2.2 and 6.2.2.3, feeding strategy can have an impact on milk responses and, thus, on concentrate efficiency with changes in concentrate supply. In addition, because of the large herd sizes, many farms with AMS use TMR with a flat rate of concentrate offered in the milk robot. Therefore, the feed is not individually optimized, and the concentrate supply cannot be linked to their milk production response. This limits the use of the algorithm because the real benefit from it is the individual optimization of concentrate to milk responses. For TMR systems, Bossen et al. (2009) showed that the shift to a mixed ration with lower energy combined with individual concentrate feeding according to live weight changes to monitor deposition/mobilization is a promising strategy for mixed ratio feeding in AMS. The use of the algorithm with this strategy could be successful and should be tested. For TMR systems, the model shown in Figure 5.4 could be used as an alternative.

7. Conclusions and perspectives

The dairy industry needs high productivity to cover the demands of an increasing population while still remaining efficient in their use of inputs, such as feed, and having a minimal environmental impact. The current thesis contributed new feed knowledge to address these demands.

The EDOM method (Paper I) is precise and practical for the measurement of OMD of compound feeds, fitting the needs of a model for estimating energy. The developed model for NEL_{20} estimation in compound feeds (Paper II) is useful for NorFor because of a low error rate compared with other models. The model is independent of ingredient composition, passage rates, and indigestible fractions and includes OMD, CFat, NDF, and CP_{corr} as input variables. Currently, these variables need to be obtained through chemical analyses. A future perspective would be to approve the use of NIR for the analysis of these variables, reducing the costs and time for the energy determination of compound feeds.

The meta-analysis (Paper III) concluded that ECM responds curvilinearly to increased concentrate intake, and this response is lower with increased silage digestibility. Paper IV verified the lower marginal responses for high-digestible silages, concluding that high milk yields can be maintained with a low concentrate supply if high-digestible silages are fed. Thus, increasing silage digestibility or increasing concentrate intake for low-digestible silages are alternatives to increase milk production. Paper V concluded that the developed response approach can be adapted to farm data by transforming the responses variable (ECM) into concentrate efficiency responses. The developed algorithm classified silage as highly digestible because of the steeper decrease in concentrate efficiency with increased concentrate supply. This also verifies the conclusions of the meta-analysis (Paper III) and the findings in the experiment (Paper IV). The algorithm allows for the evaluation of the concentrate efficiency for a specific silage, here from a group and individual cow level. The relative economic return is useful because it shows the changes in profit and, thus, the possibilities for manipulating income. However, the algorithm to use real-time farm data can be improved, such as by having a more detailed classification of silage.

Future perspectives of this work include increased utilization of the input resources in milk production through a more precise description of energy concentration in compound feeds. In addition, the model developed for the prediction of milk production responses to diet changes creates scenarios for efficient, sustainable milk production with a low environmental impact. Moreover, the ML provides perspectives for the adaptation of response functions to real-time data, which would be valuable for maximizing the utilization of available information from the rising numbers of adopted technologies in farms.

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Precision and additivity of organic matter digestibility obtained via in vitro multi-enzymatic method

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Precision and additivity of organic matter digestibility obtained via in vitro multi-enzymatic method

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ABSTRACT

The enzymatic digestibility of organic matter (EDOM) method is an in vitro multi-enzymatic method for estimating the organic matter (OM) digestibility of feeds. The EDOM method previously showed high accuracy with in vivo values for compound feeds. The aim of this study was to evaluate the precision of the EDOM method and determine its additivity, compared with the long-assumed additive property of the chemical components of compound feeds. 149 feed samples, 70 commercial compound feeds and 79 associated ingredients, were analyzed in a laboratory (lab1) for OM digestibility measured by EDOM (OMD_{EDOM}) with 2 repetitions separated in time to estimate repeatability. Of the total samples, 49 compound feeds were further analyzed in a commercial laboratory (lab2) for OMD_{EDOM} to determine reproducibility. The 49 compounds and their 69 associated ingredients were also analyzed by lab2 for dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF), and starch. The EDOM method resulted in an intralaboratory correlation of 98.9% and an interlaboratory correlation of 92.6%, with no significant mean bias between the 2 laboratories tested. The formulation of compound feeds, total mixed rations, and mixtures in general assumes that their nutrient content can be calculated by adding together the nutrient supply of individual ingredients. This is of great importance in the feed industry for the creation of compound feeds. Additivity of OMD_{EDOM} for the compound feed samples was evaluated by comparing the sum of the digestible OM (DOM_{EDOM}) of the ingredients (predicted) with DOM_{EDOM} estimated directly in the compound feed (observed). The regression of predicted versus observed showed a coefficient of determination (R^2) of 0.93 and root mean square error (RMSE) of 1.07% of total DM, with no linear bias but with a mean bias (0.83% of DM). Additivity of CP, starch, crude fat, and NDF showed an R^2 of 0.95, 0.98, 0.95, and 0.93, and RMSE of 1.56, 1.90, 0.39, and 1.46% of DM, respectively, all presenting linear bias. Crude fat also presented mean bias. Although significant, all linear and mean bias for DOM_{EDOM} and chemical components were within the acceptable error limits for declaration of feeds. The results demonstrate the high precision of the EDOM method and its additive property, which is an advantage for the estimation of OM digestibility in compound feeds. Moreover, results of the tests of chemical components confirm their additive property.
Key words: organic matter digestibility, in vitro enzymatic method, compound feed, precision, additivity

INTRODUCTION

Organic matter digestibility (**OMD**) is of great importance, as it is the main factor determining the energy value of animal feed (de Boever et al., 1986; Beecher et al., 2015). In vivo determination of OMD of feeds is regarded as the reference value. However, alternative in vitro methods have been developed due to the high levels of labor and costs involved in determining in vivo values. In vitro methods can be divided into rumen liquor digestion (Tilley and Terry, 1963), rumen liquor gas production (Menke et al., 1979), and enzymatic digestion methods (Weisbjerg and Hvelplund, 1993). Due to the high nutritive variability and importance of ruminant diets, most OMD evaluations and comparisons of methods have focused on forage (Barber et al., 1990; Gosselink et al., 2004; Jančík et al., 2011), and limited attention has been placed on concentrate feed ingredients and compound feeds. However, due to the high level of concentrates used in modern dairy production and new feed ingredients, a precise estimation of OMD in concentrate feeds is needed to match the diet supply to energy requirements and thus avoid excess feeding or under-supply. The enzymatic

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digestibility of OM method (**EDOM**) developed by Weisbjerg and Hvelplund (1993) is a multi-enzymatic method used in Denmark in the feed unit system and in the Nordic Feed Evaluation System (NorFor, Volden, 2011) for estimating the OMD of roughage (Åkerlind et al., 2011). Weisbjerg and Hvelplund (1996) evaluated the accuracy of EDOM (the closeness of the measured values to the in vivo value) on 59 compound feeds from Belgium, Holland, and Denmark, and demonstrated that the method was an accurate estimator for OMD in vivo (correlation of determination, $R^2 = 0.90$). However, little has been studied about the precision of the method (the closeness of the measured values to each other) on compound feeds.

A fundamental assumption in the formulation of diets and compound feeds is that the nutritive values of the ingredients can be added in a simple sum to estimate final concentration in the compound feed. However, this has rarely been studied. The additive relationship of digestibility between ingredients and the compound feed has, to our knowledge, not been studied in dairy nutrition but has been reported in monogastric animals (Angkanaporn et al., 1996; Fan and Sauer, 2002; Xue et al., 2014).

The hypothesis of this study was that the OMD measured by the EDOM method (**OMD_{EDOM}**) is precise and that OMD_{EDOM} measured on individual ingredients is additive in the estimation of OMD_{EDOM} of the compound feeds. Therefore, the objective of this study was to evaluate the EDOM method and to aim to establish a relationship between the OMD of individual feed ingredients and the compound feed produced therefrom.

MATERIALS AND METHODS

Samples for the Estimation of OMD

In total 149 samples were collected from 6 feed companies from Norway, Denmark, and Sweden. Of these, 70 samples were commercial compound feeds with known compositions, and 79 were the individual concentrate feed ingredients used in the formulation of the compound feeds. All samples were analyzed for OMD_{EDOM}, DM, and ash at Aarhus University, Foulum, Denmark (**lab1**). A subset of 49 compounds feeds were also analyzed for OMD_{EDOM} in a commercial laboratory, Eurofins Agro Testing, Vejen, Denmark (**lab2**).

Estimation of OMD Using the EDOM Method

For estimation of OMD_{EDOM}, the method developed by Weisbjerg and Hvelplund (1993) was used with the following adaptation, because 2 of the originally proposed enzymes (gammanase and Novozym 188 from Novozymes Inc., Copenhagen, Denmark) are no longer produced. The sample was milled, and 0.5 g was weighed into a glass crucible with a filter plate of sintered glass (porosity 1, pore size 90 to 150 μm) and with a rubber stopper at the bottom. After the addition of 30 mL of pepsin-hydrochloric acid solution (pepsin EC 3.4.23.1, HCl EC 933-977-5, 0.1 *N*, pH 1.0), the crucible was closed with a top rubber stopper. The crucible was incubated for 24 h at 40°C in a water bath. During incubation, the crucible was shaken twice. Next, the crucible was transferred to an 80°C water bath for 45 min. The crucible was then vacuum drained and washed with distilled water to neutralize the residue. With the top and bottom stopper fitted, 30 mL of enzyme acetate buffer was added (0.1 *N*, pH 4.8). The enzyme acetate buffer was freshly made at the moment of use. It consisted of 20 mL of Celluclast 1.5 L (EC 232-734-4), 10 mL of Viscozym L (EC 3.2.1.6), 17.0 mL of Novozym 51054 (EC 253-446-5; all from Novozymes Inc., Copenhagen, Denmark), and 2.175 g of amyloglycosidase (EC 3.2.1.3, 3,200 U/mL; Megazyme, Bray, Ireland) 0.1 g of chloramphenicol (EC 200-287-4), and 800 mL of acetate buffer (pH 4.8 ± 0.1). The acetate buffer consisted of 8.16 g of sodium acetate (EC 204-823-8) dissolved in 0.5 L of distilled water and 7.5 mL of acetic acid (EC 200-580-7, 30% wt/wt, 5.8 mol/L), with additional distilled water to make up 1 L. The crucible, with the rubber stoppers, was then re-incubated in the 40°C water bath for another 24 h; thereafter the crucible was transferred to a 60°C water bath for 19 h. The crucible was shaken twice during each incubation. After the stoppers were removed, the crucible was vacuum drained and washed with boiling distilled water twice, then twice with 20 mL of acetone (EC 200-662-2). The first acetone wash was performed with the bottom plug fit for 5 min to ensure successful extraction. Finally, the crucible was dried for 16 h at 103°C and, after being weighed, incinerated at 525°C and re-weighed to determine the ash residue. Each sample was analyzed twice as true replicates with at least 1 wk between batches.

Samples for Evaluation of Additivity of OMD and Chemical Components

The subset of 49 compounds and the corresponding 69 ingredient samples, analyzed for OMD_{EDOM} in lab2, were also analyzed in lab2 for nitrogen according to the Dumas method (Dumas, 1831), and CP was estimated as N × 6.25, crude fat (**CFat**) as petrol ether extract after HCl hydrolysis according to EU 152/2009 (European Commission, 2009), NDF (ash corrected) via the amylase-treated NDF method (Mertens et al., 2002), starch via the enzymatic method (Åkerlind et al., 2011) using heat-stable amylase and, glucose using a YSI 2900D apparatus (Yellow Springs, OH). Ingredients with a maximum inclusion lower than 2% of total DM in compound feed samples, minerals, and vitamins were not analyzed, and values from NorFor Feed Table were used. For many ingredients, several samples were included, corresponding to the different feed companies. Ingredient samples from the same feed company could be included in several compound feeds from that company. Table 1 shows a summary of samples per ingredient and numbers of compound feeds formulated with each ingredient. Digestible OM on DM basis (**DOM$_{EDOM}$**) was calculated based on OMD$_{EDOM}$ and OM (in DM) concentration in all the samples. This parameter was used to evaluate the additivity of OMD$_{EDOM}$.

Statistical Analysis

All statistical analysis was performed using R software (version 3.6.0, R Core Team, 2019).

Precision of the EDOM Method. Following analysis according to Bartlett and Frost (2008), repeatability of the EDOM method was evaluated via OMD$_{EDOM}$ through intraclass correlation (**ICC**) and repeatability coefficient (**rcoef**). The rcoef is the standard deviation (SD) between 2 measurements on the same sample $\left(\sqrt{2} \times 1.96 \times SD_{within\_sample}\right)$. The ICC was estimated using the rpt function from the rptR package and the linear mixed model method (Stoffel et al., 2017), considering the sample as random effect. The OMD$_{EDOM}$ of 149 samples measured at lab1 was used for the calculation, with 2 repetitions per sample, 298 observations in total.

Reproducibility was considered as the agreement of measurements between laboratories. The OMD$_{EDOM}$ of the 49 compound feeds analyzed in the 2 different laboratories (lab1 and lab2) was used, accounting for a total of 98 observations (49 observations per laboratory). Each observation was the average of 2 repetitions within the same laboratory. Agreement between the 2 laboratories was inferred using the Bland–Altman plot,

Table 1. Summary of the ingredient composition of the 49 studied compound feeds (minimum and maximum share in % of total DM) and number of samples per ingredient; individual ingredients are shown only if present in a compound feed at more than 10% of total DM

Ingredient	No. of samples per ingredient	Share of compound feeds[1] (% of DM)		Compound feeds (no.)[2]
		Min.	Max.	
Soybean meal	5	0.6	40.4	32
Wheat	5	1.9	31.3	31
Sugar beet pulp	4	1.4	29.1	29
Rapeseed meal	3	3.2	38.4	28
Rapeseed cake	4	2.0	49.5	27
Wheat bran	5	2.0	26.8	27
Barley	5	1.9	48.2	26
Distillers grains	2	2.1	35.7	23
Oats	4	1.0	24.6	22
Rye	1	2.8	31.1	19
Sunflower meal	2	1.7	10.4	17
Soybean hulls	1	2.2	16.4	12
Maize	3	1.9	14.9	11
Citrus pulp	1	2.0	15.7	10
Soypass[3]	3	0.6	32.4	9
Maize gluten meal	2	1.0	22.7	8
ExPro[4]	1	10.7	51.1	8
Malt sprout[5]	2	1.3	12.1	6
Wheat middling	1	5.0	14.9	5
Palm kernel cake	2	2.2	12.5	4
Alkaline barley[6]	1	19.5	19.5	1
Other[7]	12	<10	<10	49
Total	69			49

[1]Minimum and maximum percentages of inclusion in the compound feeds containing the ingredient.
[2]Number of compound feed samples that include the ingredient.
[3]Rumen-protected soybean meal (Denofa, Gamle Fredrikstad, Norway).
[4]Steam-processed non-GMO rapeseed meal (AAK AB, Malmö, Sweden).
[5]By-product of malting industry.
[6]Barley treated with urea and reaction promoters (Norgesfor AS, Oslo, Norway).
[7]Ingredients with a maximum inclusion lower than 10% of DM.

as suggested by Bartlett and Frost (2008), and the differences in mean between laboratories was evaluated using the *t*-test, with a level of significance of 0.05. The interlaboratory coefficient (**ILC**) was also estimated using the rptR package, with sample as random effect.

Additive Property. The additive property was examined for the 49 compound feeds containing the 69 corresponding ingredients. The relationship between the compound feed and their ingredients was tested using the following 2 approaches.

Pure additivity (**Model A**): This model is based on the hypothesis that individual feedstuffs can be added linearly to achieve a certain value in the final compound feed, called additivity. For a given nutrient, additivity was evaluated by comparing the nutrient concentration (% of DM), measured directly in the compound feed sample, with the value calculated by the sum of ingredients, considering the ingredients proportion in the compound feed (weighted sum of ingredients). Additivity was analyzed for DOM_{EDOM}, CP, starch, CFat, and NDF. Model A for all parameters was evaluated by regressing the predicted weighted sum of ingredients against the observed values measured directly in the compound samples and analyzing the R^2 and root mean square error (**RMSE**). Moreover, the predicted values were regressed on residuals. Predicted values were centered around the mean, making the slope and the intercept independent and orthogonal, for mean and linear bias evaluation, respectively (St-Pierre, 2003).

Ingredient effect (**Model B_i**; $i = 1, 2, \ldots$): We developed Model B_i assuming that not only was the DOM_{EDOM} of the compound feed explained through additivity but also that it could include effects of specific ingredients and interactions between them. Principal component analysis was used to identify possible interactions via the correlation matrix between the contribution of DOM_{EDOM} of each ingredient in the compound feeds. This means that the OMD_{EDOM} of the ingredient and its proportion of inclusion were both taken into consideration. If 2 or more ingredients were assigned to the same principal component (**PC**), the individual ingredients, as well as the interaction, were included as additional variables to model A; these models were denoted B_i ($i = 1$ to n), where i was the number assigned to a new model when a correlation between ingredients detected in the PC was significant ($P < 0.05$). Principal component analysis was performed using the pca function from package stats (R Core Team, 2019).

Models A and B_i were estimated via linear models using the linear model function (lm) from package stats (R Core Team, 2019). Effect of company was evaluated by including company as random effect and comparing fit with the models including only fixed effects of RMSE and Bayesian information criterion statistic (**BIC**). Mixed models were created using the lmer function in the lme4 package (Bates et al., 2015). The level of significance for inclusion of an effect in the model was 0.05. Model fit comparison was performed using BIC statistics. The prediction ability of the models was obtained through the predicted correlation coefficient $\left(\bar{R}^2\right)$, this being the ratio between the predicted sum of squares, calculated using the leave-one-out technique (predicted residual error sum of squares, **PRESS**), according to Allen (1974), and the total sum of squares.

RESULTS

Table 2 shows the summary of the OMD_{EDOM} for the 149 samples (70 compound feeds and 79 ingredients) used to estimate repeatability and reproducibility. Chemical compositions and OMD_{EDOM} of the 118 samples (49 compound feeds and 69 ingredients) are also shown in Table 2. The range of all nutrients was larger for the ingredient samples than for the compound feeds. Compound feeds consisted of both protein and energy supplements; 14 of the 49 compound feeds consisted of more than 25% of CP in DM and could be attributed as protein supplements, and almost half of the compound feed samples (24 compound samples) contained more than 25% starch.

Shares of the ingredients constituting the 49 compound feeds are summarized in Table 1. Soybean meal was the most frequent ingredient, included in 32 compound feeds (32), although its concentration varied considerably, from 0.6 to 40.4% of DM. Other frequently used protein-rich ingredients included rapeseed meal (28), rapeseed cake (27), and wheat bran (27). Cereals wheat (31), barley (26), oats (22), and rye (19) were the most frequent carbohydrate sources. Beet pulp was the most-used fiber-rich ingredient (29).

Repeatability

The ICC from the 149 samples and 298 observations was 0.989. This means that 98.9% of the variation between the measurements was attributable to genuine differences between the feed samples, with the 1.1% remaining variance being due to errors in measurements. The estimated SD of OMD_{EDOM} within samples was 0.8% OM, and the corresponding SD between samples was 7.7% OM. The estimated rcoef was 2.2% OM with a 95% confidence interval (the maximum future difference of OMD_{EDOM} between 2 measurements in 95% of cases).

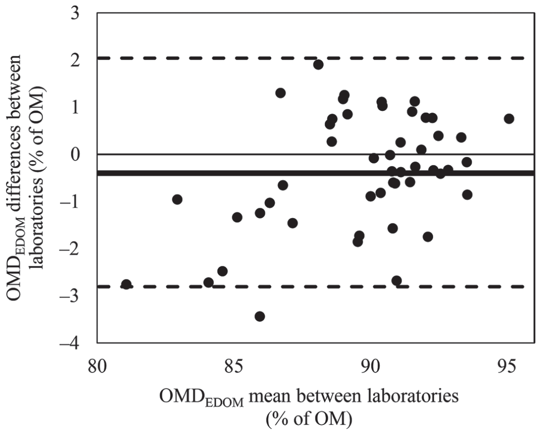

Figure 1. Differences in measures between the 2 laboratories of OM digestibility measured by enzymatic digestibility of OM (EDOM) method (OMD_{EDOM}) against the mean value. Solid line = mean differences between laboratories; upper dashed line = mean +1.96 SD; lower dashed line = mean −1.96 SD, with 95% CI.

Reproducibility

Reproducibility of the method measured by ILC was 0.926, meaning that 7.4% of the variation of OMD_{EDOM} was due to measurement errors. The relationship between the 2 laboratories can be seen in the Bland–Altman plot (Figure 1). The estimated OMD mean of the differences between lab1 and lab2 was −0.4% OM, suggesting that, on average, lab2 provided higher values than lab1 but that this difference was not significant ($P = 0.57$). The absolute maximum difference in OMD between future measurements in these 2 laboratories will be 2.8% OM in 95% of cases (lower limit, Figure 1).

Relation Between Ingredients and Compound Feeds: Additivity

The effect of company only improved model fit for starch. Thus company effect was not included, and only fixed effects were considered in the models.

Additivity of the method was evaluated through DOM_{EDOM}. Our DOM_{EDOM} regression showed an R^2 of 0.93 and RMSE of 1.07% DOM between DOM_{EDOM} values measured directly in the compound feeds (observed) and those calculated through addition of the ingredients (predicted; Figure 2a). The residual analysis (Figure 2b) showed a mean bias ($P < 0.001$), so that the added DOM_{EDOM} of the ingredients tended to underpredict DOM_{EDOM} values of the compound feed by 0.83 percentage points on average. We found no linear bias (−0.01, $P = 0.80$).

The CP regression (Figure 3a) showed an R^2 of 0.95 and RMSE of 1.56% CP in DM between observed and predicted values. The residual analysis (Figure 3b) showed no mean bias (0.26, $P = 0.25$), but a linear bias was revealed (−0.07, $P = 0.02$). However, the bias was only 0.82 percentage points at the minimum predicted value and −0.92 percentage points at the maximum.

Starch estimated by ingredient addition (Figure 4a) showed an R^2 with the starch analyzed in the compound feed of 0.98 and RMSE of 1.90% starch in DM. Residual analysis (Figure 4b) showed no mean bias (−0.34 $P =$ 0.23) but did show a linear bias (−0.04, $P = 0.04$). Biases at the minimum and maximum predicted values were 0.50 and −1.2 percentage points, respectively.

For the CFat analysis, 2 compound feeds were excluded as outliers. Crude fat estimated by additivity and CFat measured directly in the compound feed sample presented an R^2 of 0.95 and RMSE of 0.39% CFat in DM (Figure 5a). The residual analysis (Figure

Table 2. Summary statistics for the chemical composition of compound feeds and their ingredients

	Compound feeds			Ingredients		
Item[1]	Average	Minimum	Maximum	Average	Minimum	Maximum
OMD_{EDOM} (% of OM)[2]	89.3	78.2	96.0	87.3	50.2	100.0
OMD_{EDOM} (% of OM)[3]	89.4	78.2	95.8	87.4	50.5	99.6
DOM_{edom} (% of DM)	82.6	70.6	91.9	78.1	45.7	94.4
Ash (% of DM)	7.3	4.2	14.4	5.3	0.2	26.5
CP (% of DM)	24.0	16.3	42.7	22.8	0.1	66.7
CFat (% of DM)	6.0	2.2	12.1	9.1	1.0	98.9
NDF (% of DM)	23.1	13.4	36.2	25.8	0.0	71.6
Starch (% of DM)	22.0	1.6	46.9	22.4	0.5	72.6

[1]OMD_{EDOM} = OM digestibility by enzymatic digestibility of OM (EDOM) method; DOM_{EDOM} = digestible OM by EDOM method; CFat = crude fat.

[2]Feed n = 70; ingredient n = 79. Used for calculation of repeatability [analyzed in lab1 (Aarhus University, Foulum, Denmark)].

[3]Here and below, feed n = 49; ingredient n = 69. Used for calculation of reproducibility and additivity of DOM_{EDOM} [analyzed in lab1 and lab2 (Eurofins Agro Testing, Vejen, Denmark)].

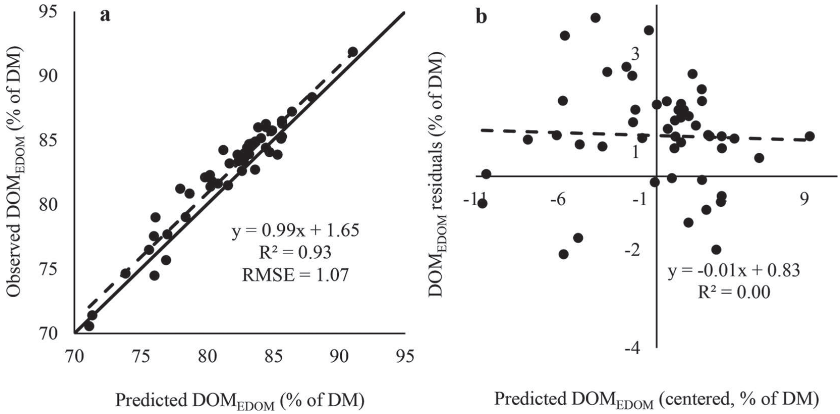

Figure 2. (a) Regression between digestible OM on DM basis (DOM_{EDOM}) calculated by the weighted sum of ingredients (Predicted DOM_{EDOM}) and DOM_{EDOM} measured in compound feeds (Observed DOM_{EDOM}). (b) Residual analysis of the relationship between DOM_{EDOM} estimated by weighted sum of ingredients (Predicted DOM_{EDOM}), centered to the mean, and DOM_{EDOM} measured in compound feed (Observed DOM_{EDOM}). DOM_{EDOM} residuals = Predicted DOM_{EDOM} − Observed DOM_{EDOM}. RMSE = root mean square error.

5b) showed that the predicted values overestimated the CFat values in the compound feed (mean bias −0.22, $P < 0.001$), and the difference increased with increasing values of CFat (linear bias −0.11, $P < 0.001$). Bias at the minimum predicted value was −0.08 percentage points and 0.56 at the maximum.

The NDF regression (Figure 6a) showed an R^2 between the predicted and observed values of 0.93 and RMSE of 1.46% NDF in DM. The residual analysis (Figure 6b) showed no mean bias (0.25, $P = 0.25$) but a linear bias (−0.12, $P = 0.001$). Bias at the minimum was 1.79 percentage points and at the maximum predicted value was −2.01 percentage points.

