Modelling nitrogen use and excretion in dairy cattle herds grazing temperate, semi-natural grasslands

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Summary

Grazing-based dairy cattle systems exhibit several benefits, such as preserving biodiverse grassland habitats, improving animal welfare, or turning grassland protein into human-edible protein. However, grazing-based diets are prone to greater nitrogen (**N**) losses via urine than balanced stall-fed diets, leading to a greater risk for N emissions. Strategies for improving the N use in grazing-based systems are predominantly investigated on homogenous clover-ryegrass pastures with high yields and nutritional quality. In contrast, grazing-based systems reliant on less external inputs (e.g., synthetic fertilisers or concentrates) using semi-natural grassland as main feed source received less attention. The present thesis addressed the knowledge gap on the N use of such low-input grazing-based systems by adapting an existing dynamic, process-based herd model (i.e., the LIVestock SIMulator, **LIVSIM**) for simulating animal performance and N use and excretion of dairy herds. For this, a broad dataset was gathered on nine commercial organic dairy cattle farms in Baden-Württemberg during two grazing periods (2019, 2020). This dataset fulfilled two purposes: firstly, to get a basic understanding on N use and excretion of dairy cows under low-input grazing conditions (study 1); secondly, to serve as reference dataset for adapting and evaluating LIVSIM for such production systems (studies 2 and 3).

The reference dataset represented the wide range of grazing and production factors found on commercial farms in South Germany using semi-natural grasslands for grazing. The dataset applied for study 1 covered n = 323 individual animal observations with mean (± one standard deviation) milk production, dry matter intake (**DMI**), and pasture DMI (**PDMI**) of 23.9 (± 5.35), 21.0 (± 3.21), and 11.3 (± 4.83) kg/d, respectively. Milk N use efficiency (**MNE**) averaged 24.7 g/100 g N intake (± 5.91), which is greater than observations in temperate, high-input grazing-based systems but lower than in cows receiving balanced stall-fed diets. Nevertheless, MNE and other indicators of N use and excretion varied greatly among farms and seasons, highlighting the need to identify the drivers for this variation. Supplement feeding had the greatest potential for manipulating the N use and excretion. Increasing shares of fresh forages as well as of hay of total supplement DMI increased N use (e.g., MNE) and decreased urinary N excretion (e.g., urinary N to creatinine ratio), while increasing shares of concentrates of supplement DMI were related to lower N losses via urine. Study 1 highlighted that using semi-natural grasslands for grazing can potentially reduce environmentally harmful N losses compared to high-input grazing systems.

For future research endeavours, a modelling approach may simplify the investigation of more feeding scenarios, their interactions, different local conditions, and considering the spatial and temporal variation of pasture herbage quality and yield. Hence, studies 2 and 3 focused on adaptating LIVSIM for low-input grazing-based dairy farms. The DMI and N intake are among the

most decisive factors for determining animal performance and N excretion. Therefore, a module for predicting the PDMI of cows grazing semi-natural grassland was identified in study 2, using a subset of the reference dataset (n = 233 individual animal observations). Among the thirteen tested models, behaviour-based and semi-mechanistic models specifically developed for grazing animals had the lowest prediction adequacy. Their underlying empirical equations likely did not fit the grazing and production conditions of farms employing semi-natural grasslands. Modelling performance of a semi-mechanistic model developed for stall-based feeding situations (Mertens II) with slight modifications was best (relative prediction error = 13.4%) when evaluated based on the mean observed PDMI (i.e., averaged across animals per farm and period (n = 28)).

Consequently, the modified Mertens II model was integrated in LIVSIM in study 3. Additionally, the modules for energy requirements, lactation, N excretion, and herd management were adopted, and breed-specific model coefficients added to represent Simmental, Brown Swiss, and Holstein-Friesian cattle breeds. Dairy cow characteristics, herd composition, annual milk yield, and DMI were predicted accurately (i.e., with a relative difference ≤ 10 % between observed and predicted outputs for the majority of outputs). The absolute total N excretion (g/d) was underpredicted by 23 % (= relative difference between observed and predicted values) mainly due to the underprediction of urinary N excretion by 43 %. The relative differences in N excretion between farming systems, in contrast, were predicted reliably. The observed faecal, urinary, and total N excretion (in % of N intake) differed by 30, -23, and -7 %, respectively, between the two reference herds, which is similar to the respective relative differences for the predicted faecal, urinary, and total N excretion of 32, -36, and -4 %. Further model improvements should focus on increasing the prediction accuracy of N excretion and its partitioning due to the varying degree of susceptibility of faecal or urinary N to volatilisation and leaching. The scenario and sensitivity analyses further confirmed that the adapted LIVSIM plausibly simulated differences in animal performance and nutrient excretions based on differences in supplement feeds and pasture herbage. Core input and model coefficients are the dietary ME, CP, and rumen-undegradable CP concentrations, as well as the available herbage biomass on pastures, for which precise measurements are thus needed. The findings of studies 2 and 3 demonstrate that existing models can be adopted for low-input grazing-based dairy production systems. There is further potential for adapting LIVSIM for production systems beyond the ones investigated in the present study, and/or for adding more outputs (e.g., enteric methane) and scales (e.g., grassland) to better capture the multifaceted aspects determining farm sustainability.