Table 3 shows the proportion of the 49 compound feed samples that, as predicted by additivity, lay in the 2% and 5% range for error. The highest proportion of predicted values below 2% error was for DOM_{EDOM}: 85.7% of the samples (42 samples) fell inside the 2% error range, and 100% lay in the 5% error range. Starch resulted in the lowest percentage of samples in the 2%

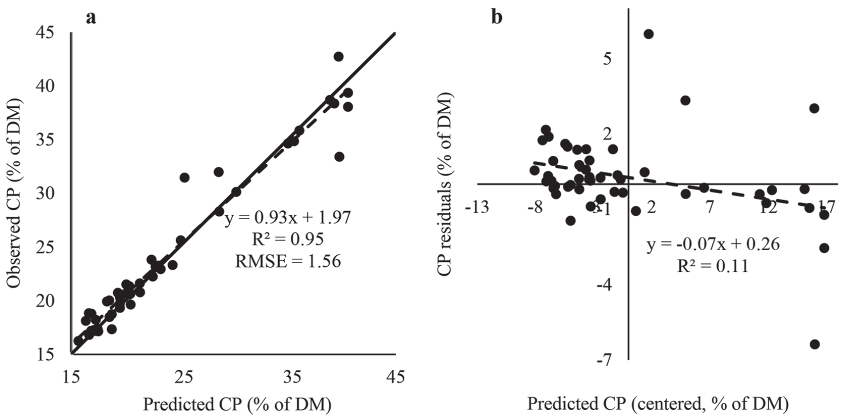

Figure 3. (a) Regression between CP calculated by weighted sum of ingredients (Predicted CP) and CP measured in compound feeds (Observed CP). (b) Residual analysis of the relationship between CP estimated by weighted sum of ingredients (Predicted CP), centered to the mean, and CP measured in compound feed (Observed CP). CP residuals = Predicted CP − Observed CP; RMSE = root mean square error.

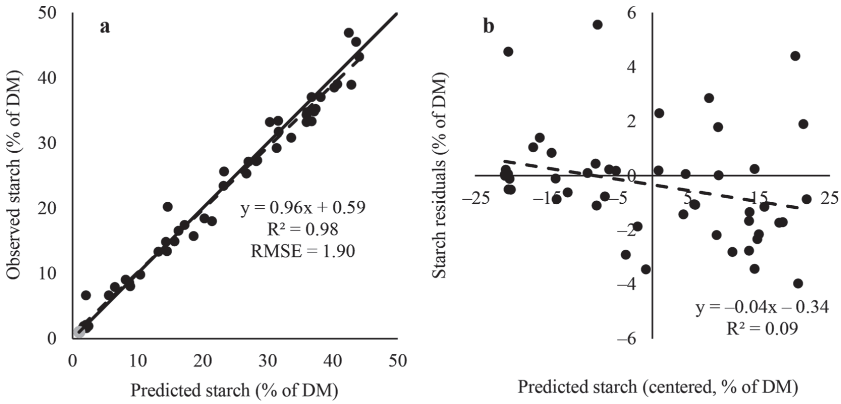

Figure 4. (a) Regression between starch calculated by weighted sum of ingredients (Predicted starch) and starch measured in compound feeds (Observed starch). (b) Residual analysis of the relationship between starch estimated by weighted sum of ingredients (Predicted starch), centered to the mean, and starch measured in compound feed (Observed starch). Starch residuals = Predicted starch − Observed starch; RMSE = root mean square error.

error range (26.5%, 13 samples) and in the 5% error range (46.5%, 23 samples). For CP, CFat, and NDF concentrations in DM, more than 50% of the samples lay within the 5% error range.

Relation Between Ingredients and Compound Feeds: Ingredient Effect for DOM

The principal component analysis showed that the first 6 principal components (**PC1** to **PC6**) explained 78% of the variance, and the correlation matrix of these PC was analyzed to detect the effects of the ingredients. Table 4 shows nested models, from the reduced model showing simple additivity (Model A), to model B_3, showing all the identified effects of ingredients (full model). The correlation matrix of PC1 showed a correlation between soybean meal, wheat, and barley. Model B_1 was created based on PC1 and showed an interaction only between soybean meal and wheat ($P = 0.03$) and an effect of wheat as an independent variable ($P = 0.05$), although soybean meal and barley had no effect as independent variables ($P = 0.17$ and $P = 0.13$,

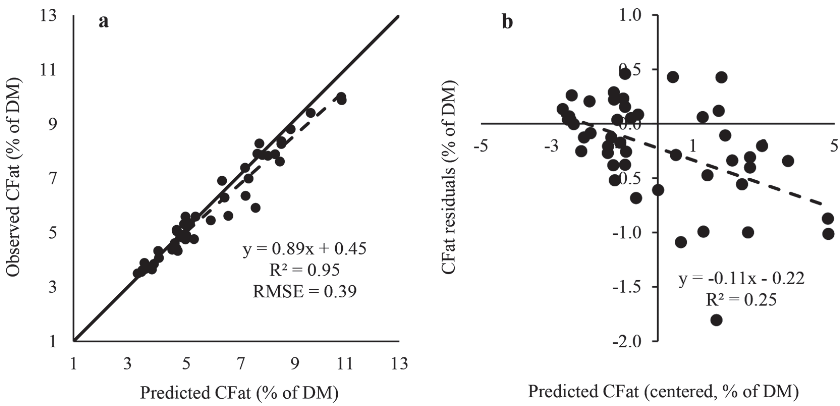

Figure 5. (a) Regression between crude fat (CFat) calculated by weighted sum of ingredients (Predicted CFat) and CFat measured in compound feeds (Observed CFat). (b) Residual analysis of the relationship between CFat estimated by weighted sum of ingredients (Predicted CFat), centered to the mean, and CFat measured in compound feed (Observed CFat). CFat residuals = Predicted CFat − Observed CFat; RMSE = root mean square error.

Table 3. Number and percentage of predicted digestible OM, CP, crude fat (CFat), NDF, and starch values by weighted sum of ingredients (additivity) within 2 and 5% error ranges

	Error range[1]			
	2%		5%	
Item	N	%	N	%
DOM_{EDOM}[2] (% of OM)	42	85.7	49	100.0
CP (% of DM)	25	51.0	35	71.4
CFat (% of DM)	14	29.8	27	57.4
NDF (% of DM)	9	18.4	26	53.1
Starch (% of DM)	13	26.5	23	46.9

[1]A compound feed was included in the 2% level if the predicted value, by additivity, fell within the range of the observed value ± 2%. A compound feed was included in the 5% level if the predicted value, by additivity, fell within the range of the observed value ± 5%. N = number of samples within the error range; % = proportion of samples within the error range (49 total compound feeds).

[2]DOM_{EDOM} = digestible OM by enzymatic digestibility of OM (EDOM) method.

respectively). The next PC (PC2) showed a correlation between rapeseed cake, soybean meal, and barley, but neither these ingredients nor their interactions were significant; thus no model was created. We found that PC3 showed a correlation between wheat, rye, and rapeseed meal. Model B_2, based on PC3, showed a triple interaction between rapeseed meal, rye, and wheat ($P = 0.02$), as well as effects of rapeseed meal ($P = 0.02$) and soybean meal ($P = 0.04$). In PC4 we discovered a correlation between wheat, soybean meal, and oats, but no effect of the ingredients nor of their interaction was found when these variables were tested in the model. We found that PC5 showed a correlation between wheat and sugar beet pulp. Based on PC5, model B_3 showed an interaction between wheat and sugar beet pulp ($P = 0.02$) as well as an effect of sugar beet pulp as an independent variable ($P = 0.03$). Both variables were included to create B_3. Finally, PC6 presented a correlation between wheat and rye, but these variables were already taken into consideration in B_2. All the models presented a nonsignificant intercept, and all the variables included in the regression had a significance of at least $P < 0.05$. The R^2 was highest for model B_3 (0.96) and lowest for model A (0.93). However, when analyzing the $\bar{R}^2$ differences between model A and B_3, this decreased from 3% to 1%. The BIC was lowest for model A (157) and highest for model B_1 (165).

DISCUSSION

The EDOM method was developed for concentrates and is accepted by NorFor for estimating the OMD of forages (Åkerlind et al., 2011). However, little has been reported regarding the use of this method on compound feeds. With the present study, we intended to evaluate the precision of the EDOM method through calculation of repeatability and reproducibility. Moreover, we examined whether the relationship between ingredients and the compound feed was purely additive or whether, in addition, the effects of ingredients and their interactions should be considered when formulating compound feeds. The additive relationship was also examined for the traditional chemical parameters (CP, CFat, starch,

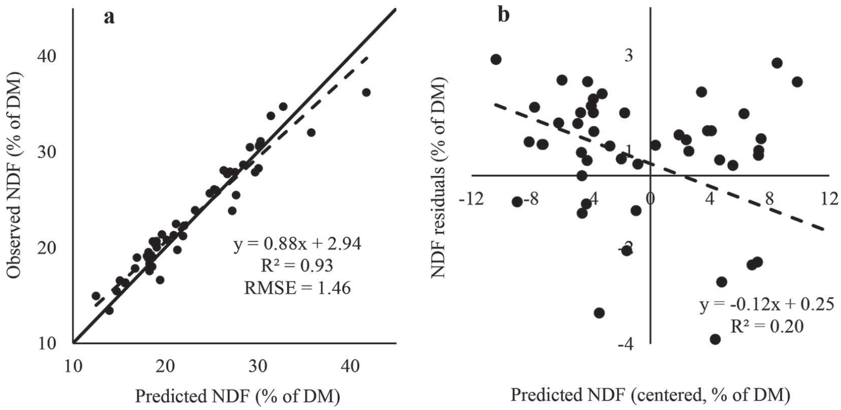

Figure 6. (a) Regression between NDF calculated by weighted sum of ingredients (Predicted NDF) and NDF measured in compound feeds (Observed NDF). (b) Residual analysis of the relationship between NDF estimated by weighted sum of ingredients (Predicted NDF), centered to the mean, and NDF measured in compound feed (Observed NDF). NDF residuals = Predicted NDF − Observed NDF; RMSE = root mean square error.

and NDF), which have long been assumed to be additive, although published evidence is difficult to find.

The high correlation between OMD_{EDOM} and in vivo values has been demonstrated (Weisbjerg and Hvelplund, 1996), and the EDOM method showed better performance than the gas production (Palić and Leeuw, 2009) and rumen fluid methods (Søegaard et al., 2001; Weisbjerg et al., 2007). Thus, EDOM could have an advantage over other in vitro methods, as the various steps could better mimic animal digestion. This high accuracy, meaning close measurements to the reference value (in vivo), is a desirable characteristic in all laboratory methods. Nevertheless, precision—here meaning how close repeated measures are to each other—is also fundamental for a method to be adoptable in practice.

The large variation of in OMD_{EDOM} and chemical components in our data set represent the variations seen in the Scandinavian feed industry. This is also reflected by the frequent use of soybean meal and rapeseed byproducts as main protein sources and cereals as starch sources. The achieved variations were of great importance for our study, as these allowed us to evaluate the precision of OMD_{EDOM} in a large range of values and evaluate how additivity performs in different nutrient concentrations. Additionally, if our results show that the EDOM method is precise, this will support the easy adoption of this method in more countries.

Precision of the EDOM Method

The results of this study indicate that the EDOM method is precise enough for the analysis of OMD in compound feeds. Repeatability defines the correlation between repeated measures of the same sample using the same measurement procedure, laboratory team, and experimental setup (JCGM, 2012). In other words, repeatability measures the agreement between repetitions in the same laboratory. The high ICC (98.9%) and low expected difference of OMD_{EDOM} between future repetitions (rcoef = 2.2% OM) showed that the EDOM method was highly repeatable within the laboratory. Higher repeatability of OMD_{EDOM} of this study agrees with lower variation of OMD_{EDOM} between measurements on forage samples compared with OMD measured via a rumen fluid method (Tilley and Terry, 1963), 0.63 and 1.87% OM (Søegaard et al., 2001), and 0.7 and 2.4% OM (Weisbjerg et al., 2007), respectively. A similar effect was found using another enzymatic method, when digestibility of compound feeds was evaluated by de Boever et al. (1986). The rumen fluid method showed higher OMD variation between repetitions (1.1% OM) compared with a pepsin cellulase method developed by Iowerth et al. (1975) and adapted for compound feeds by Aufrère and Michalet-Doreau (1983), 0.69 and 0.84% OM for the 2 different cellulase enzymes tested. This pepsin cellulase method has similarities to the EDOM method.

Although repeatability is a necessary parameter to evaluate the precision of a method, it should not be viewed as a solitary result, as the variability between laboratories can be greater than the variability within one laboratory (Parker et al., 2018). Reproducibility of 100% is desirable, meaning that different laboratories obtain the same result with the same method using different tools and operators (JCGM, 2012). The ILC in this study (92.6%) was lower than ICC (98.9%). This was expected, because more sources of variation are involved, such as different technicians and equipment available in each laboratory (Taylor and Kuyatt, 1994). This study included only 2 laboratories; however, the dismissible difference between laboratories (0.4% OM) for OMD_{EDOM} in this study agrees with 2 ring tests, each involving 4 laboratories, performed for concentrate ingredients (De Clerck, 2018, 2019). Compared with the rumen fluid method, the same tendency was found for reproducibility as for repeatability, where EDOM

Table 4. Models describing the relationship between observed digestible OM measured using the enzymatic digestibility of OM (EDOM) method (DOM_{EDOM}, % of DM) and various significant regressors: model A contains weighted sum of DOM_{EDOM} ($DOM_{EDOM}Add$); models B_i also contain effects of ingredients [DOM_{EDOM} (% of DM) × share of ingredient in compound feed (% of DM)]

Item	Models for DOM_{EDOM} (% of DM)[1]	Model fit[2]			Model prediction[3]	
		R^2	RSE	BIC	PRESS	R^2
A	$1.65 + 0.99DOM_{EDOM}Add$	0.93	1.09	157	61.04	0.93
B_1	$-4.01 + 1.06DOM_{EDOM}Add - 0.01(SBM \times Wh) + 0.04Wh$	0.94	1.06	165	66.17	0.93
B_2	$0.94 + 0.99DOM_{EDOM}Add - 0.01(SBM \times Wh) + 0.08Wh - 0.001(Wh \times Ry \times RSM) - 0.04RSM + 0.04SBM$	0.95	0.98	163	55.28	0.94
B_3	$0.11 + 1.00DOM_{EDOM}Add - 0.01(SBM \times Wh) + 0.12Wh - 0.001(Wh \times Ry \times RSM) - 0.04RSM + 0.04SBM - 0.01(Wh \times SBP) + 0.06SBP$	0.96	0.93	163	48.93	0.94

[1]$DOM_{EDOM}Add$ = digestible OM calculated by the weighted sum of ingredients; SBM = soybean meal; Wh = wheat; RSM = rapeseed meal; Ry = rye; SBP = sugar beet pulp.

[2]RSE = residual standard error of the model; BIC = Bayesian information criterion.

[3]PRESS = predicted residual error sum of squares.

showed lower variation of OMD between laboratories than did the rumen fluid methods for forages: 0.97 versus 2.33% OM (Søegaard et al., 2001) and 0.70 versus 2.10% OM (Weisbjerg et al., 2007).

A possible explanation for the higher precision (repeatability and reproducibility) of enzymatic methods might be the use of commercial enzymes and synthetic solutions that might translate into more stable results. In contrast, when rumen fluid is used, the stability of the solution might be compromised due to donor variation (Church and Petersen, 1960; Holden, 1999); therefore the method is difficult to standardize across multiple laboratories. On the other hand, a possible disadvantage of enzymatic methods could lie in the dependability of production of these enzymes from a third party. The importance of a precise method is that its precision makes it adoptable in practice, as this makes implementation of the method in different laboratories and regions worthwhile. Results from this study are of great importance for the use of EDOM, as this method could potentially be used in the NorFor system for compound feeds, expanding to other countries (it is already official in Denmark) and other regions using the system.

Relation Between Ingredients and Compound Feeds

Scarce literature exists on the additive properties of digestible organic matter. For ruminant feeds, Prestløkken (1999) found that effective protein degradability as predicted by the addition of ingredients overpredicted the values measured directly in the mix feeds by up to 5 percentage points. However, we found the contrary for $\mathrm{DOM_{EDOM}}$: additivity of $\mathrm{DOM_{EDOM}}$ underpredicted the measured $\mathrm{DOM_{EDOM}}$ of compound feeds by less than 1 percentage point (0.83% DM, Figure 2b).

The effect of specific ingredients beyond additivity was identified for the relationship between ingredients and compound feeds (Table 4). However, the numerical magnitude of the effect of ingredients and their interactions was minor. The model considering only pure additivity showed the best fit with the lowest BIC (BIC = 157). Moreover, the prediction ability $\left(\bar{R}^2\right)$ showed minor improvement with the inclusion of ingredients. Further, it is not clear whether the effect of ingredients included in these models was due to intrinsic characteristics of the ingredients or to the proportions in which they were included in the compound feed, as the ingredients detected coincide with the most frequently used ingredients in the compound feed samples (Table 1). Our results suggest that $\mathrm{DOM_{EDOM}}$ has an additive property. No evaluation of the effect of ingredients was performed for chemical components, as this was beyond the scope of the present study.

The high correlation between amounts of nutrients calculated from additivity of ingredients and amounts of nutrients measured directly in compound feeds agrees with the correlation found for $\mathrm{DOM_{EDOM}}$. The $\mathrm{DOM_{EDOM,}}$ as well as the CFat, showed an average difference between calculated and measured values that accounted for 0.83% DOM in DM and 0.25% CFat in DM, respectively. Both errors are within the acceptable limits (5% limit for $\mathrm{DOM_{EDOM}}$ corresponds to the energy tolerance limit and 1% CFat limits, respectively) established for compound feed declarations (European Commission, 2010). All chemical components presented linear bias, meaning that the prediction would have higher errors in extreme values. Despite this, the largest linear bias was reported for NDF (2.01% DM) at the maximum predicted value (36.2% of DM), which is lower than the acceptable limit in EU regulations (3.5%). Although all nutrients fell within the tolerance limits, the bias presented in this study should be taken as a guideline for the creation of compound feeds to match the targeted concentration of nutrients. Moreover, CFat presented the highest coefficient of determination between residuals and the predicted CFat content of compound feeds (Figure 5b, $\mathrm{R}^2 = 0.25$). This suggests greater overprediction of CFat by additivity of ingredients at higher CFat concentrations. Difficulties with extraction of CFat due to the diverse nature of the different components could be a potential explanation (Palmquist and Jenkins, 2003), although it is difficult to explain our finding of overestimation of CFat by additivity of ingredients.

To our knowledge, although the additive property has long been assumed in chemical components, no evidence supporting this assumption has been reported. Thus, the evaluation of additivity of the chemical parameters in this study is fundamental for several reasons. First, it is vital to evaluate and report the property's existence. Second, our study has allowed us to compare the additive property of the chemical components with the results of the $\mathrm{DOM_{EDOM}}$ method, using additivity results from chemical components as reference, and to conclude whether $\mathrm{DOM_{EDOM}}$ additivity could be potentially useful in practice. Moreover, validation of the assumption of additivity of the chemical components is central not only to the formulation of commercial compound feeds but also to research, where additivity of this parameters is commonly assumed. Our results suggest that chemical parameters are additive. Moreover, $\mathrm{DOM_{EDOM}}$ additivity was comparable with the additivity of the chemical parameters, confirming the additive property for this parameter as well.

CONCLUSIONS

The EDOM method showed high repeatability (ICC = 98.9%) and reproducibility (ILC = 92.6%) and is therefore a precise method for estimating OM digestibility in compound feeds. We found a high coefficient of determination ($R^2 = 0.93$) between DOM_{EDOM} on compound feed and DOM_{EDOM} measured in feed ingredients and weighted by the composition of ingredients in the compound feed. Therefore, the EDOM method showed a convincing additive property. Chemical components (CP, starch, CFat, and NDF) also showed additivity, with R^2 ranging from 0.93 for NDF to 0.98 for starch. However, the bias shown in this study, especially for CFat, indicates that possible bias should be considered when formulating compound feeds to match target concentrations.

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A static model for estimating energy content of compound feeds in a dynamic feed evaluation system

Paper II

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A static model for estimating energy content of compound feeds in a dynamic feed evaluation system

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ABSTRACT

The objective of the study was to develop a static empirical model for the estimation of net energy content of compound feeds in a dynamic feeding system using net energy for lactation at 20 kg of dry matter intake/d (NEL20) values calculated by the Nordic Feed Evaluation System (NorFor) model. In the NorFor system, NEL20 is a standardized value used to describe net energy content of feeds. The static model would allow prediction of the net energy value of compound feeds without access to the input data needed for the dynamic models. Our hypothesis was that NEL20 values of compound feeds can be predicted using organic matter digestibility (in vitro) and chemical components of the compound feeds as input variables. For this, 75 compound feeds and their 108 associated ingredients were collected across Scandinavia for model development. The proposed best model for prediction of compound feed NEL20 included crude fat, neutral detergent fiber, digestible organic matter measured in vitro, and crude protein (urea corrected) as independent variables. Lack of additivity of chemical components between values analyzed directly in the compound feed and values calculated by the weighted sum of ingredients was detected as the main source of error in the model, emphasizing the importance of accurate chemical analysis and sampling practices. Results from practical use of the model show that it may be a valuable tool that could be used by several actors in the feeding sector using the NorFor system. Feed manufacturers could use it to monitor the net energy content in their final product, and farmers could use it to check the net energy content of the purchased compound feed. However, validation of this model against an independent set of samples is lacking in this study and its prediction performance should be further evaluated. The model will need recalibration if the feed parameters used in the dynamic model for the estimation of reference values change, as this would not be reflected in the predicted values of the created model.

Key words: energy estimation, additivity, in vitro digestibility, concentrate ingredient, dairy cow

INTRODUCTION

In recent decades, several dynamic mechanistic models have been developed for feed and ration evaluation, such as the Cornell Net Carbohydrate and Protein System (Tylutki et al., 2008), the Nordic Feed Evaluation System (**NorFor**; Volden, 2011), and the INRA Feeding System for Ruminants (Sauvant et al., 2018). Although these models incorporate time as a variable with detailed biological interactions, they have limitations in practice, such as a lack of available data or poor data quality to use as input for the model (Tedeschi et al., 2005).

Net energy content estimation for compound feeds in the NorFor system is a good example of a dynamic model. The NorFor system does not have a fixed net energy of lactation value for feedstuffs because dietary interactions between feedstuffs, such as nutrient degradation and passage rate with feed intake level, are considered. However, for purchasing decisions and feedstuff ranking, a comparison of the energy contents of feedstuffs is essential; thus, standard net energy values for individual feedstuffs were formulated in NorFor (Åkerlind and Volden, 2011). The net energy of lactation at 20 kg of DMI/d (**NEL20**) is the most used standard energy value. The NEL20 values (MJ/kg of DM) are created for all feedstuffs, considering the same fixed input parameters, such as animal characteristics (600 kg of weight, 20 kg of DMI/d), 50% concentrate proportion in the diet, and passage rates for CP, starch, and NDF, among others. Other variables are feedstuff specific, such as chemical composition, degradation rates, and indigestible fractions. For raw materials (e.g., grains and by-products), in sacco characteristics,

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such as degradation rates and indigestible fractions, are taken from the NorFor Feed Table (NorFor, 2020) based on Nordic databases and in situ characteristics. These input values are unavailable for commercial compound feeds, so NEL20 is calculated by the weighted sum of the ingredients (Åkerlind and Volden, 2011), making it reliant on ingredient composition. However, in the European Union (**EU**), declaration of the ingredient proportion of compound feeds is not mandatory. Hence, to have an accurate diet formulation, an alternative method for estimating the net energy of compound feeds (NEL20 in NorFor), independent from ingredient composition, is required.

Several feeding systems have static empirical equations for the energy prediction of compound feeds based on OM digestibility (**OMD**) in vitro and chemical composition as explanatory variables; see, for example, the INRA system, the English Feed Into Milk system (Thomas, 2004), the German system (GfE, 2009), and the Danish system (Weisbjerg and Hvelplund, 1993). The objective of our study was to develop a static empirical model for the estimation of net energy content of compound feeds in a dynamic feeding system using NEL20 values calculated by the NorFor model. A successful static model would allow accurate prediction of the energy value of compound feeds without access to the input data needed for the dynamic models. Our hypothesis was that NEL20 values of compound feeds can be predicted using OMD (in vitro) and chemical components of the compound feeds as input variables.

MATERIALS AND METHODS

Samples

We collected a total of 75 compound feed samples together with their associated ingredients (108 ingredient samples) over 2 yr from 6 feed companies in Denmark, Sweden, and Norway (2 companies from each country). Collected ingredients were sampled from the batch from which the compound feed samples were produced, and the exact recipe for compound feeds samples was provided. To ensure variation between years, we gathered samples from different feed companies and different feed mills from the same company (with different ingredient sources) over a period of 2 yr.

Compounds and ingredient samples included at 2% of DM and higher were analyzed for OMD using the enzymatic digestibility of OM method (**EDOM**; Weisbjerg and Hvelplund, 1993), a multienzymatic method described in detail by Álvarez et al. (2020). Digestible OM (**DOM_{EDOM}**) was estimated as EDOM (%) × OM (g/kg of DM). Ash was determined by sample incineration at 550°C, nitrogen (N) was determined by the Dumas method (Dumas, 1831) using a Leco instrument (Leco Corp.), and CP was estimated as N × 6.25. Crude fat (**CFat**) as petrol ether was extracted after HCl hydrolysis according to EU 152/2009 (European Commission, 2009), and NDF (ash corrected) was determined using the amylase-treated NDF method (ISO 16472:2006; ISO, 2006). Starch (**ST**) was analyzed as described by Kristensen et al. (2007). After hydrolyzation by α-amylase and amyloglucosidase, glucose is converted to hydrogen peroxide (by glucose oxidase) and measured electrochemically using a silver–platinum probe (YSI Inc.). Organic raw materials with an inclusion lower than 2% of DM in sampled compound feeds, minerals, and vitamins were not analyzed, so values from the NorFor Feed Table (NorFor, 2020) were used.

The NEL20 value of ingredients was estimated using degradation rates and indigestible fractions from the NorFor Feed Table (NorFor, 2020) and the measured chemical composition (Åkerlind and Volden, 2011). For compound feeds, NEL20 values were calculated according to NorFor by adding the NEL20 values of each ingredient according to their proportion in the mixture, referred to here as the weighted sum. These methods were used to calculate NEL20 of ingredients and compound feed used as the reference value for model development (referred to here as the NEL20 reference).

Statistical Analysis

All statistical analysis was performed using R software (version 3.6.0; R Core Team).

Model Development. Three data sets were used to develop models: a data set of 75 compound feed samples (referred to here as the compound data set), a data set of 108 ingredients (referred to here as the ingredient data set), and a data set of both 75 compound feeds and 108 ingredients, in total 183 samples (referred to here as the all data set). The dependent variable was NEL20 (MJ/kg of DM). Independent variables for potential inclusion in the models were DOM_{EDOM}, CP, CFat, NDF, ST, and ash (% of DM). The CP content of compound feeds was corrected for urea by subtracting the urea CP proportion from the compound feed CP content (**CP_{corr}**). The order of inclusion for variables in models was determined through stepwise forward selection by small sample size corrected Akaike information criterion (**AICc**; Hurvich and Tsai, 1989) using the "stepAIC" function and the "AICc" criteria from the MASS package (Venables and Ripley, 2002). Models were developed using a sequential approach, including one variable at a time. Models were created with and without feed company or year as random effects. Models with random effects were developed using the "lmer" function from the "lme4" package (Bates et al., 2015). Models without random effects were developed using the "lm" function from the "stats" package in R (R Core Team).

The variance inflation factor (**VIF**) assessed the multicollinearity of independent variables in models (Zuur et al., 2010) with 3.3 as the limit criterion (Kock and Lynn, 2012). If a VIF value higher than 3.3 was detected, the variable with the highest VIF was discarded until the VIF for all variables met the criterion. Model fit was evaluated by AICc and root mean squared error (**RMSE**). No mean or linear biases were evaluated, as no independent data were used to evaluate model fit.

Model Validation. As models were created with different data sets, they were compared by evaluating prediction performance on compound feed samples only. For models developed with the all data set and compound data set (as compound feed samples were part of the development data set), validation was performed by cross-validation (the leave-one-out technique) using predicted residual error sum of squares (**PRESS**) according to Allen (1974). For models developed with the ingredient data set, where compound feeds were not part of the data set, validation was performed by using compound feed samples as an independent data set. Root mean squared error of prediction (**RMSEP**) was calculated for all models to evaluate prediction performance. For cross-validation, RMSEP was calculated with PRESS as $\sqrt{\frac{\text{PRESS}}{\text{no. of samples}}}$. For models validated with compound feeds as an independent data set, RMSEP was calculated as $\sqrt{\sum\frac{(\text{predicted} - \text{reference})^2}{\text{no. of samples}}}$.

Additive Property. For DOM_{EDOM}, ash, CP_{corr}, CFat, NDF, and ST, additivity was evaluated to test its effect on NEL20 prediction. Additivity was calculated according to Álvarez et al. (2020) by regressing the weighted sum of ingredients for the corresponding value (predicted) against the value directly measured in the compound feed (observed). Differences between predicted and observed values are referred to as additivity residuals. For regressions, the "lm" function from the "stats" package in R (R Core Team) was used. The additivity property was compared with EU-permitted tolerances for compositional labeling of compound feeds (European Commission, 2010). The EU tolerance levels define for all chemical components the acceptable differences between declared content and actual content in concentrates (for tolerances specifications, see Figure 1). Residual analysis was performed by regressing model-predicted values against residual values. For this analysis, model-predicted values were centered around the mean, making slope and intercept independent and orthogonal for mean and linear bias evaluation, respectively (St-Pierre, 2003).

To evaluate the effect of additivity residuals on NEL20 prediction by the model, for each compound sample additivity residuals of all chemical components were evaluated together. This was done by including the additivity residual of each chemical component as input in the selected model. Using this approach, all components were evaluated at the same time and weighted by their importance by the model's coefficient, also referred to as the weighted sum of additivity residuals. The weighted sums of additivity residuals were regressed against their NEL20 residual (NEL20 predicted − NEL20 reference), and Pearson correlation coefficient (r) was evaluated.

Model Use in Practice. To exemplify how the selected model could be used in practice, 30 independent compound feed samples (referred to here as the example data set) were collected from 4 companies with their corresponding NEL20 values as declared by each company (referred to here as NEL20 declared). Ingredient composition was supplied by each company, but no ingredient samples were collected; therefore, NEL20 reference values were not calculated. The NEL20 declared values were calculated in the NorFor software and reported by each company by including ingredients of the compound feed and their chemical composition and proportion. The values used for the NorFor calculations by the companies were a combination of measured and table values, with the proportion being company dependent and unknown for this study. The compound feed samples were analyzed for DM, ash, DOM_{EDOM}, CFat, NDF, CP, and ST as well as CP_{corr} calculated by the same methods used to analyze samples for model development. The NEL20 values predicted by the best model were regressed against the values declared by the company. Tolerance limits of EU (±5%; European Commission, 2010) were used as criteria to determine significant differences in this regression.

RESULTS

Characteristics of Feed Samples

Chemical composition, digestibility, and NEL20 reference of compound feeds and ingredient samples are summarized in Table 1. Of the 75 compound feed samples, 40 contained more than 25% DM ST and could be referred to as energy supplements, whereas 21 were protein supplements with more than 25% DM CP_{corr}. The NEL20 range for compound feed presented a minimum of 6.06 MJ/kg of DM and a maximum of

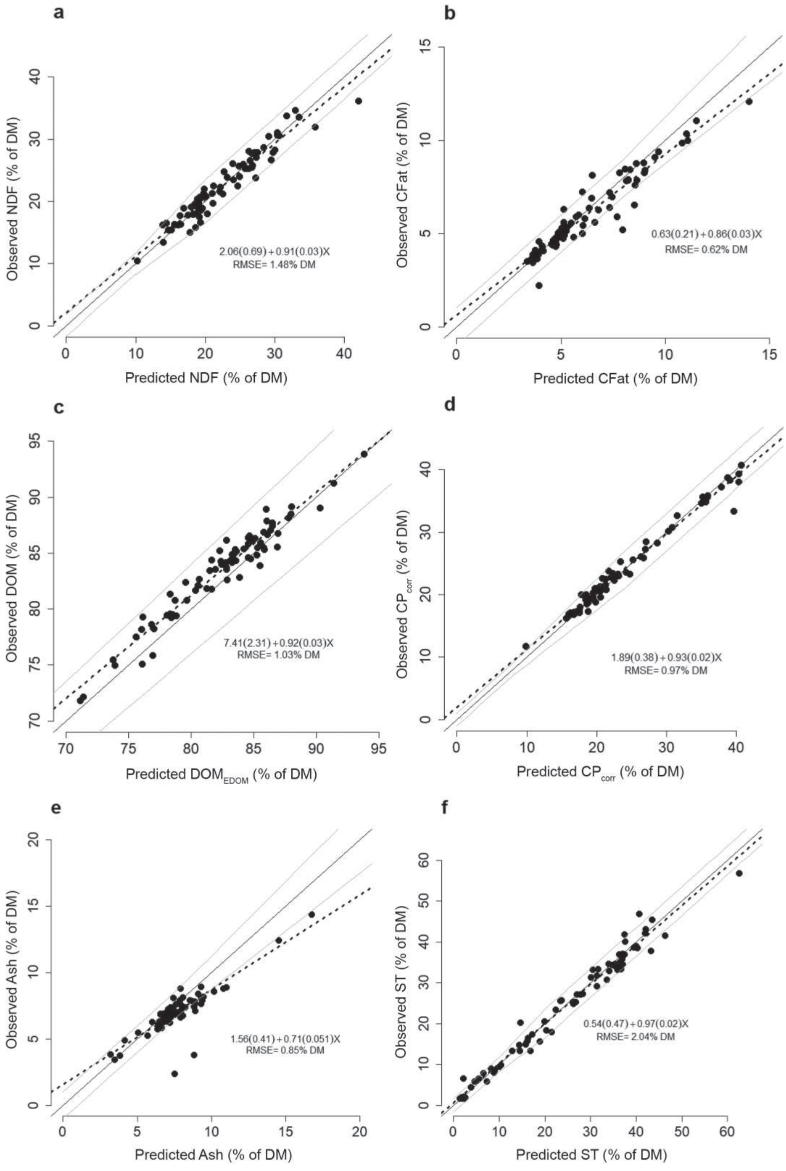

Figure 1. Regression between predicted nutrients (calculated by the weighted sum of ingredients; x-axis) and measured nutrients in compound feed (y-axis) for (a) NDF, (b) crude fat (CFat), (c) digestible OM by enzymatic digestibility of OM method (DOM_{EDOM}), (d) CP corrected by urea (CP_{corr}), (e) ash, and (f) starch (ST). Solid line: x = y; gray lines: European Union (EU) tolerance (allowed difference between declared value and actual content for the corresponding nutrient). Tolerance levels used for NDF (correspond to EU tolerance for crude fiber) and ST: ±3.5% of total mass or volume for contents of 20% or more; 17.5% of the content for contents of less than 20% but not less than 10%; ±1.7% of the total mass or volume for contents of less than 10%. Tolerance levels used for CFat, CP_{corr}, and ash: ±3% of the total mass or volume for contents of 24% or more; 12.5% of the content for contents of less than 24% but not less than 8%; ±1% of the total mass or volume for contents of less than 8%. Tolerance levels used for DOM_{EDOM} (correspond to EU tolerance for energy): ±5% of the content. Dashed lines: regression line. Regression equation: coefficient (SE). RMSE = root mean squared error.

8.43 MJ/kg of DM, with 7.14 MJ/kg of DM as the mean value.

Ingredient samples presented a wider range than compound feeds for all variables (Table 1). Ingredient samples were used to produce compound feed samples, and a detailed description of their composition, frequency of inclusion in the compound feed samples, and quantity of samples included in the data set is shown in Table 2. Rapeseed cake followed by soybean meal and rapeseed meal were the most frequently used protein-rich ingredients in compound feeds. Barley was the most frequently used starch source, followed by wheat, oats, and rye. Sugar beet pulp and wheat bran were the most included fiber-rich ingredients. Other frequently used ingredients were distillers grains, maize, and maize gluten meal.

Model Development and Validation

Stepwise AICc selection for prediction of NEL20 showed the same order of variable inclusion for the all data set and ingredient data set, with CFat as the first variable included, followed by NDF, CP_{corr}, DOM_{EDOM}, ash, and ST. For the compound data set, NDF was the first variable, followed by CFat, DOM_{EDOM}, CP_{corr}, ST, and ash. Models with ST presented a VIF higher than 3.3; thus, ST was removed from those models. After ST was removed, VIF met the criteria. Models including company and year as a random effect presented higher AICc in all model comparisons, so models only including fixed effects were chosen. Table 3 shows AICc and RMSE for all models used for model fit evaluation and RMSEP used to evaluate prediction performance. Model I presented the best fit (AICc = −71.8, RMSE = 0.149 MJ/kg of DM) and predictive performance (RMSEP = 0.149 MJ/kg of DM), followed by model J. Model E, developed with the largest data set (183 samples) and NEL20 range (3.05–18.8 MJ/kg of DM) presented a fit and predictive performance comparable with that of models J and I. Therefore, models E and I were chosen as the best models for NEL20 prediction in compound feeds, and further analysis was conducted based on these models.

Twelve out of 75 compound feed samples contained urea. Urea inclusion in these 12 samples was 1.21% of DM on average, with a minimum inclusion of 0.341% of DM and a maximum inclusion of 3.30% of DM. The effect of not correcting CP for inclusion of urea in the compound feeds was tested in models E2 and I2. Models E and I presented better fit than their corresponding models not corrected for urea (Table 3).

Additivity

The additive properties of NDF, CFat, DOM_{EDOM}, CP_{corr}, ash, and ST are shown in Figure 1a to f for the 75 compound feed samples. Of the 75 samples, 49 were also evaluated by Álvarez et al. (2020). To assess additivity, EU tolerance limits for each chemical component were used as criteria (for specific tolerance limits, see Figure 1). For NDF, the EU does not specify tolerances; thus, limits defined for crude fiber were used. Of the 75 samples, only 2 were outside these limits (Figure 1a). From residual analysis, intercept (P = 0.76) and slope (P = 0.49) presented no mean or linear bias, respectively. For CFat, 8 out of 75 samples were outside the EU tolerance limits (Figure 1b). From residual analysis, additivity of CFat presented a signifi-

Table 1. Summary statistics of the chemical composition of compound feeds and their ingredients used for model development of NEL20

	Compound feeds				Ingredients			
Item[1]	Mean[2]	Min[3]	Max[4]	SD	Mean	Min	Max	SD
Number of samples	75	75	75	75	108	108	108	108
DOM_{EDOM} (% of DM)	83.5	71.8	93.9	4.2	83.1	35.5	96.6	11.1
Ash (% of DM)	7.02	2.39	14.37	1.63	4.73	0.20	22.60	3.04
CP (% of DM)	23.9	11.7	42.7	7.21	23.7	0.131	66.7	16.4
CP_{corr} (% of DM)	23.4	11.7	40.7	6.79				
CFat (% of DM)	6.03	2.21	12.1	2.07	7.95	0.56	98.9	16.1
NDF (% of DM)	22.6	10.4	36.2	5.52	26.3	0.00	71.6	16.7
ST (% of DM)	23.6	1.60	56.8	14.1	24.0	0.00	72.6	26.6
NEL20 reference (MJ/kg of DM)	7.14	6.06	8.43	0.49	7.36	3.05	18.8	2.24

[1]DOM_{EDOM}: digestible OM by enzymatic digestibility of OM method; CP_{corr}: CP corrected by urea content in compound feeds; CFat: crude fat; ST: starch; NEL20 reference: net energy of lactation at 20 kg of DMI/d. NEL20 reference of ingredients: calculated using table values and measured chemical composition. NEL20 reference of compound feeds: calculated by the weighted sum of its ingredients.

[2]Average content.

[3]Minimum content.

[4]Maximum content.

Table 2. Summary of ingredient samples by frequency of inclusion, number of samples, share, and chemical composition[1] (values are shown as mean, with minimum–maximum in parentheses)

Ingredient[2]	Freq	No. of samples	Share (% of DM)	DOM_{EDOM} (% of DM)	Ash (% of DM)	CP (% of DM)	CFat (% of DM)	NDF (% of DM)	ST (% of DM)	NEL20_ref (MJ/kg of DM)
Rapeseed cake	48	7	12.2 (1.5–66.5)	79.0 (77.4–81.2)	6.7 (6.2–6.9)	34.0 (32.0–37.2)	14.3 (12.2–16.5)	25.9 (22.2–28.0)	2.2 (1.6–3.0)	7.66 (7.36–8.16)
Barley	46	8	23.3 (1.0–56.7)	89.6 (88.3–91.6)	2.1 (1.9–2.7)	11.7 (10.2–15.0)	3.0 (2.8–3.4)	17.3 (15.3–20.4)	59.9 (55.1–63.6)	7.27 (7.13–7.41)
Sugar beet pulp	46	7	11.1 (1.5–30.4)	89.1 (83.4–93.7)	6.3 (3.5–12.5)	9.6 (8.7–10.6)	1.1 (0.6–1.4)	38.5 (33.0–43.4)	2.0 (0.0–8.9)	6.11 (5.56–6.47)
Soybean meal	45	7	12.5 (0.6–42.4)	91.8 (89.8–92.8)	7.0 (6.2–8.1)	53.3 (49.7–56.0)	2.7 (2.2–3.0)	10.1 (6.8–12.7)	1.4 (0.5–2.1)	8.58 (8.44–8.71)
Wheat	44	8	11.3 (1.5–26.0)	95.0 (93.9–96.4)	1.6 (1.4–1.7)	12.9 (10.4–15.2)	2.5 (2.1–2.8)	10.9 (10.1–11.7)	66.5 (61.7–72.2)	7.96 (7.81–8.02)
Rapeseed meal	44	5	17.9 (3.2–79.1)	77.6 (75.2–79.2)	7.7 (7.2–8.5)	39.1 (37.6–40.8)	4.9 (4.1–6.9)	27.5 (24.7–29.4)	2.2 (1.6–2.6)	6.77 (6.64–6.91)
Wheat bran	37	8	8.9 (0.9–27.1)	77.5 (73.9–80.5)	5.0 (4.2–5.8)	16.9 (16.3–17.3)	4.7 (4.2–5.5)	44.3 (38.8–52.8)	21.0 (12.3–28.3)	5.59 (5.10–5.96)
Oats	35	7	7.9 (1.0–31.1)	74.6 (69.0–78.6)	2.8 (2.2–3.5)	12.5 (10.3–15.9)	6.3 (4.6–10.9)	31.1 (20.2–37.8)	45.4 (36.2–52.8)	6.47 (5.70–7.24)
Distillers grains	32	4	12.2 (1.1–35.7)	83.2 (80.3–85.5)	5.3 (4.9–5.6)	33.2 (30.6–36.15)	8.4 (6.8–12.5)	31.4 (28.8–35.4)	2.9 (2.1–4.4)	7.30 (7.11–7.44)
Rye	26	2	15.6 (2.9–48.7)	94.5 (93.8–95.4)	1.6 (1.4–1.7)	11.1 (8.7–13.0)	1.9 (1.8–2.2)	13.1 (12.2–14.1)	65.2 (62.9–67.6)	7.70 (7.64–7.76)
Maize	25	6	11.8 (1.8–44.9)	95.8 (93.7–96.6)	1.3 (1.1–1.6)	9.1 (8.5–9.6)	4.5 (4.2–4.8)	8.4 (7.0–9.6)	69.9 (67.4–72.6)	7.38 (7.37–7.40)
Maize gluten meal	20	3	7.0 (1.1–41.8)	91.3 (88.6–94.9)	2.1 (1.3–2.6)	66.2 (65.9–66.7)	5.4 (4.9–6.1)	4.4 (3.9–5.0)	17.8 (16.6–19.1)	9.70 (9.60–9.79)
Sunflower meal	19	3	6.1 (1.7–10.5)	73.5 (70.1–76.4)	7.1 (6.4–7.8)	39.6 (38.6–40.4)	2.6 (1.9–3.5)	30.2 (26.9–33.9)	1.3 (0.5–1.8)	6.67 (6.39–6.91)
Malt sprouts[3]	17	3	7.7 (1.3–15.3)	66.1 (61.4–71.5)	6.6 (6.0–7.4)	24.1 (21.4–26.1)	2.8 (2.4–3.1)	45.1 (41.0–51.2)	10.6 (6.7–15.4)	5.53 (5.23–5.81)
Soypass[4]	14	3	5.8 (0.5–32.7)	92.1 (91.6–92.5)	6.5 (6.4–6.6)	49.8 (48.6–51.4)	2.3 (2.3–2.4)	24.3 (23.6–25.0)	1.7 (1.6–1.8)	7.88 (7.86–7.90)
Soybean hulls	12	1	7.5 (2.2–16.4)	85.4	7.2	11.7	2.8	63.2	0.6	4.99
Expro[5]	12	2	22.7 (3.1–50.8)	75.6 (75.5–75.7)	7.6 (7.5–7.7)	37.8 (37.4–38.2)	4.1 (3.9–4.2)	30.2 (29.2–31.1)	1.3 (0.9–1.6)	6.82 (6.74–6.90)
Citrus pulp	10	1	4.4 (2.0–15.7)	91.2	6.2	7.4	2.6	21.0	1.8	6.81
Wheat middling	9	2	6.7 (1.3–14.9)	86.5 (86.3–86.8)	3.1 (2.9–3.3)	15.9 (15.5–16.4)	4.2 (3.8–4.6)	30.1 (29.0–31.1)	35.7 (33.6–37.9)	6.67 (6.60–6.74)
Palm kernel cake	6	4	7.6 (2.2–12.5)	63.3 (57.0–70.8)	4.6 (4.0–5.4)	16.9 (15.9–18.8)	7.3 (6.2–8.1)	70.5 (68.5–71.6)	1.4 (0.5–1.8)	4.62 (4.45–4.69)
Alkakorn[6]	1	1	17.2	89.0	5.0	36.1	4.0	16.0	45.9	7.33
Other[7]	75	16	<10	76.3 (35.5–94.5)	5.60 (0.2–22.6)	14.3 (0.1–37.7)	28.0 (1.5–98.9)	26.8 (0–71.3)	16.4 (0–67.6)	9.22 (3.0–18.8)
Total	75	108								

[1]Freq = number of compound feed samples that include the ingredient; No. of samples = number of samples of the ingredient; Share = range of proportion of inclusion of the ingredient in the compound feed samples; DOM_{EDOM} = digestible OM by enzymatic digestibility of OM method; CFat = crude fat; ST = starch; NEL20_ref = reference value of net energy of lactation at 20 kg of DMI/d.

[2]Individual ingredients are shown only if present in a compound feed at more than 10% of total DM. For ingredients with only 1 representative sample, no ranges are expressed.

[3]By-product of malting industry.

[4]Rumen-protected non-genetically modified organism (GMO) soybean meal (Denofa AS).

[5]Steam-processed non-GMO rapeseed meal (AAK AB).

[6]Barley treated with urea and reaction promoters (Norgesfôr AS).

[7]Ingredients with a maximum inclusion lower than 10% of DM.

Table 3. Regression equation models to predict NEL20 content (MJ/kg of DM) of compound feeds based on stepwise AICc selection

	Model fit[2]			Model prediction[3]		
Model for prediction of NEL20[1] (MJ/kg of DM)	AICc	RMSE (MJ/kg of DM)	RMSE (%)	PRESS	RMSEP (MJ/kg of DM)	RMSEP (%)
All data set (n = 183; mean NEL20 = 7.27 MJ/kg of DM)						
A: 6.43 + 0.117CFat	522.3	0.977	13.29	11.42	0.390	5.47
B: 8.17 + 0.102CFat − 0.0664NDF	214.0	0.442	5.84	5.44	0.269	3.77
C: 7.61 + 0.107CFat − 0.0622NDF + 0.0185CP_{corr}	149.0	0.487	4.87	4.15	0.235	3.30
D: 3.62 + 0.112CFat − 0.0413NDF + 0.0219CP_{corr} + 0.0402DOM_{EDOM}	43.5	0.281	3.63	2.24	0.173	2.41
E: 4.47 + 0.113CFat − 0.0429NDF + 0.0251CP_{corr} + 0.0328DOM_{EDOM} − 0.0496ash	2.8	0.246	3.22	1.84	0.157	2.20
E2: 4.17 + 0.102CFat − 0.0453NDF + 0.0195CP + 0.0352DOM_{EDOM} − 0.00568ash	11.5	0.252	3.30	2.03	0.175	2.46
Compound data set (n = 75; mean NEL20 = 7.14 MJ/kg of DM)						
F: 8.53 − 0.0620NDF	58.3	0.349	4.89	9.16	0.349	4.89
G: 7.68 − 0.0610NDF + 0.135CFat	−20.6	0.208	2.90	3.23	0.208	2.90
H: 5.07 − 0.0470NDF + 0.144CFat + 0.0272DOM_{EDOM}	−33.9	0.191	2.67	2.72	0.191	2.67
I: 3.69 − 0.0435NDF + 0.0997CFat + 0.0393DOM_{EDOM} + 0.0234CP_{corr}	−71.8	0.149	2.08	1.66	0.149	2.08
I2: 3.96 − 0.045NDF + 0.104CFat + 0.037DOM_{EDOM} + 0.019 CP	−59.0	0.162	2.26	2.02	0.162	2.26
J: 4.22 − 0.0449NDF + 0.0957CFat + 0.0346DOM_{EDOM} + 0.0244CP_{corr} − 0.0145ash	−70.2	0.150	2.11	1.69	0.150	2.11
Ingredient data set (n = 108; mean NEL20 = 7.36 MJ/kg of DM)						
K: 6.430 + 0.116CFat	354.8	1.230	16.71		0.388	5.44
L: 8.366 + 0.101CFat − 0.0687NDF	164.7	0.518	7.04		0.359	5.03
M: 7.77 + 0.105CFat − 0.0640NDF + 0.0181CP_{corr}	121.1	0.435	5.91		0.313	4.39
N: 3.99 + 0.111CFat − 0.0437NDF + 0.0214CP_{corr} + 0.0376DOM_{EDOM}	61.6	0.329	4.48		0.226	3.17
O: 4.45 + 0.113CFat − 0.0429NDF + 0.0253CP_{corr} + 0.0333DOM_{EDOM} − 0.0499ash	43.0	0.297	4.04		0.161	2.26

[1]Order of models in the table reflects stepwise inclusion of parameters for each data set. NEL20 = net energy of lactation at 20 kg of DMI/d; CFat = crude fat (% of DM); NDF (% of DM); CP_{corr} = CP, urea corrected in compound feed samples (% of DM); DOM_{EDOM}: digestible OM measured by enzymatic digestibility of OM (EDOM) method (% of DM), calculated as EDOM (%) × OM (g/kg of DM); ash (% of DM). Models A to E were developed with a data set including compound feeds and ingredient samples. Models F to J were developed with a data set including only compound feed samples. Models K to O were developed with a data set of ingredient samples only. n: number of samples in each data set.

[2]AICc = Akaike information criterion corrected; RMSE = root mean squared error.

[3]PRESS = predicted residual error sum of squares on compound feed samples; RMSEP = root mean squared error of prediction. For models A to J, $\text{RMSEP} = \sqrt{\frac{\text{PRESS}}{\text{no. of samples}}}$; for models K to O, $\text{RMSEP} = \sqrt{\sum\frac{(\text{predicted} - \text{reference})^2}{\text{no. of samples}}}$. PRESS was not calculated for models K to O as compound feeds were an independent data set; thus, no cross-validation was performed.

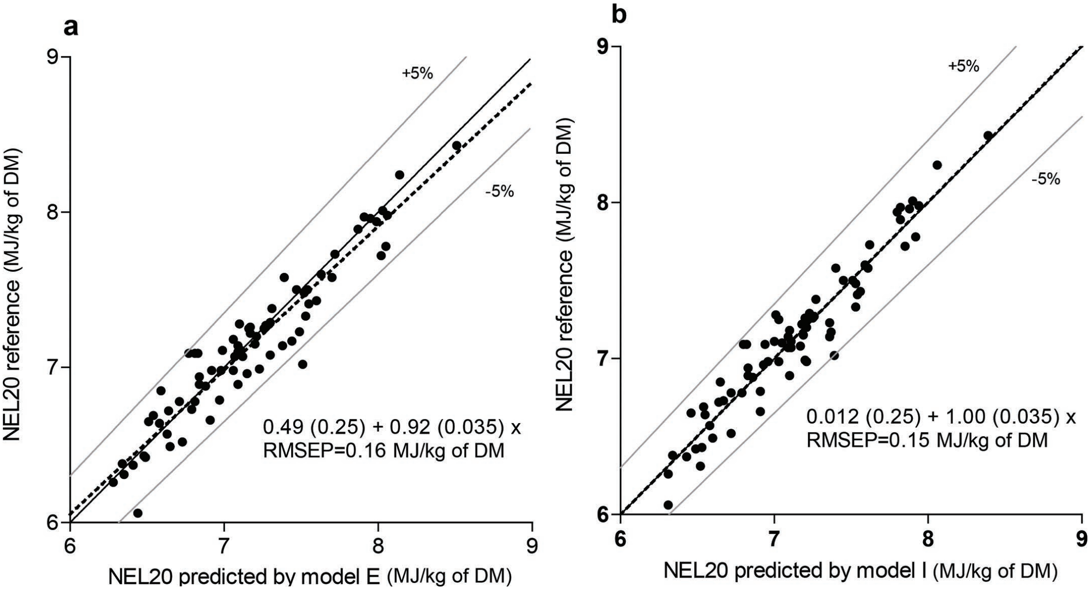

Figure 2. (a) Regression between net energy for lactation at 20 kg of DMI/d (NEL20) values of 75 compound feed samples predicted by model E (x-axis) and their respective NEL20 reference values (y-axis). (b) Regression between NEL20 values of 75 compound feed samples predicted by model I (x-axis) and their respective NEL20 reference values (y-axis). Solid lines: x = y; dashed lines: regression line; gray lines: European Union tolerance (difference between energy declared value and actual energy content; ±5% of the content). Regression equation: coefficient (SE). RMSEP = root mean squared error of prediction.

cant negative mean bias (−0.23% DM, $P < 0.05$) but no linear bias ($P = 0.17$). The EU does not provide a tolerance limit for DOM_{EDOM}, and tolerance stated for energy was used. No samples were outside these limits (Figure 1c), but residual analysis showed a significant positive mean bias (1.03% DM, $P < 0.05$) but no linear bias ($P = 0.61$). For CP_{corr}, 2 samples were outside EU tolerance limits. Residual analysis showed a significant linear bias ($P < 0.05$), meaning overestimation of low observed CP_{corr} values and underestimation of high observed CP_{corr} values (Figure 1d). No significant mean bias was detected for CP_{corr} ($P = 0.15$). Ash showed the highest number of samples (17) outside EU tolerance limits. Residual analysis showed significant mean bias (−0.63% DM, $P < 0.05$) and linear bias ($P < 0.05$) overestimating low observed values and underestimating high observed values (Figure 1e). The additivity relationship for ST showed that 8 samples were outside EU tolerance limits (Figure 1f). Starch did not show mean bias ($P = 0.28$) or linear bias ($P = 0.46$).