Zusammenfassung

Die weidebasierte Milchrinderhaltung bietet mehrere Vorteile im Vergleich zu rein stallbasierten Systemen, wie z. B. den Erhalt biodiverser Graslandschaften, gesteigertes Tierwohl oder die Umwandlung des Proteins der Weidevegetation in für den Menschen verzehrbares Protein in Form von Milchprodukten. Allerdings sind weidebasierte Fütterungssysteme auch anfälliger für höhere Stickstoff-(**N**)-Verluste über den Urin als stallbasierte Systeme mit ausgewogenen Rationen, was zu einem höheren Risiko für N-Emissionen führt. Strategien zur Verbesserung der N-Nutzung unter Weidebedingungen wurden überwiegend auf homogenen Klee-Weidelgras-Weiden mit hohen Erträgen und hoher Nährstoffqualität untersucht. Weidebasierte Systeme, die weniger externe Inputs (z. B. synthetische Dünger oder Kraftfutter) und semi-natürliches Grünland als Hauptfuttergrundlage nutzen, erhielten dahingegen weniger Aufmerksamkeit. Die vorliegende Arbeit befasste sich daher mit der N-Nutzung solcher low-input Weidemilchviehsysteme, mit dem Fokus auf der Anpassung eines bestehenden dynamischen, prozessorientierten Herdenmodells (LIVestock SIMulator, **LIVSIM**) zur Simulation der Tierleistung sowie der N-Nutzung und -Ausscheidung von Milchviehherden. Hierzu wurde ein breiter Datensatz auf neun kommerziellen Bio-Milchviehbetrieben in Baden-Württemberg über zwei Weideperioden (2019, 2020) erhoben. Dieser Datensatz erfüllte zwei Zwecke: erstens, ein grundlegendes Verständnis für die N-Nutzung und -Ausscheidung von Milchkühen unter low-input Weidebedingungen zu gewinnen (Studie 1); zweitens sollte er als Referenzdatensatz für die Anpassung und Bewertung von LIVSIM für solche Produktionssysteme dienen (Studien 2 und 3).

Der Referenzdatensatz gab das breite Spektrum an Beweidungs- und Produktionsfaktoren wieder, das auf kommerziellen Milchviehhöfen mit semi-natürlichen Grünlandflächen in Süddeutschland zu finden ist. Der für Studie 1 verwendete Datensatz umfasste n = 323 Einzeltierbeobachtungen mit einer mittleren (± eine Standardabweichung) Milchproduktion, Futteraufnahme (**DMI**) und Weide-DMI (**PDMI**) von 23,9 (± 5,35), 21,0 (± 3,21) bzw. 11,3 (± 4,83) kg/Tag. Die Milch-N-Nutzungseffizienz (**MNE**) betrug durchschnittlich 24,7 g/100 g N-Aufnahme (± 5,91), was höher ist als Beobachtungen aus temperaten, high-input Weidemilchviehsystemen, aber niedriger als bei Kühen, die eine ausgewogene Stallration erhielten. Dennoch variierten MNE und andere Indikatoren der N-Nutzung und -Ausscheidung je nach Betrieb und Saison stark. Dies unterstreicht die Notwendigkeit, die Ursachen für diese Variation zu ermitteln. Die Zufütterung im Stall hatte das größte Veränderungspotential für die N-Nutzung und -Ausscheidung. Steigende Anteile an frischem Raufutter sowie an Heu an der gesamten Zufütterung erhöhten die N-Nutzung (z. B. MNE) und verringerten die Urin-N-Ausscheidung (z. B. das Verhältnis von N zu Kreatinin im Urin), während steigende Kraftfutteranteile in der Zufütterung den N-Verlust über den Urin

verringerten. Somit betont Studie 1, dass die Beweidung semi-natürlicher Grünlandflächen im Vergleich zu high-input Weidemilchviehsystemen unter den richtigen Zufütterungsbedingungen die umweltschädlichen N-Verluste reduzieren kann.