Regression between NEL20 values predicted by models E and I against NEL20 reference for compound feeds is shown in Figure 2a and b, respectively. Differences between predicted and NEL20 reference values were observed. From these differences, 2 samples were outside the EU tolerance lower limit (−5%) for model E, whereas no samples were outside the limits for model I. Evaluation of the effect of additivity differences on the NEL20 prediction of models E and I (Figure 3a and b for models E and I, respectively) show a strong association between weighted additivity residuals and NEL20 residuals (r = −0.73, $P < 0.001$ for model E and r = −0.69, $P < 0.001$ for model I).

Prediction of ingredient NEL20 by models E and I is shown in Figure 4. For both models, the RMSEP of ingredients was higher than for compound feeds. Figure 4 shows that ingredients such as wheat bran, maize, rapeseed meal, and rapeseed cake were overpredicted by the models (positive residuals), whereas ingredients such as sugar beet pulp, barley, rye, wheat, soybean meal, and maize gluten meal were underpredicted by the models (negative residuals). Moreover, these residuals are higher for model I than for model E. Figure 4 also shows that ingredients in the extremes of the graph (e.g., oat hulls, whole rapeseeds, and fat supplements) showed the highest residuals for model I.

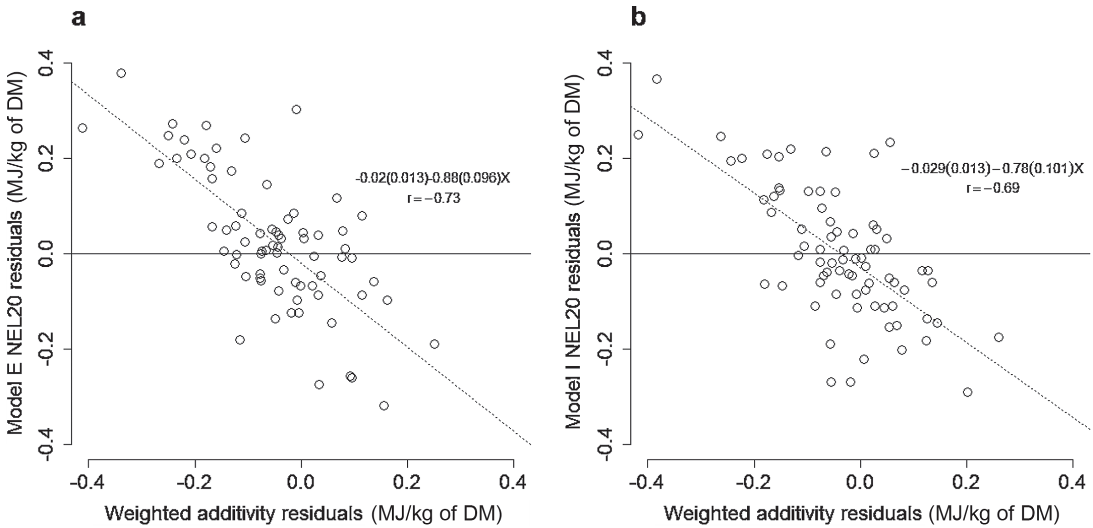

Figure 3. (a) Regression between weighted additivity differences (x-axis) and net energy for lactation at 20 kg of DMI/d (NEL20) residuals from compound feeds using model E (y-axis). (b) Regression between weighted additivity residuals (x-axis) and NEL20 residuals from compound feeds using model I (y-axis). Weighted additivity residuals: additivity differences of the chemical components weighted by including them in model E (a) and model I (b); dotted lines: regression line. Regression equation: coefficient (SE).

Model Use in Practice

Only compound feed samples were collected; therefore, no NEL20 reference values were obtained and only NEL20 declared values by the manufacturers were evaluated, simulating the practical use of models E and I. Chemical composition and NEL20 declared values are shown in Table 4. Chemical composition showed similarities to compound feeds used for model development (Table 1), although maximum values of ash, CP_{corr}, and NEL20 were higher in the example data set, but DOM_{EDOM} and ST were lower. However, as model E was developed using the all data set (including both compound and ingredients) for model development, the range of all variables was larger than in the example data set. Comparison between the NEL20 values predicted by models E and I and the NEL20 declared by companies is shown in Figure 5a and b, respectively. Mean NEL20 declared value was 7.64 MJ/kg of DM. For model E, mean NEL20 predicted value was 7.33 MJ/kg of DM, and 11 compound feeds samples out of 30 lay outside the upper 5% EU tolerance level. For model I, mean NEL20 predicted was 7.30 MJ/kg of DM, and 12 compound feed samples lay outside the upper 5% EU tolerance level. For both models, for all but 1 sample, declared NEL20 values were higher than the values predicted by models E and I.

DISCUSSION

In the NorFor system, NEL20 is the most used standard net energy value. For compound feeds, NEL20 calculation depends on ingredient composition. However, manufacturers are not obliged to declare energy content or ingredient share. Therefore, a method to measure NEL20 directly from the compound feed is essential for accurate diet formulation using NorFor, as compound feeds can represent more than half of the feed ration. With this study, we intended to formulate static empirical equations to predict the NEL20 of compound feeds independent of their ingredients, based on chemical composition and in vitro enzymatic OMD (EDOM), determined according to Weisbjerg and Hvelplund (1993) and evaluated by Álvarez et al. (2020). If the method has sufficient accuracy and all the actors using the NorFor system (farmers, advisors, feed companies, and NorFor itself) agree, a common method will enable final company verification of products, allow farmers to confirm declared net energy values, and improve trustworthiness of the industry.

Other feed evaluation systems feature empirical models for the energy prediction of compound feeds. The INRA system uses an equation proposed by Sauvant et al. (2002) based on chemical composition and OMD. In this system, OMD is predicted by

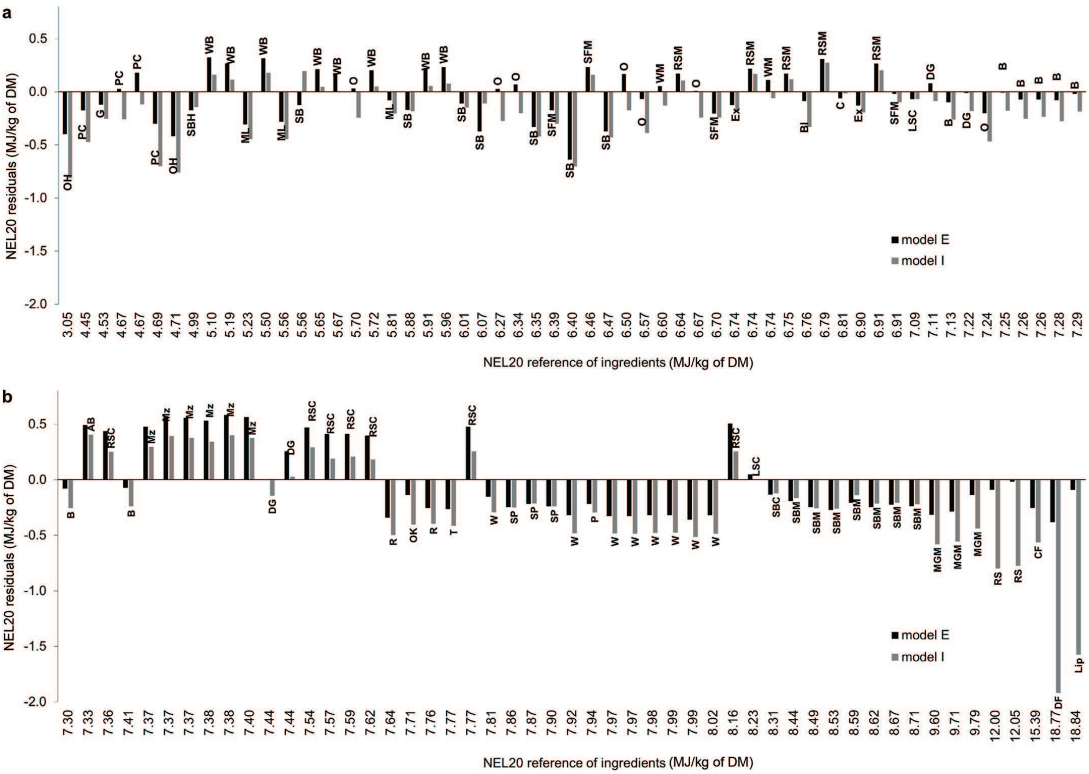

Figure 4. Net energy for lactation at 20 kg of DMI/d (NEL20) residuals of ingredients predicted by models E and I (predicted − observed; MJ/kg of DM; y-axis) against NEL20 reference values (MJ/kg of DM; x-axis): (a) NEL20 reference from 3.05 to 7.29 MJ/kg of DM; (b) NEL20 reference from 7.30 to 18.84 MJ/kg of DM. AB = alkaline barley; B = barley; Bl = mix of grains; C = citrus pulp; CF = calcium fat; DF = dry fat; DG = distillers grains; Ex = ExPro heat-treated rapeseed meal (AAK AB); G = dry Lucerne pellets; Lip = Lipitec (saturated fat; NLM Vantinge AS); LSC = line seed cake; M = maize; MGM = maize gluten meal; ML = malt sprouts; O = oat; OH = oat hulls; OK = oat kernel; P = peas; PC = palm kernel cake; R = rye; RS = rapeseed (crushed); RSC = rapeseed cake; RSM = rapeseed meal; SB = sugar beet pulp; SBC = soybean cake; SBM = soybean meal; SFM = sunflower meal; SP = Soypass (Denofa AS); T = triticale; W = wheat; WB = wheat bran; WM = wheat middling. Root mean squared error of prediction (RMSEP) of ingredients for model E = 0.29 MJ/kg of DM; RMSEP of ingredients for model I = 0.41 MJ/kg of DM (see models in Table 3).

ADF and ADL or measured by enzymatic digestibility (Baumont et al., 2018). The Feed Into Milk system (Thomas, 2004) recommends an equation based on compound feed enzymatic digestibility (Thomas et al., 1988). Germany uses prediction equations based on an enzymatic method for OMD determination (GfE, 2009). Denmark established an official prediction equation also based on OMD, measured by the EDOM method, for the Danish feed-unit system (Weisbjerg and Hvelplund, 1993) and chemical composition. Thus, chemical composition and OMD were included as potential variables in this study. Selection of DOM_{EDOM} for OMD representation was based on the premise that models should be not only accurate and precise but also easy to adopt. The EDOM method has been proven accurate (Weisbjerg and Hvelplund, 1996) and precise (Álvarez et al., 2020). Moreover, it is already a familiar method used in commercial laboratories in Scandinavia, as it is the official method for energy declaration in compound feeds in the static Danish feed-unit system (Danish Veterinary and Food Administration, 2020) and is used for OMD determination in some forage types. In this study, inclusion of DOM_{EDOM} as a variable agrees with the results of Cottyn et al. (1984) and De Boever et al. (1994), who found that models that only included chemical composition were less accurate than models that also included OMD as a variable (Table 3).

Prediction of NEL20

Two years of sample collection from several Scandinavian feed companies revealed a large variation in samples' net energy values (6.06–8.43 MJ/kg of DM in compound feeds and 3.05–18.8 MJ/kg of DM in ingredient samples), representative of the industry. Industry representation was also reflected in the collected samples, as most ingredients used in compound feed production, such as cereals, rapeseed by-products, and soybean meal were represented by the highest sample number in our data set (Table 2). This variation allowed the model development to be as representative of the Scandinavian feed industry as possible, supporting the adoption of the selected model in practice.

The use of 3 data sets in this study allowed models to be created and evaluated using different net energy ranges and feed types (compounds, or ingredients, or both), providing a more solid base for model evaluation. However, inclusion of ingredients as part of the data set is debatable, as the objective is to use the model on compound feeds. Moreover, including the ingredients could result in dependency in the data set, as compound feeds included in the data set are produced by the ingredients. Nevertheless, we decided to include the evaluation of model E to compare its performance with the best fitting model, model I.

The equation error of model I (RSD = 1.99% of mean) was lower than the average error of 3.97% of mean (average of 2.57 and 5.36% for normal and fiber-rich concentrates, respectively) reported by De Boever et al. (1994) and 4.32% of mean reported by Giger-Reverdin et al. (1994). The German system (GfE, 2009), static and empirical, reported an equation error of 2.04% of mean (RMSE), similar to model I. The Danish feed-unit system (Weisbjerg and Hvelplund, 1993), which is also static and empirical, showed a higher RSD than model I for digestible energy (3.13% of mean).

Better prediction of compound feeds of model I (RMSEP = 2.08% of mean) compared with model E (RMSEP = 2.20% of mean) could be explained by the inclusion of the same compound feeds for model development and for this evaluation. On the contrary, model E included a larger range, as not only these compound feed samples but also the ingredient samples were included.

Higher error of prediction for ingredient NEL20 of model I was expected, as ingredients were not included in the data set for development of this model but were included in the development of model E. Prediction error for model E was lower than the EU 5% limit (3.98% of mean) and therefore could potentially be used to estimate NEL20 of ingredients. However, to be included as a useful model for ingredient prediction, validation against an independent set of ingredients should be performed. As the prediction error of ingredients of model I was higher than the EU tolerance limit (5.58% of mean), model I could potentially not be useful for prediction of NEL20 of ingredients, although an independent validation is also recommended. When ingredients were not included in the developing data set, as for model I, extreme contents of chemical components had an effect on the prediction of ingredients. For example, oat hulls with high content of NDF, rapeseed with high content of CFat, and pure fat supplements showed a significant underestimation of NEL20 values by model I but not by model E. This could be due to the NEL20 of these ingredients being outside the NEL20 range used for development of model I. High prediction error of ingredients supports the use of detailed characteristics in the dynamic model for prediction of ingredients, such as indigestible fractions and passage rates, if available. These characteristics are available for ingredients in most cases.

The additivity results from this study can be compared with the results of Álvarez et al. (2020), although they share 49 out of 75 compound feeds. Additivity errors (RMSE) in our study showed errors similar to those identified by Álvarez et al. (2020) for DOM_{EDOM} (1.03% vs. 1.07% of DM), NDF (1.48% vs. 1.46% of DM), and ST (2.04% vs. 1.90% of DM), whereas CP_{corr} showed lower error in our study (0.97% vs. 1.56% of DM) but higher error for CFat (0.62% vs. 0.39% of DM). Additivity values for ash were only evaluated in our study and showed high differences between weighted sum of ingredient calculation and ash analyzed directly in the compound feed. These differences could be allocated to the siliceous proportion of the ash, as dry incineration could produce a cover over the sample, preventing

Table 4. Chemical composition (% of DM) and NEL20 values (MJ/kg of DM) of compound feeds collected from 4 feed companies in Scandinavia and used as an example of models E and I

Item[1]	Mean	Minimum	Maximum	SD
No. of samples	30	30	30	30
DOM_{EDOM}	82.5	76.1	87.9	2.62
Ash	7.91	6.20	16.6	1.91
CP	27.6	18.6	44.2	6.93
CP_{corr}	27.4	18.6	44.2	6.62
CFat	7.53	5.20	12.0	1.62
NDF	23.4	14.9	28.4	3.13
ST	15.2	1.60	31.8	10.6
NEL20 declared	7.64	6.97	9.00	0.443

[1]DOM_{EDOM} = digestible OM by enzymatic digestibility of OM method; CP_{corr} = CP corrected by urea content in compound feeds; CFat = crude fat; ST = starch; NEL20 declared = net energy of lactation at 20 kg of DMI/d, declared by the feed companies. Calculated by each company as weighted sum of NEL20 of ingredients using table and measured chemical composition. Proportion of table and measured values depended on each company and is unknown for this study.

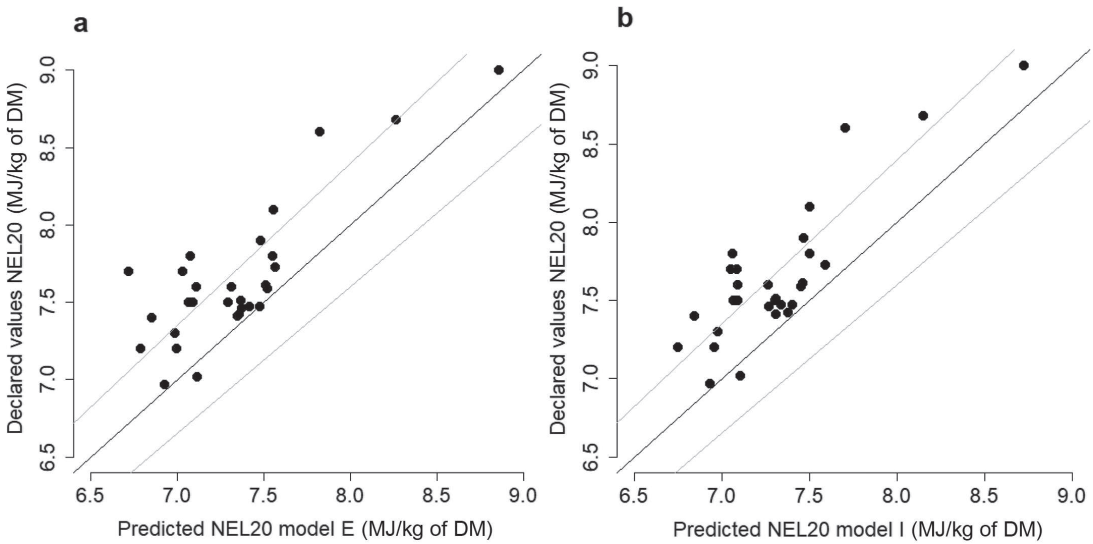

Figure 5. (a) Relationship between net energy for lactation at 20 kg of DMI/d (NEL20) values in 30 compound feed NEL20 values predicted by model E (x-axis) and declared by the feed companies (y-axis). (b) Relationship between NEL20 values in 30 compound feed NEL20 values predicted by model I (x-axis) and declared by the feed companies (y-axis). Solid lines: x = y; gray lines: European Union declaration limit of ±5.0%. NEL20 predicted by model E = 7.33 MJ/kg of DM; NEL20 predicted by model I = 7.30 MJ/kg of DM; NEL20 declared = 7.64 MJ/kg of DM.

complete combustion (Nørgaard Pedersen, 1962; Liu, 2019). As ingredients samples get analyzed in their pure form or mixed in the compound feed, the siliceous proportions of the analyzed samples get modified, thus potentially affecting the combustion performance. Higher incineration temperatures (600°C) and longer incineration time could be used in the reanalysis (Liu, 2019).

Although additivity correlation for all variables was high, some differences in additivity were found (Figure 1a to f). The effect of the lack of additivity of chemical analyses on residual values for NEL20 was expected and showed a correlation of −0.73 for model E (Figure 3a) and −0.69 for model I (Figure 3b). This indicates that, although not completely, a large proportion of the differences between NEL20 reference and predicted values could be attributed to differences between ingredients and compound feed analysis. The effect of lack of additivity can be explained by the fact that NEL20 reference values are calculated with ingredients, whereas the NEL20 values predicted by model I are based on nutrients analyzed in compound feeds. Thus, any difference in chemical components between the ingredient weighted sum and compound feeds (lack of additivity) will be reflected in NEL20 residuals. Identification of this error source in the model is meaningful because it can explain potential differences between declared and predicted NEL20 values. Moreover, these differences can be easily detected by performing an additivity comparison, as shown in Figure 1a to f. Lack of additivity could be caused by analytical errors, potential interactions among ingredients, sampling errors, or mixing errors when creating the compound feed. Another potential error is the methods used for analyzing chemical components. For nitrogen analysis, NorFor recommended the Dumas or Kjeldahl methods; thus, using different methods could potentially result in differences. However, these methods have shown high correlation (0.99) in grain and other feedstuffs (Hansen, 1989; Watson and Galliher, 2001), although it was not tested in our study. For the other chemical components, NorFor recommends only one method for each, and those methods were the ones used in this study. Moreover, intrinsic errors of the model will also contribute to the differences between NEL20 reference and predicted values.

Model Use and Maintenance

Dynamic ration evaluation systems such as NorFor can be supplemented by static empirical models. This type of static model could serve as a proxy when data for calculating the reference method is time consuming and expensive to measure. It could be used as a corroboration method by feed manufacturers to determine whether the final compound feed matches the planned energy content. It could also be used at the farm level to evaluate a concentrate mix produced on the farm. The model would reduce farmer expenses, as analysis could be done on the final product instead of on all ingredients. Moreover, the model could be used by the purchaser to ensure that the purchased compound feeds correspond to the declared energy values. The example given in Figure 5a and b shows, for both models, that the net energy content in 12 out of 30 samples lay outside the EU acceptance limit of ±5%. However, that this is an example for the model use in practice and we are not comparing NEL20 reference values, as no ingredients were analyzed. The finding suggests that not only differences in additivity or the intrinsic model error could be sources of error. Deviation between declared and real inclusion of ingredients, direct mixing errors, and variation in chemical composition and digestibility of ingredients could result in differences between NEL20 values predicted by these models and declared by the companies. In this respect, feed companies could use a systematic evaluation method such as this as a useful tool for quality control of compound feeds. Moreover, a systematic evaluation method could be a useful tool for authorities, as it would improve reliability in the feed industry. However, these models predict NEL20 based on estimates, not in vivo values.

Implementation of the model could encounter challenges related to specific ingredients, such as urea content. In the NorFor system, urea has a net energy content of zero; therefore, CP was corrected for the CP originating from added urea in compound feed samples used to develop the model. If not corrected, CP would include the NPN from urea; thus, the regression factor for CP in the model would be underestimated. This is reflected in model I2, where the coefficient of CP is lower when urea was not corrected, with a higher prediction error. Nevertheless, the need for urea correction challenges the objective of this study, which was to develop a model that is independent of ingredient composition, as the urea content needs to be known for it to be corrected. European and US laws require urea content on their feed labels; therefore CP_{corr}, could be easily obtainable. An alternative approach is to analyze for ammonium-N in the compound feed and use this for correction of urea-N. It is important to point out that compound feed containing urea will contain higher and erroneous predicted values if not corrected. Therefore, the use of models on samples with urea would require further analyses of the urea or ammonium content.

For developing a static model, it was central for this study to detect variables that were independent from the resource-demanding kinetic feed variables, such as nutrient degradation rates and indigestible fractions. However, this also challenges the models because the rate of degradation and digestibility of ingredients may change in the future due to new varieties, feedstuffs, studies, or technology. Such changes would modify the reference values but would not affect the parameters used in the empirical model; therefore, the changes would not be reflected in the predicted values.

Maintenance of the model's predictive performance should be done frequently. Model maintenance can be accomplished by collecting and analyzing representative compound feeds and their corresponding ingredients to calculate the reference value to be used for model recalibration. Recalibration of the static model would be required whenever the reference dynamic model is changed and updated.

CONCLUSIONS

This study developed a static empirical model for the prediction of net energy content of compound feeds in NorFor by using OMD measured by EDOM and chemical components measured directly in compound feeds. The proposed best model, model I, included CFat, NDF, DOM_{EDOM}, and CP_{corr} as independent variables. The model was developed using 75 compound feed samples as a data set, representative of the compound feeds used in the feed industry in Scandinavia today. The model could allow estimation of net energy concentration of compound feed samples when input data for the dynamic model are lacking. However, independent evaluation is required for the proposed method to be adopted as a valuable tool in practice.

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Effect of digestibility of silage and concentrate intake on milk yield: a meta-analysis

Paper III

Effect of digestibility of silage and concentrate intake on milk yield: a metanalysis

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Abstract

Our study evaluated the effect of grass silage digestibility and concentrate intake on milk yield of dairy cows. Eighty group mean treatments comprising different grass silage digestibility and concentrate levels were collected from nine published studies. Linear mixed models were developed with study as random effect. Silage quality was included in the models as organic matter digestibility (OMDs) or energy concentration. Concentrate intake (DMIc), concentrate proportion of total dry matter intake or energy intake were further tested. Best fit model included curvilinear effect of DMIc and linear effect of OMDs. Moreover, there was an interaction between DMIc and OMDs, where higher digestible silages resulted in higher milk yield, but marginal response in milk to increase of DMIc was lower. For low digestible silages, highest milk production was achieved with higher DMIc as compared to high digestible silages.

Keywords: milk production, substitution rate, dairy cow, meta-analysis

Introduction

Due to long winters, northern European countries depend on conserved roughage, mainly grass silage, for winter feeding (Bernardes *et al.*, 2018). Prioritising the use of grass silage over concentrate in rations for dairy cattle is important for several reasons: grasslands have higher carbon sequestration (Soussana *et al.*, 2004), and more dry matter (DM) and protein yield per hectare than most annual crops. Further, grass is often cheap compared with other crops. Grass silage quality varies significantly due to management strategies. Maturity at harvest is one of the most important factors affecting digestibility due to changes in cell wall content and lignification (Rinne *et al.*, 1997). We hypothesise that lower concentrate levels can be used with highly digestible silages, without compromising milk production. For this, the objective of this study was to evaluate the relationship between grass silage digestibility and concentrate level on milk production based on previous experiments evaluating these two parameters.

Materials and methods

The dataset included 80 group means with mixed parity from nine published studies conducted after 1990. The criteria to be included in this metanalysis study were grass silage as the predominant forage source, at least two levels of silage digestibility and two levels of concentrate within digestibility. A summary of the studies included is shown in Table 1. The evaluated dependent variable was average daily milk production (kg per day) expressed as energy corrected milk (ECM, 3.14 MJ kg^{-1}). Feed ration values were calculated based on feedstuff and animal characteristics of each experiment using the NorFor model (Volden, 2011). Data were analysed using mixed models using 'lmer' package in R software with study as random effect. Evaluated independent variables included silage organic matter digestibility (OMDs) or energy concentration (NELs), concentrate intake in kg DM/day (DMIc) or energy/day (NELc), concentrate proportion of total DM intake (DMIt) and chemical components of the ration. The level of significance for inclusion of an effect in the model was 0.05. Model fit comparison was done using Bayesian information criterion (BIC) statistic, R squared statistics (R^2) for mixed models and root mean square error (RMSE).

Table 1. Summary of trails included in the dataset.

Reference	Cows	Breed[2]	OMD silage (%)[3]	Concentrate level[4]
Aston *et al.* (1994)	40	H	68.8, 82.5	3, 6, 9, 12 kg
Rinne *et al.* (1999)[1]	32	FA	68.2, 74.9, 77.9, 79.6	7, 10 kg
Ferris *et al.* (2001)	48	H	71.2, 81.4	Hi: 10, 30, 50, 70%
				Me: 32, 48, 64, 80%
Kuoppala *et al.* (2008)	24	FA	67.3, 68.9, 69.2, 72.7, 73.4, 76.7	8, 12 kg
Prestløkken *et al.* (2008)	32	NR	74.1, 74.7, 81.4, 81.6	4, 10 kg
Randby *et al.* (2012)	66	NR	69.3, 76.3, 80.6	0, 4, 8, 12, 16 kg
Sairanen and Juutinen (2013) exp 1	36	FA	72.7, 74.2, 77.3	9, 11, 13 kg
Sairanen and Juutinen (2013) exp 2	40	FA	69.2, 76.3	4, 6.5, 8, 11.5 kg
Alstrup *et al.* (2016)	24	H	73.9, 74.2, 76.2, 79.1	20, 50%

[1] The treatments without rapeseed effect were taken into consideration.

[2] H: Holstein, FA: Finnish Ayrshire, NR: Norwegian Red.

[3] Range of *in vivo* organic matter digestibility tested in each study.

[4] Concentrate levels presents as % correspond to total mixed ration feeding systems. Hi: High digestible silage. Me: medium digestibility silage.

Results and discussion

The model which fitted best the ECM response presented a curvilinear effect of DMIc, a linear effect of OMDs and an interaction between DMIc and OMDs. Predicted ECM from this model to increased concentrate level using 3 different OMDs is shown in Figure 1. Maximum milk yield for 82% OMDs (32.2 kg ECM) is achieved with 10.0 kg DMIc and 12.4 kg silage intake (22.4 kg DMIt), which corresponds to a 0.45 concentrate: forage ratio. For the lowest digestibility silage, maximum milk yield was lower (29.8 kg ECM) and achieved with DMIt of 21.2 kg DM, of which 12.0 kg DM is DMIc, resulting in a concentrate: forage ratio of 0.57. Results confirm our hypothesis, showing higher milk yield in higher digestibility silages achieved with lower concentrate levels. Higher digestibility silages reported higher DMIt, agreeing with Huhtanen *et al.* (2007), and increased linearly with DMIc. However, silages with higher digestibility showed lower marginal response to increased concentrate level. The curvilinear response of ECM to DMIc could be explained by the decreased digestibility of the fibre (Kristensen and Aaes, 1989) together with a shift of nutrient partitioning to body weight gain (Ferris *et al.,* 2001; Randby *et al.,* 2012). Silage intake decreased by 0.53 kg DM per kg DMIc (substitution rate), which is similar

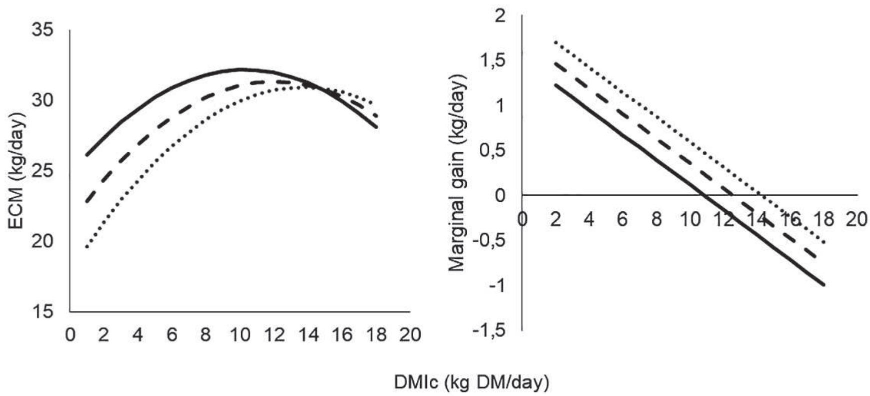

Figure 1. Predicted milk production (ECM) from the resulted model to increase concentrate intake (DMIc in kg) for 3 different silage digestibilities (OMDs in %): 82% OMDs (——), 75% OMDs (- - -), 68% OMDs (••••). Model: $y=-16.1+4.2\ DMIc - 0.07\ DMIc^2 + 0.50\ OMDs - 0.03\ DMIc \times OMDs$.

to the value of 0.47 kg DM reported by Huhtanen *et al.* (2008) although in our study substitution rate was not affected by concentrate level. Moreover, it was not affected by silage digestibility, in contrast to Faverdin *et al.* (1991).

Conclusions

Milk yield showed a curvilinear response to concentrate level, with decreasing increments. The response in milk was lower in silages with higher digestibility, achieving the same milk yield with lower concentrate level as compared with lower digestible silages. Thus, practices that increase silage digestibility also increase milk yield and reduce the need for concentrates in dairy cow diets.

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High-digestible silages allow low concentrate supply without affecting milk production

Paper IV

INTERPRETATIVE SUMMARY

High-digestible silages allow low concentrate supply without affecting milk production

(Álvarez et al.)

Harvest maturity is among the most important factors altering silage digestibility. Moreover, concentrate fed with silages can impact production and the environment, but its magnitude depends on silage digestibility. This study showed that lower concentrate levels can be supplied to cows when feeding high-digestible silages without compromising milk production. In addition, increasing concentrate showed a higher milk production response in cows fed low-digestible silages, which agrees with the tested meta-analysis. Regardless of silage digestibility, methane emissions increased with increasing concentrate intake, whereas methane per unit of intake and milk decreased with increasing intake.

HIGH-DIGESTIBLE SILAGES ALLOW LOW CONCENTRATE SUPPLY WITHOUT AFFECTING MILK PRODUCTION

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High-digestible silages allow low concentrate supply without affecting milk production

ABSTRACT

In this study, we tested a response function comprising responses in milk to changes in silage digestibility and concentrate supply. We studied the effect of changes in silage digestibility and concentrate supply on milk yield, feed intake, body weight, and methane production using 60 Norwegian Red cows. The experiment was a complete randomized block design comprising three periods. In the pre-experimental period (**PreP**), all the cows were fed a common silage ad libitum and concentrate according to yield. Next, in response period 1 (**RP1**), the cows were divided into two treatments, where a low-digestible silage (**LDS**) was fed to half of the cows, and the other half were fed a high-digestible silage (**HDS**). Concentrate was optimized according to the yield and type of silage offered. In this period, the effect of silage was evaluated using a mixed model, including the results from PreP, with parity as a covariate and animal as a random effect. In response period 2 (**RP2**), the concentrate level was evaluated by dividing the silage digestibility treatments further into three subgroups. Concentrate was increased by 2 kg dry matter (**DM**), decreased by 2 kg DM, or remained unchanged. In RP1, silage treatments were optimized to obtain similar yields and resulted in a lower concentrate offer to HDS treatment. However, the HDS treatment showed a 3.0 kg DM higher total feed intake due to a higher-than-expected silage intake. This resulted in 3.5 kg higher energy-corrected milk (**ECM**). Methane emissions were similar between silage treatments, but HDS showed lower methane per kg DM due to its higher intake. The effect of concentrate supply level and interaction with silage digestibility was evaluated using mixed models, including the results for RP1, with parity as a covariate and animal as a random effect. The reduction in concentrate offer by 2 kg in RP2 was compensated for by increased 1.3 kg

DM of silage intake for HDS, resulting in similar intake (22.1 kg DM and 21.7 kg DM without and with concentrate reduction, respectively) and ECM yields (29.4 and 29 kg ECM without and with concentrate reduction, respectively). However, concentrate offer reduction could not be compensated for by increased silage intake for LDS and resulted in lower milk yields (27.5 kg ECM). Increased concentrate showed a higher marginal ECM response (kg ECM per kg of additional concentrate intake) for LDS (1.8 vs. 3.3 kg ECM for HDS and LDS, respectively). Thus, the drop in milk yields could be compensated for by increased concentrate offers if LDS are fed. Total methane production increased with increased concentrate intake, regardless of silage digestibility. Methane emissions per unit of feed and milk were affected by total DM intake rather than by changes in silage digestibility and concentrate level.

Key Words: dairy cow, milk response, methane, silage maturity, meta-analysis.

INTRODUCTION

The importance of forage in ruminant nutrition has been widely discussed and accepted for several reasons. Ruminants' unique characteristics of digesting fiber, a growing human population that increases competition for cropland, and higher prices of concentrates are the most cited. Silage making is a widely used conservation strategy of forage because it ensures the possibility of preserving feed for cows (Bernardes et al., 2018) with minor energy losses compared with drying (Mahanna and Chase, 2003). However, as silage digestibility varies greatly and depends on several factors, high-producing dairy cows are often fed high levels of concentrates to secure sufficient energy and nutrient intake to meet their requirements (Abrahamse et al., 2008). Despite this, numerous studies have shown the advantages of high-digestible silages on milk yield (Randby et al., 2012; Alstrup et al., 2014; Weisbjerg and Johansen, 2017). Álvarez et al. (2020) conducted a meta-analysis study and showed that high-digestible silages can induce high milk yields with lower concentrate levels than low-digestible silages (Figure 1). At low levels of concentrate, higher milk yields were achieved with increased silage digestibility. As concentrate intake levels increase, ECM differences between silages disappear due to a lower ECM marginal response with high-digestible silages. This meta-analysis found no differences in substitution rates (**SR**) between silages, but other studies showed higher SR as silage digestibility increased (Faverdin et al., 1991; Thoomas, 1987). In addition, several studies have shown the effect of grass maturity (Johnson and Johnson, 1995; Moe and Tyrrell, 1979; Brask et al., 2013) and concentrate level (Ferris et al., 1999; Aguerre et al., 2011; Jiao et al., 2014) on methane emissions, although these effects were studied separately. Therefore, the effect of silage digestibility and concentrate level on methane emissions requires a combined evaluation.

The objective of this study was to test the meta-analysis of Álvarez et al. (2020) by challenging cows with different silage digestibility and evaluating the interaction between silage digestibility and concentrate level on cow responses. Further, the aim was to evaluate these scenarios from a climate perspective, measuring methane emissions for different silage digestibility and concentrate levels. From Figure 1, we hypothesize that lower intake levels of concentrate are needed to maintain high milk yields by increasing silage digestibility. Moreover, higher responses of milk with increased concentrate levels will be achieved with low-digestible silages. From an environmental perspective, we hypothesize that high-digestible silages with low concentrate levels will produce less methane than low-digestible silages with higher concentrate levels.

MATERIALS AND METHODS

Preparation of Grass Silages

Silage preparation aims to obtain silages with different organic matter digestibility (OMD). For this, two spaced harvest dates were performed: early harvest on May 28, 2019 and late harvest on

June 18, 2019. Harvests at both dates were performed from three fields at the Norwegian University of Life Sciences in Ås, Norway (59ºN, 10ºE). Based on seed weight, all fields comprised a mix of 50% timothy (*Phleum pratense*), 25% meadow fescue (*Festuca pratensis*), 15% meadow grass (*Poa pratensis*), and 10% white clover (*Trifolium repens*). Spring fertilization of all fields was performed on the April 9 and comprised 120 kg/ha of nitrogen (88 kg as mineral fertilizer and 32 kg as manure), 12 kg phosphorus, and 40 kg potassium. After cutting, grass silage was pre-wilted for 6 to 24 h, aiming for a DM content of 25 to 30%. The grass was ensiled in round bales using 5.1 L per ton of fresh matter of a formic acid-based additive (GrasAAT Plus, Addcon Nordic AS, Porsgrunn, Norway) and eight layers of plastic film. Table 1 shows the DM content, chemical composition, and fermentation quality of the resulting grass silages.

Diets and Experimental Design

The experiment was conducted at the Animal Production Experimental Center at the Norwegian University of Life Sciences, following the laws and regulations controlling experiments on live animals in Norway under the surveillance of the Norwegian Animal Research Authority.

Figure 2 shows a summary of the diets and experimental design. The experiment was conducted from February 28 to April 25, 2020 and comprised three periods on which the dietary treatments depended. From February 28 to March 18, the pre-experimental period (**PreP**), 60 cows were fed a common silage ad libitum, comprising a mix of 50% low-digestible silage from the late harvest (**LDS**) and 50% high-digestible silage from the early harvest (**HDS**) (fresh weight basis). Silage mixing for offering comprised six round bales, three from the LDS and three from the HDS. Within silage digestibility, one bale came from each of the three fields included in the study. The mixing duration was approximately 20 minutes using Siloking Duo 1814 (Kverneland, Bryne, Norway) and was performed three times per week. Concentrate offer was determined individually for cows according to milk yield, DIM and body weight recorded over a 7-day period before initiating PreP. These optimizations were performed using the NorFor Feed Ration Optimizer (**NFRO**, Volden et al., 2011b). In response period 1 (**RP1**), from March 19 to April 4, 2020, cows were blocked by parity and, were randomly allocated into two groups—30 of the cows were fed HDS, and the other 30 cows were fed LDS. Silage was fed ad libitum by ensuring a residue of 5 to 10% of the offer. Mixing of silage for feeding included mixing three bales per group. The HDS cows were offered three mixed bales from the early-cut silage, one from each field, and LDS cows were offered three mixed bales from the late-cut silage, again one from each field. Concentrate level was determined by optimization in NFRO with the corresponding silage digestibility and information of individual milk yield, weight, and DIM recorded from PreP. In response period 2 (**RP2**), from April 5 to 25, each group was further randomly divided into three subgroups; in this case, no diet optimization was performed. Instead, a group of 10 cows maintained the concentrate offer level from RP1 (**Standard**), another group of 10 cows increased 2 kg DM/day from the standard concentrate level from RP1 (**Plus2**), and a third 10-cow group was offered 2 kg DM/day less concentrate than the standard level from RP1 (**Minus2**). This resulted in six subgroups for RP2: three from HDS with standard concentrate (**HDS_Standard**), HDS with increased concentrate offer (**HDS_Plus2**), HDS with decreased concentrate offer (**HDS_Minus2**), and three from LDS with standard concentrate (**LDS_Standard**), LDS with increased concentrate offer (**LDS_Plus2**), and LDS with decreased concentrate offer (**LDS_Minus2**). As in RP1, silage mixing was performed for this period. For all periods, the offered concentrate was a commercial compound comprising barley, oat, maize, soybean meal, sugar beet pellets, molasses, minerals, and vitamins (Drøv energirik, Norgesfôr, Mysen, Norway). Table 1 shows the chemical composition of the concentrates.

Animals, Housing, Feeding, and Management

The 60 Norwegian red cows—26 first lactation, 22 second lactation, and 12 older cows—were loosely housed with concrete floors and rubber mats with sawdust for the whole experiment. At the end of PreP, cows had the following averages (± standard deviation): 599 ± 56.7 BW, 130 ± 28.8 DIM, 32.0 ± 6.7 kg ECM, and 21.6 ± 3.27 DMI. The silages were fed from 40 individual automatic feeders (BioControl AS, Rakkestad, Norway) equipped with vertically moving gates, where electronic cow identification ensured each cow's access to the correct silage source. For PreP, the 40 bins were filled with the mixed silage. For RP1 and RP2, half of the bins were filled with LDS and the other half with HDS. Silage was fed into the feed bins once a day, between 7 and 8 o'clock in the morning. The concentrate mixture was fed in the milking robot, three concentrate feeders and two GreenFeed Emission Monitor Systems (**GEM**, C-Lock Technology Inc., Rapid City, SD). The concentrate feeders and the GreenFeed units were located central to the barn, accessible through a Smart Gate (Delaval, Tumba Sweden). All units were equipped with a back-gate, allowing intake of concentrate without interference. The 60 cows used were introduced to the feeding and milking system in good time before the start of the experiment. Cows had access to water ad libitum, silage ad libitum, and the allowed concentrate level all day. The concentrate was distributed with 1 kg per day in the GreenFeed units, and the remaining part in the milking robot and any of the three concentrate feeders. Concentrate offering was restricted to a maximum of 4 kg per feeding and a time gap of 4 hours between the two feedings, depending on total daily allocation. In RP2, changes in concentrate for the Plus2 and Minus2 groups were made by increasing and reducing the offer in the concentrate feeders, respectively.

Experimental Measurements, Analysis, and Calculations

Feed intake was recorded daily. Silage was recorded using the bin's weighing mechanism as the difference in weight between opening and closing of the bin gate. Daily silage intake was the sum of all individual visits. Intake of concentrate was recorded by volume in the milking robot feeder, concentrate feeders, and GEMs. None of these concentrate feeding systems measured leftovers; therefore, it was assumed that the amount fed was all eaten by the animal. Samples of silage were taken every second day and subsampled into biweekly samples for analysis. Samples of concentrate were taken weekly and pooled into period samples (three samples in total) for analysis. Dry matter content for silage was determined by drying at 60ºC and adjusted for losses of volatiles, as described by Åkerlind et al. (2011). For concentrate, DM was determined by oven drying at 103ºC for 24 hours. Samples for chemical analysis were ground using a cutter mill fitted with a 1.0 mm screen. For concentrate, a proportion was also ground using a 0.5 mm screen for starch analysis. Feed samples were analyzed for nitrogen, according to the Kjeldahl method using the Kjeltec 8400 automated distillation unit (Foss Analytical, Hillerød, Denmark), and CP was estimated as N × 6.25. Soluble CP (**sCP**) was determined according to NorFor by extracting samples in a borate–phosphate buffer. After centrifugation, the sCP in the supernatant was determined using Kjeldahl method. Crude fat was determined by accelerated solvent extraction using Dionex™ ASE™ 350 Accelerated Solvent Extractor (Thermo Scientific, Waltham, USA). Analyses for NDF, ADF, and ADL were performed using the Ankom^{200} Fiber Analyzer (Ankom Technology Corporation Fairport, NY, USA). NDF (ash corrected) was determined using the amylase-treated NDF method (Mertens et al., 2002). ADF (ash corrected) was determined by incubating the sample for 60 minutes with sulfuric acid and cetyl trimethylammonium bromide, and ADL (Ankom Technologies, 2017) was determined after ADF determination by incubating the samples in sulfuric acid for 3 hours (Ankom Technology, 2020). Starch was determined by first hydrolyzation with α-amylase and amylo-glucosidase to glucose, which was finally determined spectrophotometrically by color reaction using RX Daytona + (Randox Laboratories Ltd, UK). Ash was determined by incineration at 550°C for 8 hours (European Commission, 2009). Organic matter digestibility was determined by near infrared analysis at Eurofins Agro (Moss, Norway). Calculation of standard feed values at 20 kg DM intake level was done according to NorFor (Åkerlind and Volden, 2011), giving net energy of lactation (**NEL_{20}**), amino acids absorbed in the small intestine (**AAT20**), and protein balance in the rumen (**PBV20**). Fill value (**FV**) for silages and total diet NE_L was also calculated according to NorFor (Volden, 2011a).

Daily milk yield (**MY**) was recorded in an automatic milking system (**AMS**, DeLaval International AB, Tumba, Sweden), where the yield was registered for each milking. At the end of each experimental period, milk samples were taken for each milking for a 48-hour period. Each milk sample was preserved using Bronopol tablets (2-bromo-2-nitropropane-1,3 diol, Broad Spectrum Microtabs II) and stored chilled (4°C) until analyzed for fat, protein, and lactose using Fourier Transform Infrared (FTIR) spectroscopy (Bentley FTS/FCM or Combi 150, Bentley Instruments Inc., Minnesota) at the TINE laboratory (TINE, Trondheim, Norway). The individual milk samples were linked to their respective yields and used to determine the weighted average milk composition over this 48-h period. Energy corrected milk was then calculated from this chemical composition according to Sjaunja et al. (1990). Body weight was measured after each milking with an electronic scale situated at the milking robot exit.

Methane (**CH_4**) daily emissions were recorded with two GEMs throughout the whole experiment. In the barn, the GEMs were placed between the concentrate feeders and visited by animals voluntarily; thus, recording was reliant on animal visits. For a recording to be accepted as valid, at least two minutes of proximity to the sensor of the head position were set as criteria. Hristov et al. (2015) extensively described the details of the GEM method. Average CH_4 daily values were calculated with recordings corresponding to the same day.

For all measurements, the last 10 days of each period were included as repeated measurements or used to calculate an average per cow to avoid potential carryover effects from the previous period.

Statistical Analyses

Silage Composition

As the objective of the study depended on achieving two different silage digestibility, a statistical comparison was performed between the HDS and LDS by one-way ANOVA using the "aov" function from the "stats" package (R Core Team, 2019). Four samples per silage (two samples per silage per response period) were included in the comparison, where silage was the main effect, and all nutritional components were evaluated as response variables. If significant, pairwise comparison by Tukey was performed using the "TukeyHSD" function, also from the stats package.

Response Periods 1 and 2

The experiment was a complete randomized block design, with parity as the block (primiparous vs. multiparous). All statistical analyses were performed in RStudio (RStudio Team, 2020). Randomization of the experimental unit for cow was done using the "block_ra" function from the "randomizr" package (Coppock, 2019). For RP1, the effect of silage digestibility and change in concentrate from PreP on feed intake, milk yield, and CH_4 were analyzed using linear mixed models with the "lme" function from the "nlme" package (Pinheiro et al., 2020) using RStudio software. The models evaluated milk yield (**MY)**, ECM, milk fat concentration, milk protein concentration, milk lactose concentration, fat yield, protein yield, lactose milk, silage DMI (**DMIs**), concentrate DMI (**DMIc**), DMI, weight, CH_4, CH_4/DMI, and CH_4/ECM ratio. Silage digestibility (HDS or LDS) and change in concentrate (kg DM/day) and the silage digestibility × change in concentrate interactions were included as fixed effects. A covariate was included in the model to account for variation between cows, and it was nested to parity. The covariate used for the different dependent variables corresponded to the same evaluated variable but with values from before the start of the period, which corresponded to PreP for RP1 evaluation. Therefore, change in concentrate from PreP to RP1 was selected as an independent variable as opposed to DMIc. Cow was used as random variable. An autoregressive correlation structure of CAR(1) was applied to account for correlation among repeated measurements (daily measures) within cow. For post hoc tests, the "ghlt" function from the "multicomp" package (Hothorn et al., 2008) was employed using Tukey's test for comparison. The results were considered statistically significant at $P \leq 0.05$, and P-values > 0.05 and ≤ 0.1 were considered to indicate trends.

For RP2, models for the same dependent variables as RP1 were also analyzed with linear mixed models using the same statistical software and function as for RP1. Random variables, correlation structure, post hoc test, and significance level were also the same as RP1. Models included concentrate level as the categorical main fixed effect (Standard, Plus2, and Minus2). As values for RP1 were included as covariates for this period, silage digestibility was not included as a main effect for RP2, as this was evaluated in RP1, and the effect of digestibility was thus included in the covariate. Silage digestibility was only included in the interaction term to evaluate the effect of the concentrate level on the different silage digestibilities. Substitution rate was calculated for RP2 only for the treatments where the concentrate offer was changed (Plus2 and Minus2). The SR was calculated for each individual cow as the change in DMIs per kg change in DMIc.

RESULTS

Silage Composition

Table 1 presents the chemical composition of the grass silages and concentrates. HDS showed higher DM content (*P* = 0.02), OMD (*P* = 0.0001), concentrations of CP ($P = 5.0 \times 10^{-8}$), sCP ($P = 9.0 \times 10^{-8}$), crude fat (*P* = 0.006), starch ($P = 8.3 \times 10^{-5}$), and WSC (*P* = 0.0004) than LDS. Concentration of NDF ($P = 1.2 \times 10^{-6}$), ADF ($P = 5.0 \times 10^{-6}$), and ADL ($P = 3.0 \times 10^{-6}$) were higher for LDS. Fermentation quality parameters such as lactic acid (*P* = 0.02), acetic acid (*P* = 0.02), and ammonium-N (*P* = 0.0005) concentrations were higher for LDS, while HDS showed higher pH (*P* = 0.02) and formic acid concentration (*P* = 0.01). Energy standard feed value, NEL20, and protein balance in the rumen (PBV20) were higher for HDS ($P < 0.001$), while no difference in AAT20 was found (P = 0.63). Fill value was higher for LDS ($P = 1.5 \times 10^{-5}$).

Response Period 1

Of the 60 cows included in the experiment, six were taken out during RP2 and excluded from the whole experiment. From these excluded cows, three were excluded due to stealing HDS and three due to low milk yield not related to treatments. This resulted in 27 cows for the HDS and 27 cows for the LDS, respectively. Table 2 shows the results for intake and yield for RP1.

Intake: Total DMI was, on average, 3.0 kg DM higher for cows in the HDS than those in the LDS ($P = 2.7 \times 10^{-12}$). Also, DMI increased 0.53 kg DM with each increase in kg DM of concentrate offer ($P = 2.145 \times 10^{-10}$), regardless of silage digestibility treatments, as no interaction was found between silage digestibility and change in concentrate offer (*P* = 0.76). Higher DMI for the HDS group could be explained by DMIs, which was, on average, 3.8 kg DM higher for the HDS group than for the LDS group ($P < 2.2 \times 10^{-16}$). No interaction was found for DMIs between silage digestibility and concentrate offer (P = 0.13). Regardless of silage digestibility, DMIs were reduced by 0.26 kg DM with an increase in each kg DM of concentrate offer (*P* = 0.0008). Concentrate intake was lower for HDS (*P* = 0.039) and increased for both silage digestibility treatments by 0.82

kg DM when concentrate offer was increased by 1 kg DM ($P < 2.2 \times 10^{-16}$). Residual concentrate, defined as the concentrate allowed but not fed out to the animals, showed no difference between silage digestibility (P = 0.51) or concentrate offer change (P = 0.19).

Milk Production and Composition: Milk yield was, on average, 3.0 kg higher for the HDS than the LDS treatment (P = 0.001) and increased linearly by 0.49 kg of milk per kg increased offer of concentrate (P = 0.009), regardless of silage digestibility, as no interaction was found (P = 0.90). Similar response was found for ECM, with, on average, a 3.5 kg higher yield for the HDS than the LDS treatment. Irrespective of silage digestibility, ECM showed a linear increase in 0.66 kg per kg DM of concentrate offer increase, as no interaction was found (P = 0.13). Silage digestibility did not affect fat and protein concentrations (P = 0.97 and P = 0.79), likewise change in concentrate offer (P = 0.60 and P = 0.63). For both silage digestibility treatments, the lactose concentration increased with an increased concentrate offer ($P < 0.01$). Fat, protein, and lactose yield were highest for the HDS treatment (P = 0.03 for fat yield and $P < 0.01$ for protein and lactose yield) and were affected by concentrate offer ($P < 0.05$), although no interaction was detected for any of these parameters ($P > 0.1$). Weight was not affected by silage digestibility (P = 0.31) or change in concentrate offer (P = 0.79). Silage digestibility treatments did not affect milk and ECM per kg DMI (P = 0.97 and P = 0.77), likewise change in concentrate offer (P = 0.92 and P = 0.47).