Ein Modell, welches die Untersuchung weiterer Fütterungsszenarien, ihrer Wechselwirkungen, unterschiedlicher lokaler Bedingungen und die Berücksichtigung der räumlichen und zeitlichen Variabilität der Weidequalität und des Weideertrags ermöglicht, könnte zukünftige Forschungsvorhaben vereinfachen. Daher konzentrierten sich die Studien 2 und 3 auf die Anpassung von LIVSIM für low-input Weidemilchviehbetriebe. Die DMI und N-Aufnahme zählen zu den entscheidendsten Faktoren für die Leistung und N-Ausscheidung von Milchvieh. Daher wurde in Studie 2 ein Modul zur Vorhersage der PDMI für weidende Kühe auf semi-natürlichem Grünland anhand eines Teildatensatzes der Referenzdaten (n = 233 einzelne Tierbeobachtungen) identifiziert. Unter den dreizehn getesteten Modellen wiesen verhaltensbasierte oder semi-mechanistische Modelle, die speziell für Weidetiere entwickelt wurden, die geringste Vorhersagegenauigkeit auf. Vermutlich konnten die ihnen zugrunde liegenden empirischen Gleichungen die Weide- und Produktionsbedingungen von low-input Weidemilchviehbetrieben nicht abbilden. Stattdessen erreichte ein semi-mechanistisches Modell, das für stallbasierte Fütterungssysteme entwickelt wurde (Mertens II), die höchste Vorhersagekraft (relativer Vorhersagefehler = 13,4 %).

Das in Studie 2 identifizierte Modell (Mertens II) wurde daraufhin in LIVSIM integriert (Studie 3). Darüber hinaus wurden die Module zur Vorhersage des Energiebedarfs, der Milchleistung, der N-Ausscheidung und des Herdenmanagements angepasst und rassespezifische Modellkoeffizienten hinzugefügt (Fleckvieh, Braunvieh, und Holstein-Friesian). Die Milchviehkennzahlen (z. B. Laktationstage), die Herdenzusammensetzung, die jährliche Milchleistung und die DMI wurden mit hoher Genauigkeit vorhergesagt (d. h. mit relativen Differenzen von ≤ 10 % zwischen beobachteten und vorhergesagten Werten). Die Gesamt-N-Ausscheidung (g/Tag) wurde um 23 % unterschätzt (= relative Differenz), hauptsächlich aufgrund der Unterschätzung der Urin-N-Ausscheidung um 43 %. Die relativen Unterschiede in der N-Ausscheidung verschiedener Managementsysteme wurden dagegen zuverlässig vorhergesagt. Die beobachtete Kot-, Urin- und Gesamt-N-Ausscheidung (in % der N-Aufnahme) unterschied sich zwischen den beiden Referenzherden um 30, -23 bzw. -7 %, was den jeweiligen relativen Unterschieden für die vorhergesagten Ausscheidungen ähnelte (32, -36, and -4 %). Zukünftige Modellanpassungen sollten sich dennoch darauf konzentrieren, die Schätzgenauigkeit der N-Ausscheidung zu erhöhen, insbesondere da die Differenzierung nach Kot oder Urin darüber entscheidet, ob und inwiefern N emittiert oder ausgewaschen wird. Die Szenario- und

Sensitivitätsanalysen bestätigten weiterhin, dass das angepasste LIVSIM-Modell Unterschiede in der Tierleistung und N-Ausscheidungen abhängig von Unterschieden in der Zufütterung und dem Weidefutter plausibel simuliert. Die Sensitivitätsanalyse zeigte zudem, dass die Konzentrationen an umsetzbarer Energie und Rohprotein, die Pansenabbaubarkeit des Rohproteins und die verfügbare Weidebiomasse wesentliche Inputs für das angepasste Modell sind und damit genau bestimmt werden müssen. Die Ergebnisse der Studien 2 und 3 bestätigen, dass bestehende Modelle für low-input Weidemilchviehbetriebe angepasst werden können. Weiteres Forschungspotential steckt in der Anpassung von LIVSIM für weitere Produktionssysteme, um eine einheitliche Ebene für Vergleiche zwischen Produktionstypen zu schaffen. Zudem könnten weitere Outputs (z. B. enterisches Methan) und Ebenen (z. B. Grünland) dazu beitragen, die vielfältigen Aspekte, die die Nachhaltigkeit landwirtschaftlicher Betriebe bestimmen, besser darzustellen.

Chapter 1 | General Introduction

1.1 Role of grazing-based dairy production in Europe

Grazing-based milk production increasingly gains attention in the public discourse on sustainable food production in Europe. Grazing-based farming systems make use of grassland-based feed resources and therefore play an important role in the preservation of grasslands and within the feed-food debate. Firstly, preserving grasslands can contribute to maintaining grassland-based ecosystem services, such as the conservation of biodiverse grassland ecosystems, the sequestration of carbon, or the provision of feed resources for ruminant production systems (Schils et al., 2022). Grazing cows, secondly, turn human-inedible grassland crude protein (**CP**) into human-edible protein, which lowers the requirements for feed grown on land which could otherwise be used for food production (Mottet et al., 2017). In addition, farmers appreciate grazing practices due to their low feed and labour cost (Becker et al., 2018). The growing consumer demand and willingness to pay premium prices for pasture-based dairy products is foremostly driven by the associated benefits for animal welfare (Schaak and Mußhoff, 2018). Dairy farmers are therefore increasingly motivated to continue or begin integrating grazing in their dairy production systems (Lessire et al., 2019). The extent to which grazing-based dairy production systems can fulfil these social and environmental benefits expected by the public, however, depends on the type of grazing system.

A wide range of grazing practices exist throughout Europe. For the present thesis, a rough classification into low-input and high-input grazing-based dairy cattle production systems is suggested, acknowledging that a range of varieties between these two extremes exist (Figure 1.1; adapted from Moorby and Fraser, 2021, p. 2). These two categories can be distinguished by their use of external resources and the types of grassland applied for grazing. High-input grazing-based systems usually depend on improved permanent or temporary grasslands, typically cultivated with perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), and fertilised with high rates of synthetic nitrogen (**N**) to ensure sufficient levels of high-quality biomass on pasture (Peeters et al., 2014). In addition to offering high-quality pasture herbage, a high animal productivity is also achieved by supplementation with substantial amounts of concentrates. High-input grazing-based dairy farms are therefore highly competitive and ensure an efficient use of the grassland resource in terms of a high product output per hectare (Delaby et al., 2020). However, the use of temporary pastures as well as the increased use of external feed resources to maximise milk production further adds to the pressure on arable land resources. A systematic review on permanent grasslands in Europe suggests that the increasingly intensified use of permanent grasslands is detrimental for its multifunctional benefits (i.e., ecosystem services), foremostly owing to the reduction in forage species diversity on homogenous improved grasslands or via the conversion of permanent grasslands into temporary pastures (Schils et al., 2022).

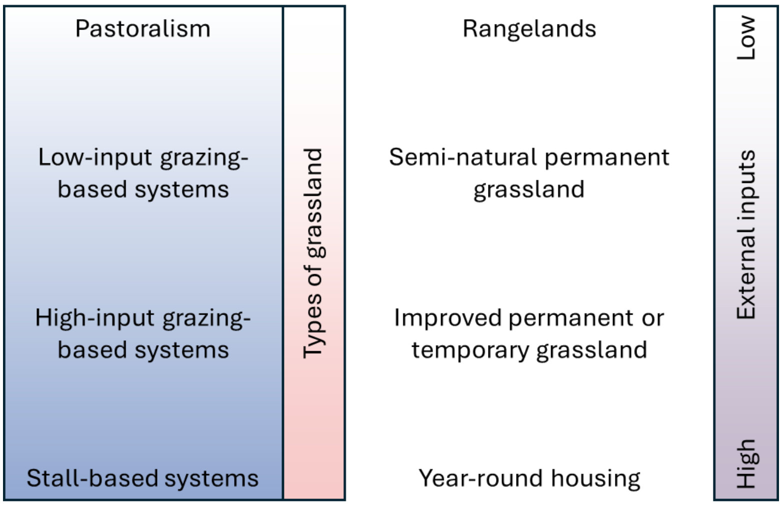

Figure 1.1. Range of cattle production systems classified by housing situation or types of grassland (classification according Peeters et al., 2014), and reliance on external inputs (e.g., bought-in fertilisers and concentrates) (adapted from Moorby and Fraser, 2021, p. 2).

Low-input grazing-based systems foremostly use semi-natural permanent grasslands which receive a lower treatment intensity than improved or temporary pastures. Semi-natural grasslands are primarily subject to animal-based impacts such as excretions, trampling, and foraging and receive no or little soil cultivation or fertilisation (Peeters et al., 2014). This low treatment intensity leads to a greater abundance of different forage species allowing for a higher multifunctionality than seen in improved grasslands (Schils et al., 2022). Trade-offs of the lower treatment intensity are, at least partially, a lower forage yield and energy concentration than in cultivated or improved grasslands. Low-input grazing-based systems pursue a low-cost strategy and accept a lower milk productivity per animal and hectare, in return. Both, organic and conventional, low-input dairy production systems are generally smaller, family-owned, and reliant on less external inputs such as bought-in synthetic fertilisers or concentrates (Scollan et al., 2017). Instead, they make greater use of own farm resources, and allocate more agricultural area to forage cultivation and grasslands. The lower dependency on external goods and a lower requirement for capital investment can enable low-input farmers to economically perform equally well as high-input dairy farms, despite the lower productivity of low-input dairy production systems (Scollan et al., 2017). Especially in regions where less favourable soil and topographic conditions signify a high share of permanent grasslands e.g., in regions with low mountain ranges or in (pre-)alpine regions, implementing low-input grazing practices depicts an economically viable production strategy (Poetsch, 2007; Reidy and Ineichen, 2014; Dentler et al., 2020). In such regions, another distinct feature is the fragmented distribution of grassland across the farming area i.e., the natural division of the farmland into individual plots via landscape elements such as hedges and streams, and by the steep and variable topography. The fragmented landscape limits the grazable grassland surrounding the milking parlour. The limited grazable area as well as the high share of low-yielding permanent grassland almost exclusively permits small-scale dairy farms to keep dairy herds on pasture. It further restricts the applicability of full-time grazing (Akert et al., 2020). Instead, part-time grazing is commonly combined with greater supplementation in barn, predominantly met with the locally available forages from permanent grasslands not suitable for grazing (i.e., fresh forages, grass silages and hay) as well as other forages from temporary grassland (i.e., grass-clover leys) and arable land (e.g., maize silage). In such grassland-dominated regions, low-input grazing systems can make optimum use of the locally available grassland resources while preserving valuable, species-rich permanent grassland (Dentler et al., 2020; Gazzarin et al., 2021).