Methane Emissions: Table 3 shows the results for methane emissions for RP1. Out of the 54 cows (27 per group), 35 cows had a continuous voluntary daily visit to the GEMs. The other 19 cows did not visit the unit voluntarily, or their visit was sporadic; therefore, access to the GEMs was prohibited before the start of PreP. This represented 15 cows, with recordings from the HDS group and 19 from the LDS. Methane emissions (l/day) were not affected by silage digestibility (P = 0.70) or a change in concentrate offer (P = 0.84). Methane related to ECM (l CH_4/ kg ECM) was not affected by silage or concentrate offer (P = 0.21), and no interaction was found between silage digestibility and concentrate offer (P = 0.36). Methane per kg of DMI (CH_4/DMI) was, on average, 3.7 l/kg higher for LDS (P = 0.002) and significantly decreased by 0.60 l/kg with an increased offer of concentrate (P = 0.02), but no interaction between silage digestibility and concentrate offer was found (P = 0.89). Average values of CH_4/DMI and CH_4/ECM for individual cows for period RP1 (Figures 3 and 4) were regressed against DMI and showed a decrease with increased DMI (P < 0.001) with no effect of silage type (P > 0.1).

Response Period 2

All cows included in RP1 continued in the experiment for RP2, where they were subdivided into the three concentrate level subgroups. Due to the cows excluded from the experiment, the number of cows per subgroup was as follows: 10, HDS-Plus2; 10, HDS-Standard; 7, HDS-Minus2; 10, LDS-Plus2; 9, LDS-Standard; 8, LDS-Minus2. Uneven distribution of cows in the subgroups was because the removal of cows was done during this period and excluded from the whole experiment. Table 4 shows the results for RP2.

Intake: Table 4 shows both the least square means (**LSmeans**) and raw means for silage, concentrate, and total DMI. Due to the use of a covariate, estimated LSmeans of intakes appear numerically similar between concentrate levels for both silage digestibility, whereas raw means show actual intakes. Concentrate intake was significantly affected by concentrate offer ($P = 2.0 \times 10^{-16}$) with Plus2 showing the highest intake and Minus2 the lowest for both silages, and no interaction was found (P = 0.39). Residual concentrate showed higher values for Plus2 than the other concentrate levels (P = 0.03), regardless of silage digestibility (P = 0.23). Silage intake was affected by concentrate level ($P = 1.3 \times 10^{-10}$), depending on the silage digestibility (P = 0.0003).

For LDS, increasing the concentrate level did not affect DMIs, but DMIs decreased by 1.3 kg DM for HDS. For both silages, there was a significant difference for DMIs between the highest and lowest concentrate levels (Minus2 vs Plus2). For HDS-Plus2, DMIs were 2.6 kg lower than for Minus2. For LDS, Plus2 showed 1.4 kg lower DMIs than for Minus2. Total DMI showed a significant effect of concentrate level (P = 0.03) with an interaction with silage digestibility (P < 0.04). While no difference was detected between concentrate levels for HDS, LDS showed lower DMI when the concentrate offer was Minus2. NE_L intake showed similar results to DMI, with an interaction between silage digestibility and concentrate level (P = 0.04). NE_L showed no differences between concentrate offer treatments for HDS, while, for lower LDS, NE_L intake was shown for LDS-Minus2 treatment.

Substitution Rate: Substitution rate was calculated only for cows allocated in the treatments where the concentrate offer was changed (HDS-Plus2, LDS-Plus2, HDS-Minus2, and LDS-Minus2). Average SR was 0.59. HDS showed a higher average SR (0.78) than LDS (0.42). Figure 5 shows the SR for each individual cow according to changes in DMIc. The HDS treatment showed a higher increase in DMIs with the reduction of concentrate intake and a higher reduction when concentrate increased, showing a variable SR. For LDS, SR was constant, showing a similar reduction or increase in DMIs when DMIc increased or decreased, respectively.

Milk Production and Composition: Concentrate level affected MY ($P = 1.1 \times 10^{-8}$), with higher MY for increasing concentrate level (Plus2) and decreased when concentrate offer was reduced (Minus2) with no interaction between silage digestibility and concentrate offer level (P = 0.48). However, ECM showed an interaction with concentrate offer (P = 0.02); HDS showed no differences for Minus2 compared to Standard, but LDS showed a lower yield for Minus2 treatment. Milk fat concentration was not different within HDS, although higher numerical concentrations were found for the Minus2 treatment. For LDS, Minus2 showed a lower milk fat concentration than Plus2 (P = 0.004). Milk protein concentration was reduced for both silages for the Minus2 treatment (P = 0.009).

Figure 6 shows a comparison of the ECM response between the predicted values according to Álvarez et al. (2020) and observed results of the present study. Predicted results were calculated for the specific OMD of the silages in this study and the raw means of DMIc. For the HDS, the predicted response of ECM by the model to increasing DMIc from Minus2 to Standard was 0.89 kg ECM, while the observed was an increase in 0.4 kg of ECM. The predicted response to an increase in DMIc from Standard to Plus2 was 1.1 kg ECM, while the observed was 1.7 kg ECM. Predicted total response for increasing DMIc from HDS-Minus2 to HDS-Plus2 was 2.1 kg ECM, while the observed was 1.8 kg ECM. For the LDS, the predicted and observed responses of ECM to concentrate intake shift from Minus2 to Standard was 1.9 kg ECM. Predicted response from Standard to Plus2 was 0.5 kg ECM, while the observed was 1.4 kg ECM. Predicted total response from Minus2 to Plus2 for LDS was smaller than the observed (2.3 vs. 3.3 kg ECM, respectively).

Methane Emissions

Table 5 presents the results for CH_4 emissions. The total number of cows with voluntary continuous daily measurements of methane was the same as for RP1 (n = 35). The number of cows with methane recordings per sub-treatment were as follows: 5, HDS-Plus2; 5, HDS-Standard; 5, HDS-Minus2; 5, LDS-Plus2; 8, LDS-Standard; 7, LDS-Minus2. Methane emissions were affected by concentrate level (P = 0.02), which was higher for Plus2 than for Minus2, regardless of silage digestibility. However, CH_4/ECM and CH_4/DMI were not affected by the concentrate level (P > 0.1).

Figures 7 and 8 present the regression of CH_4/DMI and CH_4/ECM against DMI for the average period values of individual cows, respectively. The CH_4/DMI ratio showed a decrease in 0.84 l per kg DMI increase ($P = 2.3 \times 10^{-5}$), regardless of silage digestibility, as no effect of silage digestibility was detected (P = 0.18). The CH_4/ECM ratio also showed a decrease in 1.23 per kg of DMI increase ($P = 2.0 \times 10^{-7}$), with no effect of silage digestibility (P = 0.60).

DISCUSSION

Silage Characteristics

Our results agree with the well-known fact that plants mature as harvest date is postponed, increasing cell walls and lignin fractions (Buxton, 1996). In grass silage, this results in increased NDF and ADF proportion, whereas the proportion of CP and digestibility decreases. Some studies have shown similar results (e.g., Rinne et al., 1999; Kuoppala et al., 2008; Alstrup et al., 2016). However, silage quality varies greatly depending on year, botanical composition, silage making, etc. (Buxton, 1996). Moreover, as latitude and altitude also play a part in the harvest date effect, the magnitude of the effect of harvest date should be compared within location or at least region. Studies from the same location as ours agree with the observed trends in the effect of silage composition but differences in magnitude. For example, Dønnem et al. (2011) showed a higher daily decrease in OMD for later harvest (0.83 vs. 0.57 percentual points) but a lower decrease in CP (2.43 vs. 3.55 g/kg DM per day) and a higher increase in NDF content (7.19 vs. 6.45 g/kg DM). Randby et al. (2012) showed a similar decrease in CP content per day (3.31 g/kg DM) but a higher OMD decrease rate (0.71 percentual points per day) and NDF increase (7.75 g/kg DM per day). Harvest date effect on silage composition due to differences in maturity showed, in this study again, to be an important management practice to improve silage composition (Harrison et al., 1994; Buxton, 1996).

Effect of Silage Digestibility

The use of a covariate allowed for accounting for variation between cows, making it easier to detect the significance of the evaluated variables (Lee et al., 2019) and increase statistical power (Kahan et al., 2014; Thompson et al., 2015, Jacobs et al., 2013). Moreover, by including covariate as a baseline measurement (values from before the experiment) allowed us to compare how the study variables changed to the introduced treatments. In our study, the experimental design allowed the use of a covariate from the previous period as baseline measurement. For evaluating the effect of silage digestibility in RP1, values from the previous period (PreP) were included as covariate. This allowed the comparison of cows that were previously subjected to the common silage as baseline.

The cows' diets were individually optimized according to the yield and silage assigned. As anticipated, rations optimized by NorFor attributed less concentrate offer for the HDS than for the LDS treatment due to the difference in energy concentration between the two silages. A separate feeding strategy allowed us to evaluate the impact of concentrate and silage intake on milk production. Concentrate intake was higher for the LDS and was a direct effect of a higher concentrate offer, as the residual concentrate was not different between silage digestibility treatments. Dry matter intake was expected to be similar between treatments, as initial yield was alike, and diets were optimized by NorFor to maintain these yields. Higher DMI for HDS treatment was due to higher-than-predicted values of DMIs for HDS by NorFor (Figure 9). Jensen et al. (2015) showed that the NorFor intake model overpredicts DMI at high intake levels, which contrasts our results on which NorFor underpredicted at high intake levels. In addition, this underprediction was not shown for high intakes in PreP when an intermediate mixed silage (50% LDS and 50% HDS) was fed. Therefore, this systematic underprediction could relate to silage quality rather than the intake level and should be further studied. Higher DMI probably explains the higher MY and ECM yields for the HDS treatment, as feed efficiency (milk/DMI, ECM/DMI and NE_L/kg DM) did not differ between silage treatments. Higher yield with increased intake supports the fact that total intake is the most important factor determining animal production (Waldo, 1986; Allen, 2000). Regardless of intake underprediction, the results from RP1 confirmed our hypothesis that high milk yields can be produced with low concentrate offers if high-digestible silages are fed.

Concentrate Level and interaction with Silage Digestibility

The effect of the concentrate level was evaluated in RP2, separately from the silage digestibility evaluation in RP1. The goal in this period was to evaluate responses to changes in concentrate supply. This was achieved by optimizing the concentrate level for all cows according to their individual yield and the two silages in the previous period (RP1). Therefore, to evaluate the effect of concentrate level in RP2, the results from RP1 were used as covariate. The values from RP1 included the effect of silage digestibility as it was evaluated in that period, so inclusion of RP1 values as a covariate masked the effect of silage digestibility. Therefore, only a comparison between concentrate levels within each silage digestibility treatment was performed. Thereby, the effect of silage digestibility was included only in the interaction term to detect different trends of concentrate levels between the silage groups.

Concentrate levels similarly affected DMI and NE_L intakes, with no differences between concentrate levels in the HDS but lower DMI and NE_L for Minus2 in the LDS. Low-digestible silages could not compensate for the reduction of concentrate offer with higher silage intake, probably because maximum intake potential was already reached in the optimized concentrate level. Although HDS silage intake did not significantly differ, there was a numerical increase in 1.3 kg DMIs with a decrease in the 2 kg DM concentrate offer, while the increase in LDS was 0.6

kg DM. Average SR of 0.59 kg DM in this study exceeded the 0.53 found by Álvarez et al. (2020) and 0.47 reported by Huhtanen et al. (2008). Differences in the SR values between studies could be attributed to the type of silage, level, and type of concentrate alongside cow characteristics used in the different studies. Huhtanen et al. (2008) and Álvarez et al. (2020) showed no differences in SR between silage digestibility, while, in this study, higher SR was detected for HDS. The differences in SR between silages revealed in this study agree with Thomas (1987), Faverdin et al. (1991), and Jensen et al. (2016). The lower increase in intake with increased concentrate intake due to higher SR for high-digestible silages (Huhtanen et al., 2002; Kuoppala et al., 2008) could be a possible explanation of the lower marginal ECM responses.

The differences shown in ECM between concentrate levels within silages were similar with the differences shown for DMI, which is supported by the similar ECM/DMI shown between concentrate level treatments. When concentrate was reduced, HDS maintained DMI and ECM production, while LDS showed a reduction of DMI and ECM for LDS-Minus2 treatment. The lower MY of cows fed HDS with low concentrate intake was compensated for by the numerically higher milk fat concentration, probably due to higher silage intake, showing no differences in fat yield. Reducing concentrate for the LDS showed a lower milk fat concentration and was not expected. However, Sutton (1989) showed high variability in milk fat concentration due to several factors affecting it. Thus, other factors could have affected this result. Decrease in milk protein concentration with deceased concentrate shown for both silages agrees with Alstrup et al. (2016), Kuoppala et al. (2008), and Rinne et al. (1999), reflecting the negative relationship between NDF intake and milk protein concentration (Sutton, 1989). However, differences in milk protein yield were not enough to be reflected in different ECM between concentrate treatments, as ECM for HDS was similar between HDS-Standard and HDS-Minus2. This was not expected, as Álvarez et al. (2020) predicted responses of ECM for those concentrate levels. For the LDS, reduced ECM with reduced concentrate intake corresponded to the meta-analysis (Álvarez et al., 2020). This lower MY could not be compensated for by milk fat concentration; therefore, a lower ECM was recorded for this treatment.

The results confirm our hypothesis, showing that it is possible to maintain high yields with a high proportion of silage in the diet by feeding HDS (81% proportion for HDS-Minus2). However, this is not possible by feeding silages with lower digestibility. In our study, increasing the silage proportion from 55 to 62% of an LDS in the diet could not maintain the same yields. Although the results cannot be statistically compared between silages, numerically higher ECM was achieved with HDS at similar concentrate levels (Figure 6, LDS-Minus2 vs HDS-Plus2). From another perspective, numerically similar ECM yields to HDS can only be achieved by feeding LDS with high concentrate intakes. Moreover, the marginal responses of the ECM shown in this study agree with our hypothesis based on Álvarez et al. (2020) by showing lower marginal responses to concentrate increases for high-digestible silages. This emphasizes the benefit of feeding high-digestible silages with low concentrate levels, but the advantage of high-digestible silages dilutes at higher supplies of concentrate (Ferris et al. 2001).

Methane Production

Usually, CH_4 production increases with an increased total intake (Moe and Tyrrell, 1979; Warner et al., 2017; Nielsen et al., 2013; Niu et al., 2018). Thus, similar methane production for the two silage treatments was not expected since DMI was highest for HDS treatment. However, the higher NDF content and lower digestibility of late mature silages were shown to favor acetate production (Johnson and Johnson, 1995) and increase methane production (Moe and Tyrrell, 1979). This could have counterbalanced the lower DMI of LDS, resulting in similar total methane emissions. Similar methane production but higher intake for high-digestible silages was also shown by Brask et al. (2013). Brask et al. (2013) showed lower CH_4/DMI for high-digestible silages due to higher intake, agreeing with our study. The importance of intake was also reflected in the lack of differences found for CH_4/DMI and CH_4/ECM at the same total intake level (Figures 3 and 4).

Methane production increased with an increased concentrate offer, regardless of silage digestibility. This differs from the results shown by many, although studies are also contradictory. Some studies have shown decreased methane production (Aguerre et al., 2011; Ferris et al., 1999), while others have revealed no differences (Jiao et al., 2014; Patel et al., 2011). However, differences in total methane production were not translated to CH4/DMI and CH4/ECM. The lack of difference between concentrate levels for CH_4/DMI and CH_4/ECM was probably because the differences in concentrate offer were minor (plus or minus 2 kg DM) and masked by SR, resulting in similar DMI. The negative relationship between CH_4/DMI and DMI shown for both periods agrees with most studies that intake is one of the most important factors determining total methane production (Moe and Tyrrell, 1979; Warner et al., 2017; Nielsen et al., 2013; Niu et al., 2018). Moreover, a similar relationship between CH_4/ECM and DMI was also expected due to the high relationship between milk yield and intake (Johnson and Johnson, 1995; Niu et al., 2018).

CONCLUSION

Our study evaluated the relationship between silage digestibility and concentrate supply level on intake, milk, and methane production. The results showed similar responses to the meta-analysis of Álvarez et al. (2020), showing lower responses of milk to increased concentrate supply for high-digestible silages. Unlike low-digestible silages, high yields can be maintained with low concentrate levels with HDS. This demonstrates the benefits of feeding high-digestible silages with low concentrate levels, which disappear if concentrate levels are increased. Our study also showed that methane production was not affected by feeding high-digestible silages with low concentrate levels and that methane per unit of intake and milk depends more on total intake than on silage digestibility and concentrate level when silage is fed ad libitum.

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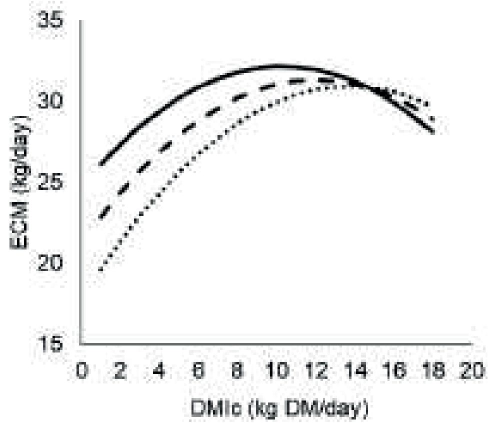
Figure 1: Álvarez et al.



ECM: Energy corrected milk (kg per day); DMIc: Dry matter intake of the concentrate (kg DM/day). Silage digestibility of the organic matter (OMDs): 82% OMDs (——), 75% OMDs (- - -), 68% OMDs (····).

Model: ECM = -16.1+ 4.2 DMIc - 0.07 $DMIc^2$ + 0.50 OMDs – 0.03 DMIc × OMDs.

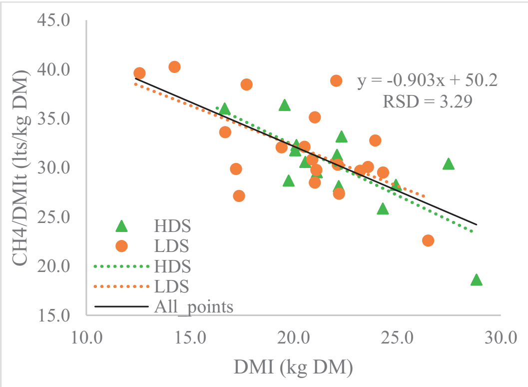
Figure 1: Responses of milk yield according to concentrate level for 3 different silage digestibility (Álvarez et al., 2020).

Figure 2: Álvarez et al.

PreP	RP1	RP2
20 days	17 days	20 days
60 cows Mix silage	High-digestible silage group (HDS) 30 cows	HDS-Plus2 group (10 cows) Increase 2 kg DM/day
		HDS-Standard group (10 cows) Same concentrate as RP1
		HDS-Minus2 group (10 cows) Decrease concentrate by 2 kg DM/day
	Low-digestible silage group (LDS) 30 cows	LDS-Plus2 group (10 cows) Increase concentrate by 2 kg DM/day
		LDS-Standard group (10 cows) Same concentrate as RP1
		LDS-Minus2 group (10 cows) Decrease concentrate by 2 kg DM/day

Figure 2: Schematic representation of experimental design and diet treatments.

Figure 3: Álvarez et al.

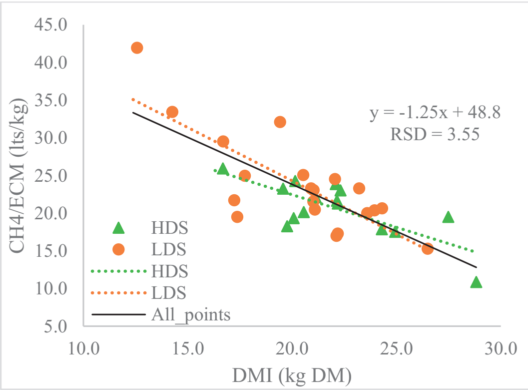


HDS: high-digestible silage, LDS: low-digestible silage, All points: regression including both silage digestibility groups.

CH_4/DMI: methane production per kg of total dry matter intake.

Figure 3: Effect of total dry matter intake on methane production per kg total dry matter intake for individual cows in response period 1.

Figure 4: Álvarez et al.

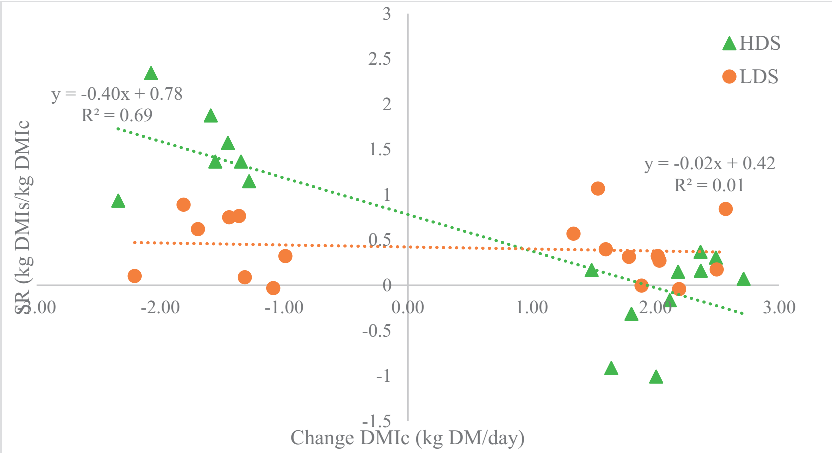


HDS: high-digestible silage, LDS: low-digestible silage, All points: regression including both silage digestibility groups.

CH_4/ECM: methane production per kg of ECM.

Figure 4: Effect of total dry matter intake on methane production per kg ECM for individual cows for response period 1.

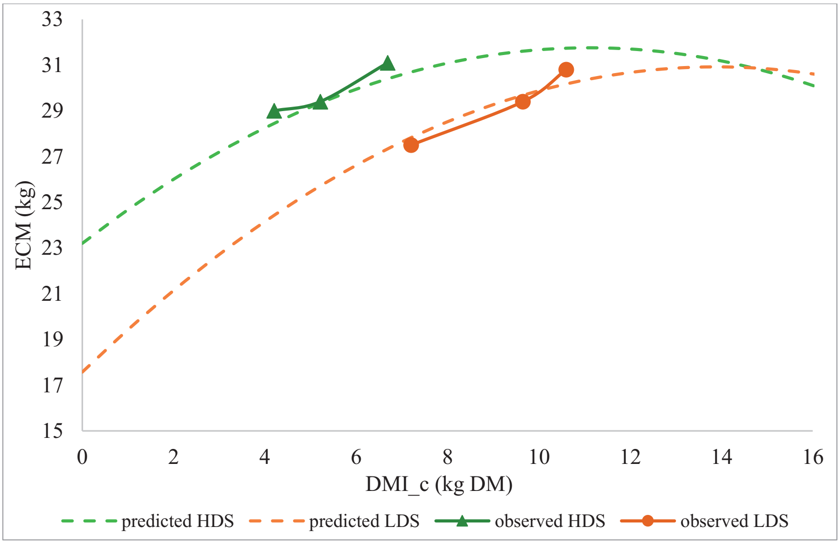
Figure 5: Álvarez et al.



SR: Substitution rate = (DMIs Response period 2 - DMIs Response period 1) / (DMIc response period 2-DMIc response period 1), HDS: high-digestible silage, LDS: low-digestible silage, Change DMIc: Change of dry matter intake concentrate = DMIc response period 2 – DMIc response period 1.

Figure 5: Substitution rate (change in silage intake with changes in concentrate intake) for individual cows for two silage digestibilities.

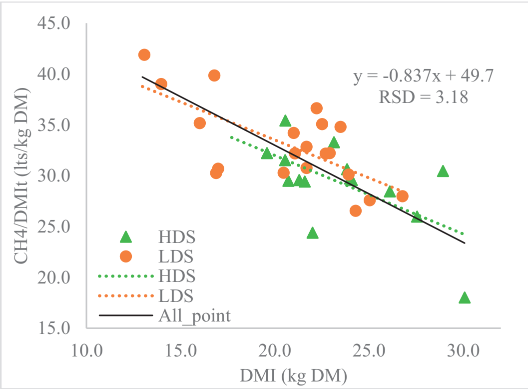
Figure 6: Álvarez et al.



Predicted HDS: predicted energy corrected milk according to Álvarez et al. (2020) for OMD = 78.9%. Predicted LDS: predicted energy corrected milk according to Álvarez et al. (2020) for OMD = 67.6%. Observed HDS: Observed energy corrected milk in this study for the high-digestible silage for 3 concentrate levels. Observed LDS: Observed energy corrected milk in this study for the low-digestible silage for 3 concentrate levels.

Figure 6: Mean energy corrected milk (kg/day) group values predicted by Álvarez et al. (2020) and observed in this study according to concentrate intake and organic matter digestibility of the silage.

Figure 7: Álvarez et al.

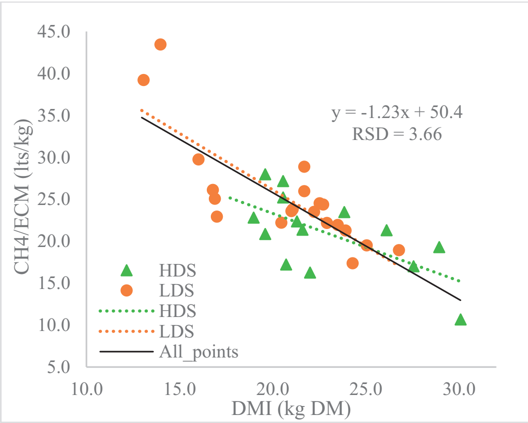


HDS: high-digestible silage, LDS: low-digestible silage, All points: regression including both silage digestibility groups.

CH_4/DMI: methane production per kg of total dry matter intake.

Figure 7: Effect of total dry matter intake on methane production per kg of total dry matter intake for individual cows for response period 2.

Figure 8: Álvarez et al.

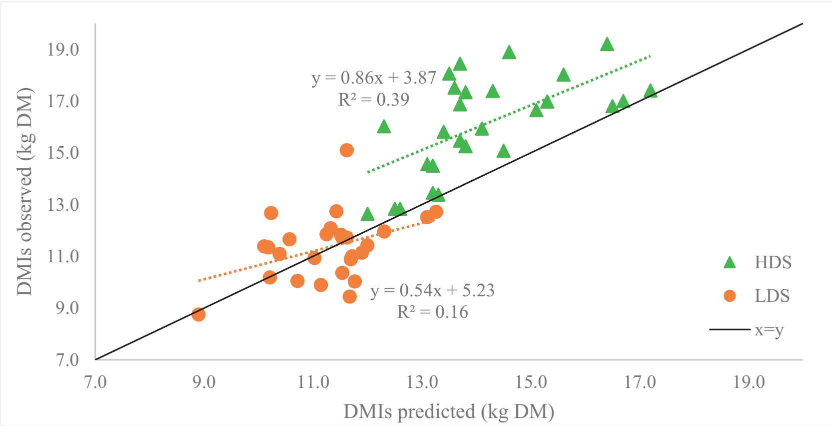


HDS: high-digestible silage, LDS: low-digestible silage, All points: regression including both silage digestibility groups.