In sum, low-input grazing-based dairy farming systems represent economically feasible production system for small-scale farms throughout the (semi-)mountainous regions in Central Europe, are able to sustain the valuable ecosystem services provided by multispecies grassland, and have a lower demand for external inputs and feed resources from arable land. The present dissertation will, therefore, focus on this particular production system. However, the efforts of the past decades to meet the growing demand for agricultural goods has given rise to extensive research on high-input grazing practices (Roche et al., 2017). Not surprisingly, less research has focused on low-input grazing systems. This dissertation, thus, aimed at filling this research gap (1) by specifically focusing on investigating the N use and excretion of dairy herds kept in low-input grazing-based systems, (2) by adopting a modelling approach to capture the performance and N use and excretion of grazing cattle, and (3) by choosing the German state of Baden-Württemberg as a case-study region with good prerequisites for implementing low-input grazing practices.

1.2 Challenge of grazing-based dairy production: nitrogen losses

While grazing-based dairy production exhibits several benefits for the environment and society, it is also known as a significant emitter of environmentally harmful N. Nitrogen emissions are one of the major environmental burdens caused by agricultural production by emitting ammonia, the greenhouse gas nitrous oxide, and causing eutrophication by nitrate leaching and surface-runoff (Powell et al., 2010). Continuous research on opportunities to mitigate N losses to the environment from agricultural production is therefore inevitable. In animal production systems, efforts to mitigate N losses to the environment typically focus on reducing N excretions via urine and faeces and increasing N utilisation i.e., the conversion of input N into N contained in animal products. Powell et al. (2010) stated that the N utilisation in dairy production was lower and subject to higher variability than in poultry and pig production, suggesting that there is a special need to discern the factors causing this high variation in N utilisation in dairy farming. In general, this disparity between monogastric and ruminant production systems is attributable to the differences in the ingestive physiology between monogastric and polygastric species. The more precise and uniform housing and feeding management among the poultry and pig production sector further favours a more efficient use of feed protein. Dairy production systems, in contrast, can vary with regards to the type and share of home-grown or external concentrates and forages, and to manure and housing management (Powell et al., 2010). With dairy production systems using balanced stall-fed diets, a possibly higher N utilisation can be achieved than in grazing systems due to a more precise control of the dietary CP inputs in the barn, whereas high variability in terms of CP degradability, concentration and intake prevail on pastures (e.g., Powell et al., 2010; Oenema et al., 2012). Young, grazed herbage is also characterised by high levels of rapidly degradable CP and low concentrations of energy, causing a surplus of ammonia in the rumen which cannot be converted to microbial protein due to the lack of energy. Since excessive ammonia concentrations are toxic for the rumen environment, surplus ruminal ammonia is absorbed at the rumen epithelium, converted to urea in the liver, and primarily excreted via urine. Urine excretion on pastures causes urine patches with locally high soil N concentrations which are in excess of plant N requirements. These excessive N loads from urine patches are especially vulnerable to N volatilisation and leaching losses (Selbie et al., 2015). Grazing-based diets are, thus, prone to greater losses of N via urinary N excretions than balanced stall-fed diets (Hoekstra et al., 2007), suggesting that there is a special need to identify strategies to reduce urinary N excretions in grazing-based dairy production systems.

Several studies have investigated strategies to mitigate the N losses from grazing-based dairy production systems. They have shown that a combination of several strategies such as the integration of alternative forage species (e.g., plantain, *Plantago lanceolata*) into the grazed pasture, use of supplemental feedstuffs to balance the supply of CP and energy, a lower N fertilisation application to the grassland, and part-time grazing can effectively reduce N losses through ammonia, ammonium, and nitrous oxide from temperate grazing systems (Christie et al., 2014; Beukes et al., 2017; Bryant et al., 2020). However, these strategies were foremostly identified for mitigating the N losses from high-input grazing systems, and fewer studies have dealt with the N use and excretion of dairy cows under organic and/or low-input grazing systems (Akert et al., 2020; Ayers et al., 2022). It can be expected that findings from high-input grazing systems are not fully applicable to low-input grazing systems because of the differences in grassland types deployed, and the diverging levels of resource use and productivity. Strategies such as the integration of alternative forage species or the reduction of N fertilisation, for instance, might not be relevant under low-input grazing conditions, due to the naturally species-rich nature and low treatement intensity of semi-natural grassland. It is therefore necessary to, firstly, establish a basic knowledge on the N use and excretion of dairy cows in low-input grazing systems and its differences to high-input grazing systems. Then, previously found strategies to reduce the N losses under grazing conditons can be re-evaluated or new strategies identified that are applicable in low-input grazing systems.

1.3 Simulating nitrogen use and excretion with mathematical models

Investigating the N flows (i.e., intake and partitioning into milk, faeces, and urine) under experimental farming conditions is time-consuming and expensive which limits the variables (e.g., dietary changes or local conditions) and time horizon that can be tested (Christie et al., 2014). An increasing number of studies, thus, apply models to evaluate strategies to reduce N losses in grazing-based dairy production systems on the animal (Gregorini et al., 2016; Dodd et al., 2019) or farm level (Vogeler et al., 2013; Christie et al., 2014; Bryant et al., 2020). While the development of a new, adequate (i.e., accurate and precise; Tedeschi, 2006) model is equally time consuming as experimental research approaches, there is a host of existing models, from which the most suitable can be chosen to be adapted and/or evaluated for the production system of the present thesis. The main objective of the present dissertation was thus the adaptation of an existing mathematical model which can be employed to evaluate opportunities to mitigate N losses from grazing-based low-input dairy farms. Among the existing models, different model types, system boundaries, and emphases can be found. The key model requirements for the issue at hand will therefore be outlined, followed by describing the candidate model chosen based on these key requirements.

1.3.1 Key requirements for modelling N use and excretion of grazing dairy herds

Types of models

Mathematical models have long been used in agricultural research to describe or forecast patterns and behaviours of certain farming aspects by translating “real-life situations into mathematical formulations” (Tedeschi, 2019, p. 1922). Different model types are applied, depending on the purpose of the model. A common distinction is made between empirical and mechanistic models. Numerous empirical (i.e., data-driven) models have been developed to estimate N use and excretion. They are mainly based on relationships between key input variables (e.g., milk urea N or milk production) and the desired output parameters (e.g., urinary N excretion) observed in stall-based dairy cows offered total mixed rations (Reed et al., 2015). A substantial limitation of such models, however, is that their application is restricted to the production conditions of the underlying empirical dataset. Empirical models, further, do not capture the feedforward and feedback interactions between different system components (e.g., animal performance and feed availability and quality; Bateki and Dickhoefer, 2020) because they commonly focus on one aspect of the production system (e.g., N partitioning; Reed et al., 2015).

In contrast, mechanistic or process-based models attempt to reproduce and link the biological, (geo-)physical and physiological processes underlying the system of interest. This hierarchical and interactive approach allows the representation of a whole system, the investigation of changes in various components and levels of the system, and a more universal application for a range of farming systems (Reed et al., 2015). Process-based models can, thus, be applied to simultaneously assess various grazing management options across different grazing or local conditions, which is a key requirement for the diverse grazing and farming systems which can be found in low-input grazing-based dairy farming.

Process-based models further allow the assessment and adaptation of separate components to focus on key parameters or processes within a system. For example, feed intake, or more specifically N intake, is one of the key predictors of N partitioning (Reed et al., 2015). The intake of pasture herbage further determines whether the available grasslands resources are used efficiently. Chobtang et al. (2017), for instance, compared the environmental sustainability of low- and high-input grazing-based dairy farms via life cycle assessment. In their study, pasture herbage intake was negatively related to all environmental impact indicators attesting that increasing pasture herbage intake is a crucial strategy for improve the sustainability of dairy farming. Identifying a module to accurately predict feed intake is therefore a key requirement of a suitable model to simulate N utilisation and resource use of grazing-based farming systems.