CH_4/ECM: methane production per kg of ECM.

Figure 8: Effect of total dry matter intake on methane production per kg ECM for individual cows in response period 2.

Figure 9. Álvarez et al.



HDS: high-digestible silage, LDS: low-digestible silage.

DMIs observed: dry matter intake of silage recorded in the study. DMIs predicted: dry matter intake of silage predicted by NorFor from optimization for RP1.

Figure 9: Individual cows observed silage dry matter intake against predicted silage dry matter intake by NorFor optimization for individual cows in response period 1.

Table 1: Mean (Standard deviation) nutritive values of experimental silages and concentrate supplement used in the study.

	Silages[1]				Concentrate[2]
Item[3]	HDS	LDS	SEM	P value[7]	
DM (g/kg)	254	237	0.404	*	861
OMD (%)	78.8	67.6	2.22	***	
		g/kg DM			
Ash	83.8	80.4	1.04	NS	73.6
CP	215	144	13.4	***	198
sCP	163	99.7	12.1	***	41.0
CFat	33.0	26.8	1.34	**	33.6
NDF	468	597	24.6	***	179.8
ADF	274	349	14.4	***	74.7
ADL	18.4	30.2	2.25	***	8.40
Starch	22.5	12.7	0.192	***	358
WSC	42.2	23.0	0.383	***	63.1
pH	4.50	4.25	0.059	*	
Lactic acid	38.8	50.2	2.81	*	
Acetic acid	7.00	7.75	0.183	*	
Propionic acid	1.38	1.53	0.093	NS	
Butyric acid	0.40	0.40	0	NS	
Formic acid	9.00	7.00	0.463	*	
Ethanol	5.78	5.40	0.178	NS	
Ammonium-N (g/kg N)	91.8	119	5.44	***	
NEL20 (MJ/kg DM)	6.25	5.41	0.169	***	7.49
AAT20 (g/kg DM)	68.2	68.8	0.463	NS	129
PBV20 (g/kg DM)	79.0	28.0	9.68	***	17
FV (FV/kg DM)	0.472	0.572	0.019	***	0.22

[1]Each silage included 4 pooled samples (2 per response period) out of every other day sampling.

HDS: High-digestible silage. LDS: Low-digestible silage. SEM: Standard error of the mean.

[2]Concentrate sample consisted in 3 pooled samples out of weekly sampling.

[3]OMD: organic matter digestibility, CP: crude protein, sCP: soluble crude protein, CFat: crude fat, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, WSC: water soluble carbohydrates, NEL20: Standard feed value for net energy of lactation at 20 kg DMI. For compound feed NEL20 vas calculated according to Álvarez et al. (2021), AAT20: Standard feed value for amino acids absorbed in the small intestine at 20 kg DMI, PBV20: Standard feed value for protein balance in the rumen at 20 kg DMI, FV: Fill value of the feedstuff

[7] Significance levels: ***: $P \leq 0.001$, **: $P \leq 0.01$, *: 0.05, .: $P \leq 0.1$, NS: $P > 0.1$

Table 2: Estimated least square means resulted of the effect of silage digestibility treatment and concentrate offer on milk yield, milk components and intake for silage digestibility and concentrate offer in response period 1.

	Silage digestibility[1]			P value[2]		
Item[3]	HDS	LDS	SEM	S	Cc	SxCc
Weight (kg)	601	607	2.75	NS	NS	NS
Milk (kg/day)	29.7	26.7	0.338	**	**	NS
ECM (kg/day)	30.9	27.4	0.330	*	*	NS
Fat (%)	4.32	4.30	0.058	NS	NS	NS
Protein (%)	3.58	3.55	0.036	NS	NS	NS
Lactose (%)	4.78	4.70	0.019	NS	**	.
Fat yield (kg/day)	1.28	1.15	0.013	*	*	NS
Protein yield (kg/day)	1. 10	0.947	0.011	**	*	NS
Lactose yield (kg/day)	1.41	1.25	0.016	**	*	NS
DMI (kg DM/day)	22.6	19.5	0.154	***	***	NS
DMIs (kg DM/day)	15.9	12.1	0.127	***	***	NS
DMIc (kg DM/day)	6.67	7.35	0.154	*	***	NS
NE_L total	142	130	3.19	*	*	NS
Milk/DMI (kg/kg DM)	1.36	1.36	0.010	NS	NS	NS
ECM/DMI (kg/g DM)	1.44	1.44	0.009	NS	NS	NS
NE_L /kg DM	6.68	6.90	0.06	NS	NS	NS
Residual concentrate	0.86	0.77	0.034	NS	NS	NS

[1]HDS: High-digestible silage, LDS: Low-digestible silage,

[2]S: silage digestibility effect, Cc: concentrate change effect, SxC: interaction between silage digestibility and concentrate change effect. Significance levels: ***: P ≤ 0.001, **: P ≤ 0.01, *: 0.05, .: P ≤ 0.1, NS: P > 0.1

[3]ECM: energy corrected milk, DMIc: dry matter intake of the concentrate, DMIs: dry matter intake of the silage, DMI: total dry matter intake. Residual concentrate: Offered but not fed out concentrate (DM offer minus DMIc).

Table 3: Estimated least square means resulted of the effect of silage digestibility treatment and concentrate offer on methane emissions in response period 1.

Item[3]	Silage digestibility[1]		SEM	P value[2]		
	HDS	LDS		S	Cc	SxCc
n	15	19				
CH_4 (l/day)	641	646	9.40	NS	NS	NS
CH_4/ECM (l/kg)	21.4	23.4	0.615	NS	NS	NS
CH_4/DMI (l/ kg DM)	28.4	32.1	0.439	**	*	NS

[1]HDS: High-digestible silage, LDS: Low-digestible silage,

[2]S: silage digestibility effect, Cc: concentrate change effect, SxCc: interaction between silage digestibility and concentrate change effect. Significance levels: ***: $P \leq 0.001$, **: $P \leq 0.01$, *: 0.05, .: $P \leq 0.1$, NS: $P > 0.1$

[3] n: number of cows with methane measurements. CH_4: total daily methane production, CH_4/ECM: methane production per kg ECM, CH_4/DMI: methane production per kg of total dry matter intake.

Table 4: Estimated least square means for response period 2 according to silage digestibility and concentrate level.

	HDS[1]			LDS				P value[2]	
Item[3]	Plus2	Standard	Minus2	Plus2	Standard	Minus2	SEM	C	SxC
Weight (kg)	617	614	611	602	612	602	2.68	NS	NS
Milk (kg/day)	28.7^{A}	27.2^{B}	26.4^{C}	28.8^{A}	27.8^{B}	25.9^{C}	0.341	***	NS
ECM (kg/day)	31.1^{a}	29.4^{ab}	29.0^{b}	30.8^{a}	29.4^{a}	27.5^{b}	0.343	***	*
Fat (%)	4.39^{a}	4.43^{a}	4.70^{a}	4.41^{a}	4.28^{ab}	4.19^{b}	0.057	NS	**
Protein (%)	3.69^{A}	3.68^{A}	3.66^{B}	3.66^{A}	3.69^{A}	3.54^{B}	0.029	**	.
Lactose (%)	4.75	4.68	4.69	4.74	4.80	4.69	0.016	NS	NS
Fat yield (kg/day)	1.27	1.20	1.22	1.25	1.16	1.12	0.014	NS	NS
Protein yield (kg/day)	1.05^{A}	1.00^{A}	0.946^{B}	1.04^{A}	1.03^{A}	0.938^{B}	0.011	***	NS
Lactose yield (kg/day)	1.36^{a}	1.27^{b}	1.23^{b}	1.37^{a}	1.35^{a}	1.21^{b}	0.017	***	*
DMI (kg DM/day)	22.0^{a}	22.1^{a}	21.7^{a}	21.8^{a}	21.2^{a}	19.7^{b}	0.173	*	*
DMIs (kg DM/day)	13.9^{b}	15.2^{a}	16.5^{a}	12.7^{b}	13.5^{ab}	14.1^{a}	0.155	***	***
DMIc (kg DM/day)	9.12^{A}	7.18^{B}	5.39^{C}	8.94^{A}	7.39^{B}	5.51^{C}	0.163	***	NS
NE_L intake (MJ/day)	143^{a}	143^{a}	144^{a}	142^{a}	137^{a}	128^{b}	3.32	***	*
DMI (kg DM/day)[4]	23.5	23.2	22.0	21.2	21.4	19.1			
DMIs (kg DM/day)[4]	16.8	18.0	17.8	10.6	11.7	11.9			
DMIc (kg DM/day)[4]	6.69	5.21	4.20	10.6	9.65	7.20			
F:C[4]	71:29	77:23	81:19	50:50	55:45	62:38			
Milk / DMI	1.46	1.20	1.29	1.29	1.28	1.29	0.016	NS	NS
ECM / DMI	1.59	1.29	1.33	1.37	1.35	1.36	0.017	NS	NS
NE_L /kg DM	6.07	6.13	6.54	6.76	6.41	6.73	0.073	NS	NS
Residual concentrate	0.602^{A}	0.438^{B}	0.570^{B}	0.709^{A}	0.432^{B}	0.318^{B}	0.029	*	NS

[1]HDS: High-digestible silage, LDS: Low-digestible silage, Plus2: increased 2 kg DM of concentrate offer, Standard: no change in concentrate offer, Minus2: decrease 2kg DM concentrate offer. [2]C: concentrate level effect, SxC: interaction between silage digestibility and concentrate level effect. Significance levels: ***: $P \leq 0.001$, **: $P \leq 0.01$, *: 0.05, .: $P \leq 0.1$, NS: $P > 0.1$. Significant differences between treatments were uppercase letters if interaction between silage digestibility and concentrate level was not significant, while lowercase letters if interaction was significant.[3]ECM: energy corrected milk, DMIc: dry matter intake of the concentrate, DMIs: dry matter intake of the silage, DMI: total dry matter intake. Residual concentrate: Offered but not consumed concentrate (DM offer minus DMIc). [4] Raw means for DMIc, DMIs, DMI. F:C : forage concentrate ratio based on raw mean intakes.

Table 5: Estimated least square means for methane emissions for silage digestibility and concentrate offer in response period 2.

	HDS[1]			LDS				P value[2]	
Item[3]	Plus2	Standard	Minus2	Plus2	Standard	Minus2	SEM	C	SxC
n	5	5	5	5	8	7			
CH_4 (l/day)	714^{A}	650^{AB}	647^{B}	702^{A}	678^{AB}	655^{B}	6.49	*	NS
CH_4/ECM (l/kg)	22.8	22.8	23.0	23.7	23.3	25.0	0.360	NS	NS
CH_4/DMI (l/kg DM)	31.2	28.7	29.9	32.0	31.8	33.0	0.305	NS	NS

[1]HDS: High-digestible silage, LDS: Low-digestible silage, Plus2: increased 2 kg DM of concentrate offer, Standard: no change in concentrate offer, Minus2: decrease 2kg DM concentrate offer.

[2]C: concentrate level effect, SxC: interaction between silage digestibility and concentrate level effect. Significance levels: ***: $P \leq 0.001$, **: $P \leq 0.01$, *: 0.05, .: $P \leq 0.1$, NS: $P > 0.1$

[3] n: number of cows with methane measurements. CH_4: total daily methane production, CH_4/ECM: methane production per kg ECM, CH_4/DMI: methane production per kg of total dry matter intake.

Machine learning is a useful tool to determine milk yield responses to diet changes from real time farm data

Paper V

INTERPRETATIVE SUMMARY

Machine learning is a useful tool to determine milk yield responses to diet changes from real-time farm data by Álvarez et al.

Big data technologies are viable tools for analyzing a large amount of data from milking robots. Feed represents the largest cost in milk production. Several studies have developed response functions that relate milk responses to feed intake to improve precision and understanding between feeding and milk production. However, none have been implemented for farm use using real-time data. Therefore, we developed an algorithm for farm scale using real-time data, which enables the classification of silage digestibility used for each farm and the evaluation of changes in concentrate for milk production.

MACHINE LEARNING IS A USEFUL TOOL TO DETERMINE MILK YIELD RESPONSES TO DIET CHANGES FROM REAL-TIME FARM DATA

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Machine learning is a useful tool to determine milk yield responses to diet changes from real-time farm data

ABSTRACT

Data obtained from milking robots offers a great potential to improve the analysis of feed's impact on milk production from farms. The objectives of this study are to 1) use milk and concentrate data from the milking robot to predict silage digestibility as an input to farm-specific response functions, 2) adapt a theoretical response function, developed from a meta-analysis, for farm scale using real-farm data, including an economical decision tool, and 3) evaluate the adaptation success by indicating areas for potential use and improvement. Data for predicting silage digestibility included the studies found in the meta-analysis we want to adapt, a new research study testing this meta-analysis, and data from two farms where the meta-analysis was also tested. The developed algorithm was applied to data from the milking robot from Norwegian farms and information about each cow and milk characteristics. Typical Norwegian feed and milk prices were used to simulate the opportunities of this algorithm as an economic decision tool. The training dataset available allowed for the binary classification regression of silage digestibility as high and low digestible silage by logistic regression. The developed algorithm showed that silages classified as high digestible showed a higher reduction of concentrate efficiency (kg milk per kg concentrate) with increasing concentrate intake than silages classified as low digestible. This is due to the lower marginal milk increases in the high digestible silages with increasing concentrate intake. The economic tool developed from the algorithm could be a useful tool to have an additional decision layer, rather than only productivity, for changes in diet. If more data with known digestibility are to be included in the training dataset, more digestibility classes could be differentiated, or even a continuous variable could be predicted, such as organic matter digestibility. Information about

silage intake would be valuable for completing the economic calculations because, until now, this information cannot be gathered from milking robots.

Keywords: big data, milking robot, dairy cow, silage digestibility, concentrate intake

INTRODUCTION

Feed represents the largest variable cost in milk production, in which concentrates comprise about 70%. Therefore, developing solutions that ensure the optimal use of concentrates is imperative. This means adapting the right concentrate level to the forage quality to achieve high feed efficiency is also essential for reducing their impacts to the environment. Several studies have developed response functions that relate milk production to changes in feed intake and nutrient supply (Huhtanen and Nousiainen, 2012; Jensen et al., 2015; Daniel et al., 2016, Álvarez et al., 2020). These response functions help in evaluating different scenarios for changes in diet, making it a flexible decision tool for different goals. In a meta-analysis based on research studies, Álvarez et al. (2020) developed response functions describing responses of milk production to changes in concentrate intake for different silage digestibility. The study showed that the absolute level and marginal response in milk to changes in concentrate depended on the silage digestibility of organic matter (**OMD**). Milk, expressed as energy-corrected milk (**ECM**), showed a curvilinear response to increased concentrate intake, with higher absolute ECM yields for high digestible silages at low concentrate intake levels; these differences reduce as concentrate intake increases due to lower marginal ECM gains for the high digestible silages. However, all these efforts have been in the theoretical field, lacking implementation in real-time data from farms.

Over the last few years, big data technologies have risen in popularity as a viable tool to handle large and increasing amounts of data. The introduction of machine learning (**ML**) to agriculture is necessary due to the dramatic increase in available data from the increased use of robots, sensors, etc. For big data, unlike traditional statistical methods, large volumes of data can be more efficiently processed using ML (Storm et al., 2019). As example, in Norway, more than 55% of the delivered milk comes from farms with automatic milking systems (**AMS**; TINE, 2020), of which information on every cow and every milking is collected into a common dataset. Liakos et al. (2018) described several attempts to use ML in livestock production. Within cattle production, estimating rumen fermentation patterns based on milk fatty acids, estimating weight trajectories with few weight recordings, and predicting carcass weight 150 days before slaughter are some examples that have shown high accuracy. An application of such response functions to real-farm situations in real time will be an excellent decision tool for farmers and advisors to decide a feeding strategy according to the available feed at the farm. Moreover, if feed and milk prices are included, financial decisions will increase the usability of this tool. Thus, we hypothesized that using big data in real time would improve feed optimization at the specific farm level.

The objectives of this study are to 1) use milk and concentrate data from the milking robot to predict silage digestibility as an input to farm-specific response functions, 2) adapt the theoretical response functions of Álvarez et al. (2020) for farm scale using real-farm data, including an economical decision tool, and 3) evaluate the adaptation success by indicating areas for potential use and improvement.

MATERIALS AND METHODS

Figure 1 shows a schematic overview of our approach.. A set of data (training data) was used to develop an ML model to predict silage digestibility based on regression parameters between ECM per kg of concentrate (**concentrate efficiency**) and concentrate intake. Concentrate was fed as a concentrate mixture, and the whole concentrate ration was fed separately from the silage. We applied the developed model to specific farms during a specific period (i.e., live farm data) and classified the average silage digestibility used during that period. From this, a response function for that specific farm was obtained, describing concentrate efficiency to changes of concentrate intake for predicted silage digestibility. Finally, milk and concentrate prices were included to stipulate earnings at different concentrate levels.

Datasets

Training dataset: For the training process, datasets with known silage OMD, concentrate level, and ECM yield were selected from three data sources. First, the data presented in Álvarez et al. (2020) meta-analysis was included as the objective to adapt this model to practice. Second, data from Álvarez et al. (unpubl.) was included. This experiment had two periods, in which two silages with different OMDs and individual concentrate levels were fed to each animal. In the first period, cows were divided into two groups and fed two silages with different OMDs. Concentrate level was optimized according to yield and silage OMD according to NorFor (Volden, 2011). In the second period, the concentrate allowance was either reduced, increased, or unchanged. Only the second stage of the experiment (after changing the concentrate levels from optimized levels) was included in the training data. Finally, data from two Norwegian commercial dairy farms for which the meta-analysis was tested were also included in the training dataset.

Each datapoint in the training dataset contained aggregated information from individual animals. Animals belonging to the same experiment were labeled according to high or low silage OMD and grouped to form the basis for one datapoint. A datapoint was only created if the group contained three or more observations. Each group was then described by the minimum and maximum concentrate intakes of the animals, together with the regression parameters and uncertainties for both linear and inverse linear trends of concentrate intake vs. concentrate efficiency. We labeled data with silage OMD above 76% as high digestibility, and below as low digestibility.

Live farm dataset: The model was subsequently applied to live farm data. Individual cow data on daily milk yield and concentrate intake from known farms were available from Nordic Cattle Data eXchange (**NCDX,** Kyntäjä et al., 2018). These data were matched to data from the Norwegian dairy herd recording system (**NHR,** TINE SA, Ås, Norway) to calculate the ECM, as milk composition for each cow was included in this database. The NHR is the Norwegian milk producers' information system and the data basis for the management tools in milk established in 1898. The system is available to all the country's milk producers and collects data on pedigree, milk production, feeding, reproduction, and health in cows. This provides a basis for herd management systems. NCDX is a cloud-based common exchange data system between Iceland, Norway, Sweden, Finland, and Denmark. It was created to standardize data from milk recordings of the on-farm equipment with national dairy information systems and other databases, launching the first version in 2016. From these datasets, information from April 2020 was selected, and only cows with days in milk (**DIM**) over 100 were included in the matching dataset.

Statistical analysis

We analyzed and visualized the relationship between concentrate efficiency and concentrate intake for each individual cow's average values from the study period (Figure 2). This is a domain that displays the changes in ECM yield vs. concentrate intake clearly. Constant ECM levels then trend as inverse concentrate intake, presented as isolines in Figure 2. This behavior is central to our classification, as will be demonstrated.

Classification algorithm: To adapt the Álvarez et al. (2020) model, silage OMD classification was necessary because this variable is unavailable in the Live farm dataset. To solve the high vs. low silage digestibility classification problem, we applied the Catboost library (Prokhorenkova et al., 2018) to the training dataset, and the analysis was implemented in Python 3.8 (Van Rossum and Drake, 2009). Catboost is based on gradient boosting (Hastie et al., 2009) and is built to handle categorical data and requires little hyper-parameter tuning. It is an iterative method that tries to match a set of features (variables) to a set of data (measurements) according to a specific loss function measuring the discrepancy. We used the so-called 'log loss' classification metric, which amounts to performing logistic regression. Logistic regression is particularly applicable when the output variable is binary, e.g., high vs. low silage digestibility, but it can be generalized to more classes. We can, without loss of generality, assign a value of 1 to high digestible silage (**HDS**) and 0 to low digestible silage (**LDS**). Following the reasoning of James et al. (2021), linear regression is unsuitable because it can easily yield values outside the (0,1) range, i.e., outside the label range. A natural choice is to use the following logistic function:

$$p(x) = \frac{e^{f(x)}}{1 + e^{f(x)}},$$

where x is a vector containing all the selected features (variables), and $f(x)$ a linear polynomial in the features selected for regression. By this, we have accomplished a mapping of all feature values to a value in the (0,1) range. An example of how the logistic function looks and why it is suitable for classification problems is shown in Figure 3. It varies from label 0 to label 1 through a smooth transition interval, with the steepness of the transition determining how well defined the separation is.

It is further straightforward to verify that:

$$\frac{p(x)}{1-p(x)} = e^{f(x)},$$

and thus

$$ln\frac{p(x)}{1-p(x)} = f(x).$$

If we interpret $p(x)$ as a probability, this equation states that the logarithm of the odds is linear under these assumptions. Compared to the original problem, it overcomes the range problem and is thus more suitable for linear regression.

This regression problem, i.e., determining the coefficients of $f(x)$, is solved by minimizing what is known as the log-loss loss function:

$$\mathcal{J}(x) = \frac{1}{N}\sum_{i=1}^{N} y_i p_i + (1-\mathrm{y_i})(1-\mathrm{p_i}),$$

where the index i runs over all N data points, y_i is the label of the datapoint, and p_i is the value of the logistic function. We observe that, for the two-class classification, only one of the terms in the sum contributes to each datapoint. The value $p_i(x)$ is interpreted as the probability of the features describing label equal to 1, and the complement $1 - p_i(x)$ becomes the probability of belonging to label 0. The minimization of the loss function ensures that we find a logistic function that maximizes the likelihood of matching the training labels. It penalizes assigning the wrong label, especially assigning the wrong label very confidently. These probabilities are very useful when assessing the confidence of predicted classifications, in our case, high vs. low silage OMD.

In each iteration, the algorithm creates a fixed depth random decision tree, and the information from each decision tree is used to optimize the loss function according to a fixed learning rate. In our case, we allowed a maximum of 10000 iterations, a learning rate of 0.02, and a decision tree depth of 6.

We selected as features the parameters describing the linear and inverse linear regression lines through concentrate against concentrate efficiency. The linear regression is described by the gradient, intercept and, in addition, their variances and their co-variance. The inverse linear regression is described correspondingly. In addition, the minimum and maximum concentrate levels for the data from each farm were used, as they indicate a range of validity of the regression lines.

We analyzed the importance of the different features in determining the label in the algorithm using SHAP methodology, as described by Lundberg and Lee (2017) and Lundberg et al. (2020). The SHAP methodology visualizes which features influence the ML model (the Shapley value) the most, and what it does to the overall result. For each datapoint, it considers each feature and describes whether a high or low value of that feature has a high or low impact (Shapley value) on the result. A high Shapley value means that the feature has a high degree of influence on the ML model.

*Economic evaluation tool: T*wo economic scenarios were created, comprising two concentrate mixture prices (0.47 USD and 0.71 USD per kg), and milk with a constant price (0.59 USD per kg ECM), which represents Norwegian prices for feed and milk. Substitution rate (**SR**) between silage and concentrate was considered with a value of 0.53 kg DM silage / kg DM concentrate, which was reported by Álvarez et al. (2020), and silage was allocated a price of 0.24 USD / kg DM. To include the substitution rate in the economic calculation, concentrate and silage were considered on a DM basis; therefore, prices for concentrates were 0.55 and 0.82 USD / kg DM by assuming 86% DM for all concentrates. With these values, earnings were calculated for individual farms based on the algorithm.

RESULTS AND DISCUSSION

Classification algorithm

The training dataset resulted in a total of 19 different datapoints on which we trained the ML algorithm (Table 1). From the live farm dataset, April 2020 was selected based on the amount and quality of data available at the time of the study. The NCDX is a new initiative, and the amount and quality of the data will increase with time, which, in turn, will help to improve our work. The DIM above 100 was selected because there was a different response to concentrate before this stage, while, after 100 DIM, the response was similar throughout the lactation. Selecting this period and stage of lactation leads to data from 1290 different animals belonging to 157 farms. Figure 2 shows the 1290 cows from 157 farms used in the live-farm data, according to concentrate efficiency, concentrate intake, and total ECM production, where the isolines show similar ECM production (**ECM-isolines**). This figure shows that, at a similar ECM, the concentrate efficiency is reduced with increasing concentrate intake and that similar milk yield can be achieved with a large range in concentrate intake. This is also demonstrated in the meta-study of Álvarez et al. (2020), where silage OMD was identified as the main indicator of different ECM yields at the same level of concentrate, which in Figure 2 is identified as the ECM-isolines.

Figure 4 shows the results of the model development, indicating the SHAP values. We observed that all the selected features affected the model behavior. The linear regression gradient is imperative for determining the silage digestibility. Gradient differences between silage digestibility can be seen in Figure 5, where data from Álvarez et al. (unpubl.) from the training dataset was used as visualization example. There is a clear difference in the gradient between the two silage digestibility labels, with a steeper reduction in concentrate efficiency with increasing concentrate intake for HDS than for LDS. The behavior of these gradients agrees with Álvarez et al. (2020). We can see that the HDS regression line has similar behavior to the ECM-isolines, showing similar ECM production with increasing concentrate. This translates into a pronounced reduction efficiency with increasing concentrate intake. The authors also showed a lower marginal milk response for HDS, which would translate into similar results. However, the LDS regression 'cuts' the ECM-isolines, which means that ECM keeps increasing with increasing concentrate intake, resulting in a lower reduction of concentrate efficiency. This matches the results shown by the meta-analysis, with higher marginal increases of ECM for this type of silage. Lower marginal yields for high digestible silages with increasing concentrate were reported by other authors. Lower increases in intake for high digestible silages due to higher SR (Kuoppala et al., 2008), high nutrient partitioning to body reserves with increasing concentrate (Randby et al., 2012), or a higher impact of increased concentrate on the fiber digestibility for these silages (Rinne et al., 1999) were the proposed reasons.

Moreover, we can see that, for the same concentrate level at low and middle levels, HDSs show higher concentrate efficiency due to higher ECM, but differences in milk production are reduced at high concentrate levels, which also agrees with the results of Álvarez et al. (2020). Therefore, high milk responses can be achieved with low concentrate levels by feeding high digestible silages, which translates into better concentrate efficiency or by increased concentrate by feeding silages with lower digestibility.