System boundaries

The representation of a farming system using process-based models can be a complex endeavour which requires precise definition of the system boundaries. Most existing process-based models for grazing-based dairy production systems are models simulating the whole farm with the focus on the environmental emissions of grazing livestock (e.g., APSIM: Holzworth et al., 2018; Orfee: Mosnier et al., 2017; PaSim: Graux et al., 2011). Such models contain livestock as well as grassland sub-models to simulate the interacting effects of animal production and excretions and grassland growth and nutritional quality on environmental impacts such as nitrous oxide emissions or nitrate leaching. To reliably apply such whole-farm models to the production system at hand, a validation of both the livestock and grassland model would be necessary. However, the additional validation of a grassland model would add another level of complexity, which was beyond the scope of the present thesis due to the substantial amount of information on soil characteristics and dynamics, as well as expertise, required for the validation of such a grassland model. Instead, the focus was set on adapting a livestock model for simulating resource use for production and excretions on the animal level.

Another central point was set on capturing the N use and excretion across the total herd, i.e., including dry cows, heifers, and calves, as opposed to solely focussing on lactating dairy cows. In 2023, male and growing animals accounted for 47 % of all cattle held on dairy farms in Baden-Württemberg (Statistisches Landesamt Baden-Württemberg, 2023a). Calves and heifers frequently spend the whole grazing season on pasture, hence, can contribute significantly to the N losses of dairy farms.

Temporal and spatial variation

Another key requirement was the ability to capture the effect of spatial and temporal variation in the feed base of grassland-dominant dairy systems. Compared to controlled stall-fed diets, grazing-based diets are generally subject to a greater variation in nutritional quality and biomass availability from pasture herbage due to changes in plant growth and the seasonal weather variability (Bell et al., 2018). Additionally, a greater spatial heterogeneity can be expected for multi-species semi-natural than improved grasslands, as a consequence of selective grazing (Tonn et al., 2019), and the heterogenous and fragmented topography. The fragmented farming area, greater forage species diversity, and heterogeneous distribution of forage species on semi-natural grassland, and climate variability favours a substantial spatial and temporal variation in the nutrient availability during the grazing period. Further, in regions where the year is separated into an exlusively stall-based winter period and a grazing period during the growing season, the interaction with the winter period needs to be considered. Grazing management decisions, such as the allocation of grassland for grazing or mowing, impact the resources (i.e., conserved forages) available for the stall-fed diet in winter. The winter diet can further determine the body condition with which animals enter the grazing period. It is therefore necessary to identify a dynamic model which captures the effect of changes in farming conditions and management over time and its effects on the long-run. A dynamic model further allows for the simulation of the relationships and trade-offs between farm resources, farm management and the productive and environmental outputs over time.

The present thesis, thus, aimed at adapting an existing dynamic process-based model to simulate the N use and animal performance of dairy cattle herds as a response to changes in grazing conditions and management under various local farming conditions.

1.3.2 The candidate model: The LIVestock SIMulator (LIVSIM)

There are several models which fulfil the above-mentioned key requirements to simulate dairy cows (e.g., Molly: Gregorini et al., 2016), dairy herds (Ruelle et al., 2016; Dynamilk: Jacquot et al., 2015) or farms within a grazing context (e.g., APSIM: Holzworth et al., 2018, and Orfee: Mosnier et al., 2017). However, they commonly require inputs which are not routinely available for low-input grazing-based systems e.g., due to their interaction with elaborated grassland models to predict pasture growth and nutritional value (e.g., APSIM or Dynamilk), or the integration of intricate rumen sub-modules (e.g., Molly). Adapting a model developed for (sub-)tropical systems represents a suitable alternative since they are generally developed for data constrained systems (Bateki, 2020). One model developed to simulate cattle systems within data constrained situations is the LIVestock SIMulator (**LIVSIM**). The LIVSIM is a dynamic herd model that simulates the productive and reproductive performance of ruminants based on feed and animal genetic resources available in grassland-based, smallholder systems in Africa (Rufino et al., 2009). The model also predicts the associated nutrient excretion (e.g., N excretion) and greenhouse gas emissions from different animal groups (i.e., calves, heifers, and cows). Although this model was developed and further modified to suit the production conditions in the Tropics, it was chosen as a suitable candidate model for low-input grazing-based systems in temperate regions, because LIVSIM was largely developed based on data gathered from temperate dairy cattle systems (Bateki and Dickhoefer, 2020). It can further be assumed that the basic biological processes underlying milk production, body weight change, and resource utilisation will be similar across production systems, where changes are predominantly required for coefficients or singular equations. The LIVSIM was also chosen for practical reasons. The former adaptation of LIVSIM took place within the working group within which the present dissertation was settled, making model code and expert insights easily accessible for the present thesis. Secondly, the expansion of LIVSIM to temperate production systems allows research groups to compare production systems (e.g., tropical versus temperate dairy farms) on a global scale using one common tool. A common tool to evaluate the resource use and animal performance of ruminant systems reduces the resources otherwise needed for different tools and provides a solid, uniform scientific basis.