We further note that the model is sensitive to the range of concentrate intake considered (Figure 4), i.e., the range of validity and the uncertainty in the regression parameters. Specifically, the upper limit of the concentrate range is important, which describes how the regression lines flatten out. The fact that the linear parameters are located at the top of the SHAP value list, and the inverse linear parameters are at the bottom, is because these parameters are tightly related. It reflects that key information is easiest accessible in the linear regression, especially the gradient, but that there is some additional information to be gathered from the inverse linear parameters. Notably, having a larger training dataset will influence these results.

In the training dataset, only the second period of the experiment presented in Álvarez et al. (unpubl) was included, i.e., after changing concentrate levels from their optimized levels. In Figure 6, we utilize the individual animal data from the first period of this experiment (as previously explained, the first period referred to all animals with optimized concentrate levels according to yield and silage OMD) to study the individual animal response to the changes in concentrate. Therefore, Figure 6 shows pre- and post-concentrate efficiency. It shows that, except for low concentrate levels (less than 4 kg/day), a change in concentrate intake results in a change in concentrate efficiency along the fitted linear regression lines. This is true for both high and low silage OMDs. This demonstrates that individual animal responses are like group responses for this algorithm. For this, we could also utilize the algorithm to evaluate individual animal responses and make further decisions to reduce or increase the concentrate level for individual cows.

In Figure 7, we present how to use the developed ML model for four commercial farms. The algorithm allows for the identification of a farm's response to changes in the diet for predicted silage digestibility (HDS and LDS). It is a useful tool to identify feeding status and marginal milk responses to concentrate on a specific farm, allowing to make feeding decisions from it. In Figures 7a and 7b, we can see a clear identification (probability of 0.99 for LDS) of an LDS used in these farms, with positive responses to increasing concentrate intake (Figure 7a) or a slight decrease in concentrate efficiency (Figure 7b). We can deduce that silage from the farm in Figure 7a has a lower digestibility than that of Figure 7b. The result of Figure 7c is referred to as 'borderline', as the algorithm labeled it as HDS but cannot be confidently distinguished from the LDS case; this can be seen by the probability of 0.66 for being HDS and 0.33 for LDS. Figure 7d indicates a clear HDS classification (probability of 0.91 for HDS), decreasing concentrate efficiency rapidly with increasing concentrate intake. Although Figures 7c and 7d look similar, the ML algorithm finds that they indeed differ. The borderline in Figure 7c is likely a silage with a relatively high OMD close to the threshold we set at 76%. Having more training data, we could have introduced more digestibility classes, such as medium digestibility, and answered this. However, regardless of silage digestibility classification, the regression lines show the pattern of how concentrate efficiency behaves at different concentrate levels.

From a production outlook, this would allow us to maximize concentrate efficiency by adjusting the concentrate level. Moreover, with this algorithm implemented in the milking robot management tool, the farmer, in real time, can predict the response pattern according to the silage programmed for feeding and predict which level of concentrate will improve concentrate efficiency. It could also allow for a comparison between farms with similar feeding strategies, of which ideas and decisions from other farms could be considered.

Economic evaluation tool

If we include concentrate and milk prices in the algorithm, economic scenarios can be an additional decision tool, as it is well known that production and economic optima can differ. In Figure 8, we present economic scenarios corresponding to Figure 7 for the two concentrate price scenarios. In Figures 8a and 8b, we see that with LDS the effect of increasing the concentrate price from 0.47 USD/kg to 0.71 USD/kg results in lower earnings per cow per day, with little change in the actual optimum. This is because, with low silage digestibility, the animals depend highly on concentrate intake to increase their energy intake and, thus, the response in milk production. For the borderline case (Figure 8c), the scenario differs. With the low concentrate price (0.47 USD/kg), earnings are not as sensitive to concentrate intake, having similar earnings for increases over 6 kg/day concentrate intake. However, with an increased concentrate price (0.71 UDS/kg DM), the earnings see a clearer economic optimum of around 5 kg/day of concentrate intake, reducing earnings after this concentrate level. Thus, this feeding strategy is quite sensitive to concentrate price. In the HDS case, Figure 8d, there is a small difference between the price scenarios at low concentrate levels because the money spent on concentrate is limited. However, as the amount of concentrate increases, the curves diverge. While the high concentrate price (0.71 UDS/kg) implicates an optimal earning reached at about 6 kg/day, and for the low concentrate price (0.47 USD/kg), the earnings curve is still increasing at that point. These figures show that, in the LDS cases, earnings increase with increasing concentrate intake because the animals have less of their energy intake covered by the silage. In the HDS case, however, earnings are more sensitive to the actual concentrate intake and the concentrate price. Although the uncertainty of the response functions, and thus also the uncertainties of the earnings, are significant and depend on the spread between the animals and the number of animals, the general trend remains. If the farmer is feeding HDS, the earnings are significantly influenced by the concentrate intake. This sensitivity increases if the concentrate price increases compared to the price of milk. Moreover, although HDS are more sensitive to concentrate prices, similar earnings for these types of silages are achieve with lower concentrate input. For LDS, the same earnings are only achieved with higher concentrate inputs, above 10 kg for Figures 7a and above 7 kg for Figure 7b.

Figure 9 also shows the economic scenario but, in this case, including SR of the silage. The SR refers to the change in silage intake when varying concentrate intake levels, which, normally, silage intake decreases with increased concentrate intake. The substitution rate is expressed on a DM basis; therefore, prices and amounts were converted to this basis. In our study, the SR used was, according to Álvarez et al. (2020) results, 0.53, which means the decrease or increase in intake by 0.53 kg DM silage per kg DM of concentrate increased or decreased, respectively. However, several authors have reported that variable SRs depend on silage quality (Thomas, 1987) and concentrate level of intake (Huhtanen et al., 2008). By including SR in the economic evaluation, the increase in concentrate limitedly affects the profits compared to Figure 8, as a reduction of silage intake is excluded from the costs, resulting in lower costs than only considering concentrate increase. However, to include SR in the equation, relative earnings must be included, choosing the average concentrate intake of each farm as it is. This is because feeding costs are incomplete, as information on total silage intake cannot be gathered from NCDX. Therefore, Figure 9 shows economic scenarios relative to the actual feeding strategy without considering total silage costs. For the economic evaluation to be complete, from a feed–productivity perspective, information about the silage intake must be obtained. This information is missing because silage is not fed individually, meaning no feeders with sensors are available at the farm level for its measure, as is the case for concentrates.

Limitations and perspectives of the algorithm

The ML silage digestibility and milk concentrate response model would benefit from having a larger dataset at our disposal. First, a larger dataset would allow us to make a meaningful split into training, validation, and test datasets, which, in turn, would help us to make a better assessment of the model's predictive strength and, thus, decide how much information we can optimally extract from the data. Second, it would also allow us to predict more digestibility classes, or if there is even more data for a regression model for silage OMD. However, we are not yet at this point. It requires either more academic or more controlled experiments, like Álvarez (unpubl.), to be available, or to obtain data from commercial farms that, for a given period, have accurate information about silage digestibility. To obtain silage's OMD from farms, there are several possibilities where collaboration with farmers is essential. Farmers could send for analysis feed samples taken from the silage bunk fed to animals, identifying the date of which this sample was taken, so we can relate it to data from AMS. Another possibility could be the use of portable NIR technology. This could improve the simplicity of the data acquisition, as no sampling or sample delivery has to be performed by the farmer. However, research is still needed regarding NIRS for digestibility measurement with portable equipment (Paz, 2019).

Moreover, information on silage intake is necessary for the economic evaluation to be complete. Average intake of forage could be calculated on-farm by considering input-output and number of cows. For individual intake information, silage-feeding bins with sensors will be ideal; however, it implicates changes in farm infrastructure and investment. Sensors monitoring eating time, ruminating time, and chewing activity could be used to predict silage intake (Beauchemin, 2018; Andriamandroso et al., 2016, Øvreeide, 2019).

As more technology is included in farms, and solutions for storing this data in an organized manner are available, models such as the one developed in this study will gain importance as real-time decision tools. Therefore, developing new models and improving first-attempt models, such as the proposed model, will be central.

CONCLUSION

The possibility of adapting a response function of milk to changes in concentrate supply to real-farm data was investigated. Farm data do not regularly provide silage digestibility data, which is essential for the response function adaption. However, ML allowed its prediction, and the first attempts at adaptation of response functions were made. To refine the model, more information on silage digestibility and intake is needed. Nevertheless, the current developed model will allow the farmer to make decisions from a production and economical perspective.

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Figure 1. Álvarez

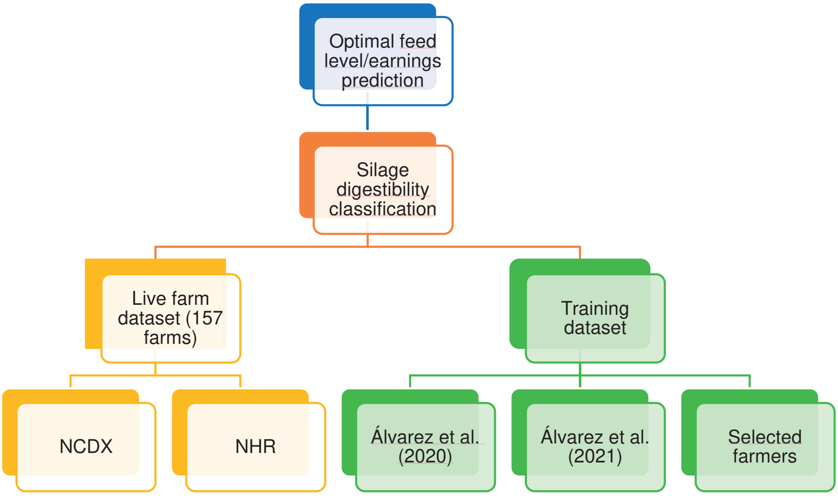

Figure 1: Summary of the machine learning approach used in the study

Figure 2. Álvarez

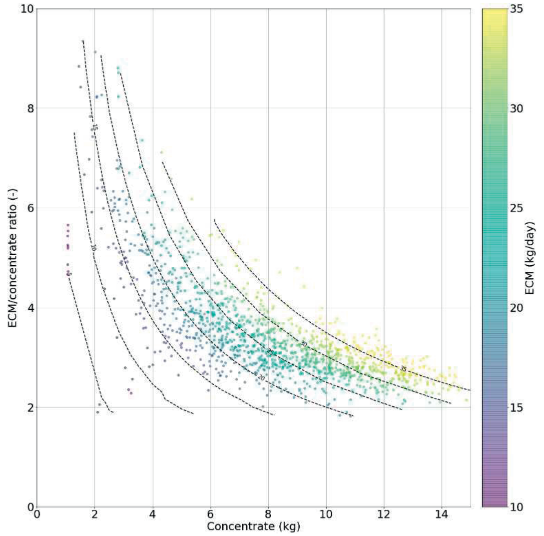

Figure 2: An overview of live farm dataset. Each point represents an individual cow with DIM above 100 and data averaged in April 2020. Black, dashed lines denote constant ECM levels (ECM-isolines).

Figure 3. Álvarez

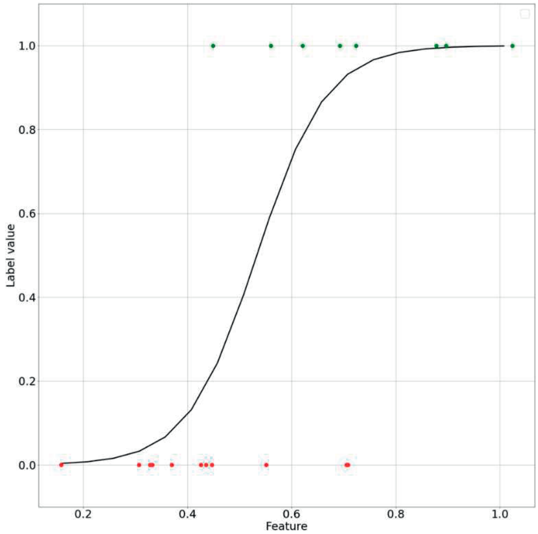

Figure 3: Example of a logistic function describing the relationship between label 0 (red) and label 1 (green).

Figure 4. Álvarez

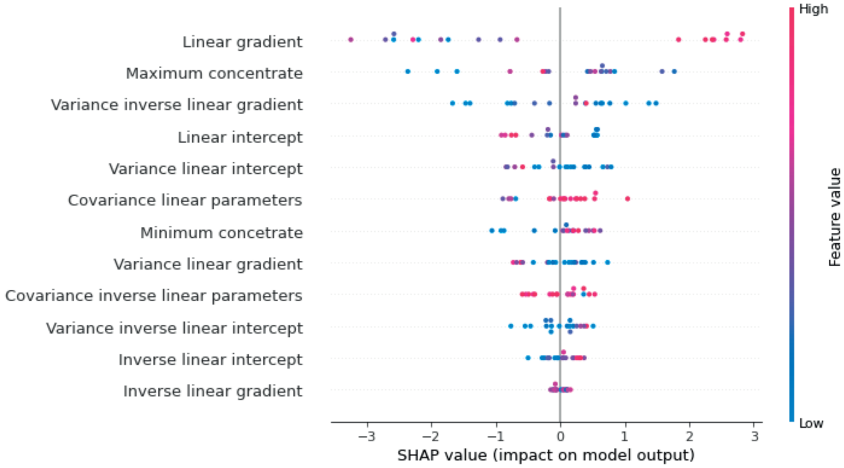

Figure 4: SHAP values for the classification of silage digestibility in the ML model.

Figure 5. Álvarez

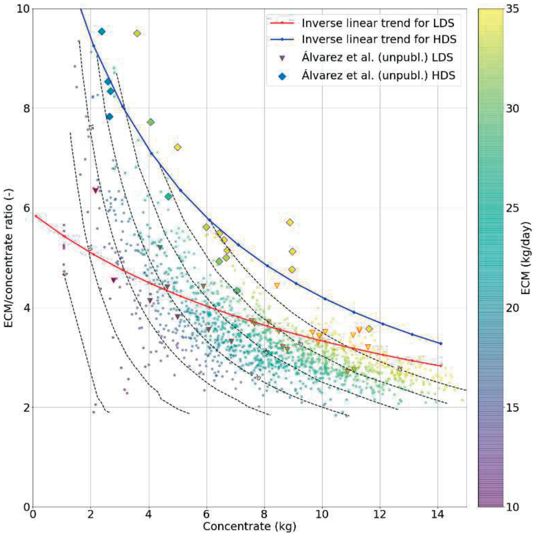

Figure 5: Example of fitted regression using data from Álvarez et al. (unpubl.) as a visualization example. Blue line: Inverse linear regression fitted to high digestible silage (HDS) data points. Red line: Inverse linear regression fitted to low digestible silage (LDS) data points.

Figure 6. Álvarez

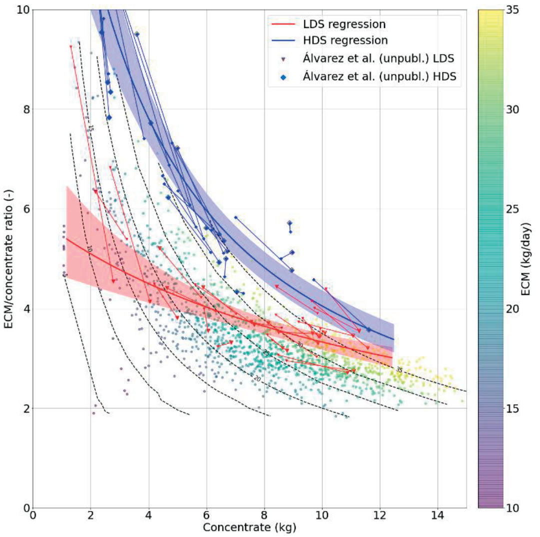

Figure 6: Individual animal responses from Álvarez (unpubl.) to the change in concentrate intake and algorithm regression line according to silage label (HDS and LDS). Pre- and post-change data for individual animals are connected by lines.

Figure 7. Álvarez

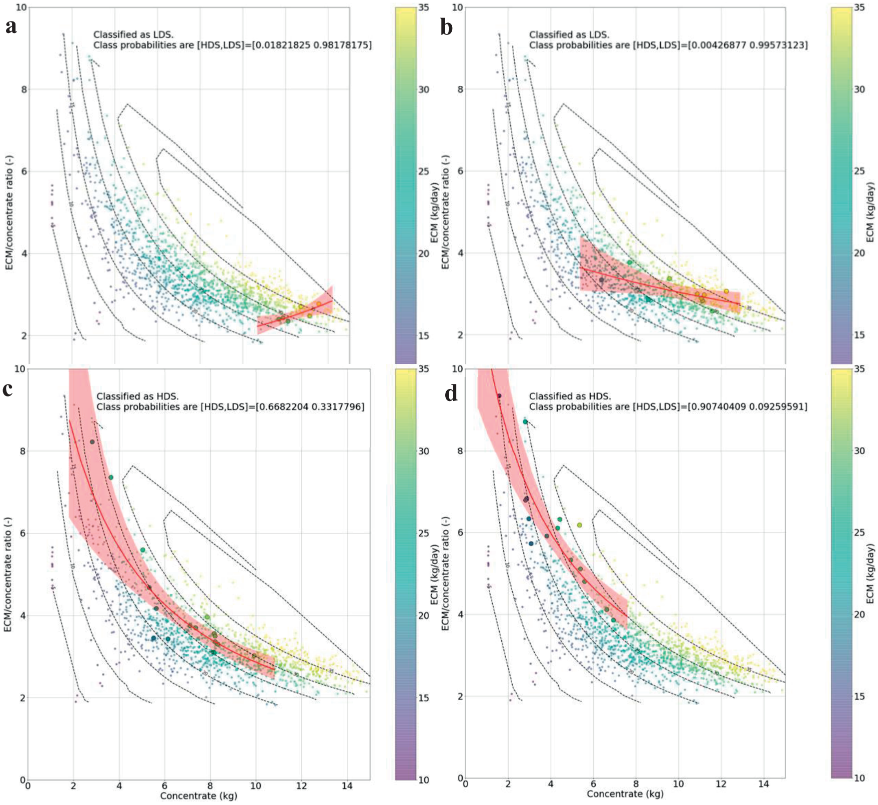

Figure 7: Examples of algorithm use for four different farms. Red line: Inverse linear regression, red area: plus/minus one standard deviation. a) Very low digestibility, b) low digestibility, c) borderline digestibility, and d) high digestibility

Figure 8. Álvarez

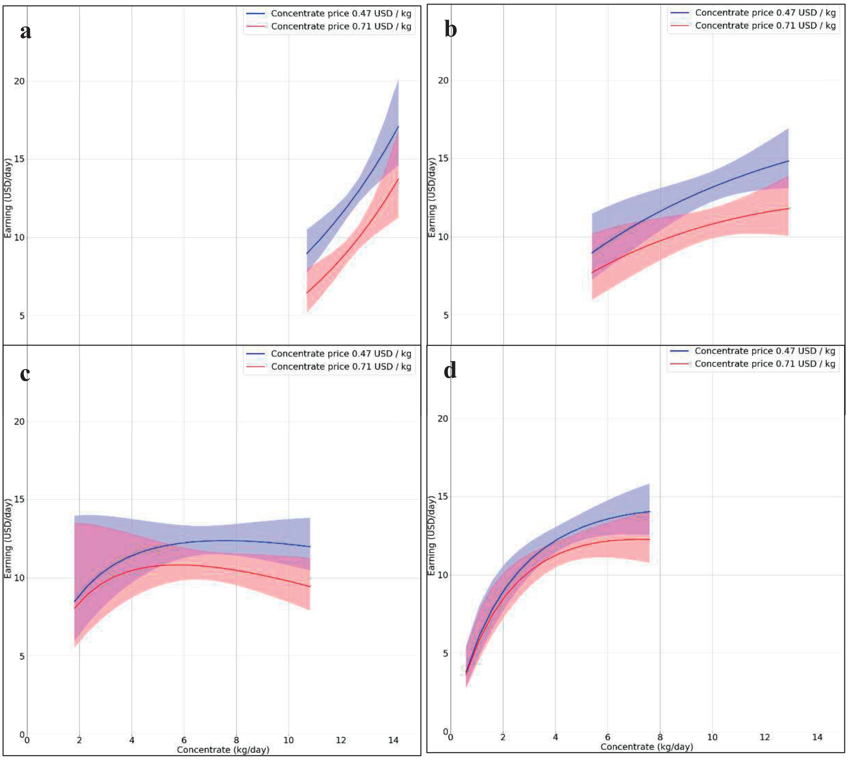

Figure 8: Predicted earnings per animal per day corresponding to the responses from Figure 7 for different silage digestibility. a) Very low digestibility, b) low digestibility, c) borderline digestibility, d) high digestibility. Milk price set at 0.59 USD / kg, and concentrate prices set at 0.47 USD/kg (blue) and 0.71 USD/kg (red), respectively.

Figure 9. Álvarez

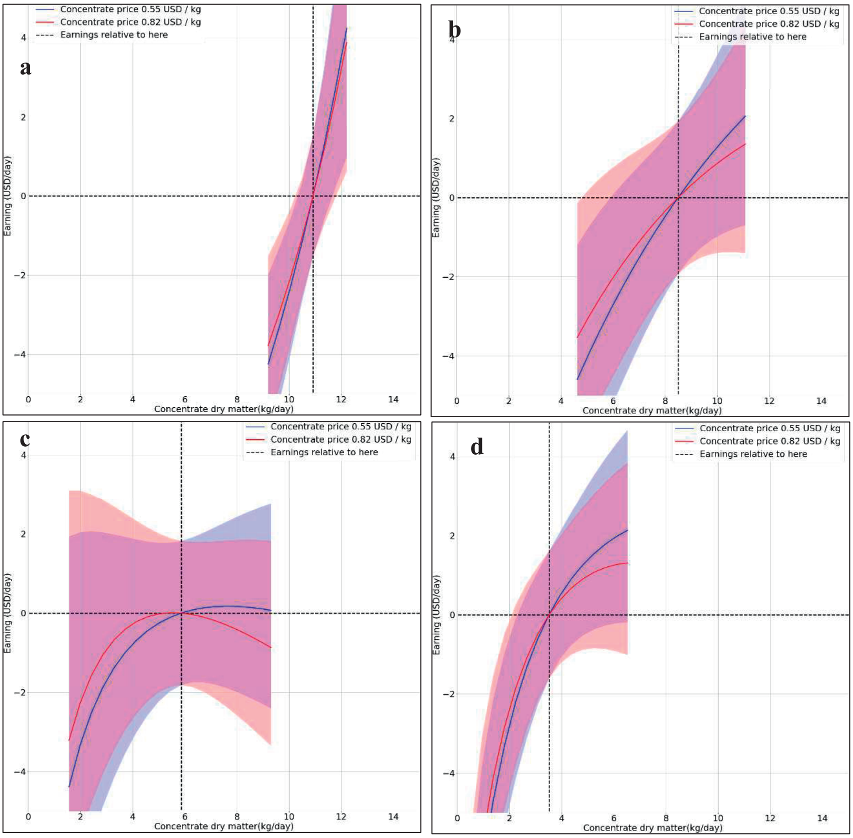

Figure 9: Predicted earnings similar to those of Figure 8 for an animal initially being fed the herd mean concentrate, considering a substitution rate of 0.53 and silage price of 0.24 USD/kg of dry matter. Dry matter percentage of 86% assumed in concentrate. a) Very low digestibility, b) low digestibility, c) borderline digestibility, and d) high digestibility. Milk price set at 0.59 USD / kg, and concentrate prices set at 0.55 USD/kg DM (blue) and 0.82 USD/kg DM (red), respectively.

Table 1: Training dataset comprising 19 points

Inverse linear gradient	Inverse linear intercept	Variance inverse linear gradient	Variance inverse linear intercept	Covariance inverse linear parameters	Linear gradient	Linear intercept	Variance linear gradient	Variance linear intercept	Covariance linear parameters	Minimum conc. intake[1]	Maximum conc. intake[1]	OMD label[2]
0.0435	0.0096	6.1e-06	0.0003599	-4.17e-05	-0.5602	8.019	0.0181	1.0773	-0.1248	2.59	12.0	HDS
0.0369	0.074	1.22e-05	0.0008206	-9.12e-05	-0.37	6.0657	0.0067	0.4497	-0.05	3.0	12.0	LDS
0.0299	0.0011	3e-07	1.94e-05	-2.1e-06	-0.6925	10.6872	0.0007	0.0471	-0.0051	4.3	11.1	HDS
0.0302	0.0083	2.7e-06	0.0001768	-1.99e-05	-0.7054	10.5733	0.0032	0.2076	-0.0234	3.8	10.8	LDS
0.0247	0.0531	2.7e-06	0.0002517	-2.56e-05	-0.3071	6.4462	0.0009	0.0834	-0.0085	7.8	11.3	LDS
0.0271	0.0413	3e-07	1.38e-05	-1.8e-06	-0.6214	9.081	0.017	0.8312	-0.1117	3.5	10.1	HDS
0.0253	0.0585	9e-07	4.65e-05	-6.2e-06	-0.5514	8.4273	0.0151	0.7378	-0.0991	3.5	10.1	LDS
0.0282	0.0049	9e-07	0.0001019	-8.2e-06	-0.8961	14.8157	0.1159	13.0772	-1.0546	1.8	16.6	HDS
0.0287	0.0097	3.9e-06	0.0006374	-4.6e-05	-0.3327	7.4611	0.0053	0.8566	-0.0618	4.7	18.4	LDS
0.0238	0.0672	3.6e-06	0.0002807	-3.12e-05	-0.3283	6.5959	0.0008	0.0646	-0.0072	6.9	10.8	LDS
0.03	0.0218	5e-07	1.88e-05	-2.8e-06	-0.8781	10.9565	0.002	0.0815	-0.0119	3.5	8.4	HDS
0.0298	0.0379	1e-07	5.5e-06	-8e-07	-0.7081	9.5071	0.0	0.0013	-0.0002	3.5	8.7	LDS
0.0298	0.0545	4e-07	3.19e-05	-3.4e-06	-0.4354	7.3417	0.0055	0.4201	-0.0449	3.65	12.0	LDS
0.0279	0.0464	5.9e-06	0.0003299	-4.37e-05	-0.4493	7.3538	0.001	0.0555	-0.0074	6.11	8.58	HDS
0.0345	0.0222	0.0001434	0.0081639	-0.0010659	-0.4469	7.0182	0.0187	1.0646	-0.139	6.13	8.79	LDS
0.013	0.1977	4.3e-06	0.0004179	-4.03e-05	-0.1576	4.7055	0.0006	0.0619	-0.006	2.5152	13.4494	LDS
0.0198	0.0566	2.2e-06	9.22e-05	-1.23e-05	-1.0237	13.2335	0.0312	1.3112	-0.175	1.1322	13.4924	HDS
0.0222	0.0874	6.9e-06	0.0002702	-4.2e-05	-0.7238	9.1157	0.0076	0.2979	-0.0463	2.2605	8.3252	HDS
0.018	0.1212	1.1e-05	0.0004767	-7.09e-05	-0.4258	7.0744	0.007	0.3028	-0.045	2.8146	10.6112	LDS

[1]Minimum and maximum concentrate intake (kg)

[2]HDS: High digestible silage, LDS: Low digestible silage.

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