1.4 The German state of Baden-Württemberg as case study region

For the present dissertation, the German state of Baden-Württemberg, including the low mountain ranges of the Black Forest and the Swabian Alb, was chosen as a case-study region representative of grassland-dominated regions favouring low-input grazing practices. In 2020, 61 % of cattle farms allowed pasture access to their cattle which amounted to 27 % of the cattle stock in Baden-Württemberg (Stütz, 2021). This state is further characterised by a high share of small-scale, family-owned and/or organic farms where grazing practices continue to play an essential role in dairy production. Here, 57.4 and 11.4 % of dairy cows are kept in herds with < 100 animals and on organic farms (Statistisches Bundesamt [Destatis], 2023a, 2023b), respectively (Germany: 40 and 6 %; Destatis, 2023c, 2023d). The state of Baden-Württemberg has a comparatively high share of permanent grassland (39% of the agricultural area; Germany: 29 %) (Destatis, 2023e, 2023f), which will also remain as high in the future, owing to the prohibition to transform permanent grassland into arable land issued by the federal state in 2011 (Hartmann, 2012). The importance of permanent grasslands within dairy farms varies regionally, and is especially high across the low mountain ranges of the Black Forest and the Swabian Alb. Here, the agricultural land consists of 60 to 100 % of permanent grassland (Hartmann, 2012). A high share of these permanent grasslands are managed semi-naturally, which is apparent from the low mean annual forage yield of 5.3 t/ha for grasslands used simultaneously for cutting and grazing (*Mähweide*) or exclusively for grazing reported for the years 2016 to 2022 (Statistisches Landesamt Baden-Württemberg, 2023b). This reported pasture biomass productivity is even lower than the mean annual pasture yield of Swiss hill and mountain dairy farms (6.5 ± 1.55 t/ha; Repar et al., 2018), and substantially lower than in high-input grazing-based dairy farms such as found in Nothern Germany (10.9 – 11.6 t/ha; Peters et al., 2022) or in Ireland (12.7 – 15.0 t/ha; O'Donovan et al., 2022).

1.5 Objectives and outline of dissertation

The overall objective of the present thesis was, thus, the adaptation of an existing dynamic, process-based livestock model i.e., LIVSIM, for predicting the animal performance, feed intake, and N use and excretion of dairy herds using temperate, semi-natural grasslands for grazing. To evaluate and adapt LIVSIM for such dairy production systems, an extensive dataset characterising the farm, herd, and grazing management, and N use and excretion of nine commercial organic dairy farms was gathered during two grazing periods (2019 and 2020) in Baden-Württemberg. The compiled dataset fulfilled two purposes: firstly, to get a basic understanding on N use and excretion of dairy cows under low-input grazing conditions (Chapter 2); secondly, to serve as reference dataset for adapting and evaluating LIVSIM for such production systems (Chapters 3 and 4).

In the first study (Chapter 2), the dataset was used to quantify the N utilisation and excretion of lactating dairy cows under low-input grazing conditions, because it was hypothesised that N utilisation in such dairy production systems would be lower and urinary N excretion greater than in high-input grazing systems. It was further stipulated that strategies to mitigate N losses to the environment established for high-input grazing conditions would not be transferable to low-input grazing systems. The second objective was, therefore, to explore the main drivers (i.e., grazing management factors) determining N use and excretion of dairy cows within these systems, ultimately, to identify the main factors which need to be included in LIVSIM.

Feed intake and related N intake are among the most decisive factors for determining animal performance (Smith et al., 2021), and urinary and faecal N excretion (Bougouin et al., 2022). Therefore, the second study (Chapter 3) aimed at identifying an adequate model to predict the intake of pasture herbage of dairy cows grazing semi-natural grassland for integration into the final adapted LIVSIM model. The differences in the biomass availability, botanical composition, and nutritional value of the grassland vegetation between semi-natural and improved grasslands potentially alters the response of the animal's ingestive behaviour to grazing conditions. It was, thus, hypothesised that existing models cannot adequately predict the intake of pasture herbage of dairy cows grazing semi-natural grasslands, due to the differences in grazing conditions between low- and high-input grazing-based systems.

It was further hypothesised that, given the demand for more readily available input variables and the differences in grazing and production conditions between low-input and high-input grazing systems, a model specifically adapted to low-input grazing-based systems would be required. Therefore, in the third study (Chapter 4), adaptations in the modules for intake, lactation, energy requirements, N excretion, and herd management were implemented to adapt LIVSIM to the investigated dairy production systems observed in the first study, This was followed by assessing the accuracy and sensitivity of predictions from the adapted LIVSIM and using scenarios to evaluate the model's capability to simulate the influence of different supplementation strategies varying in types of feedstuffs and inclusion level on animal performance and N excretion.

The present dissertation ceases with a general discussion of the N use and excretion of grazing cows in low-input grazing-based production systems and the ability of mathematical models to simulate and gauge strategies to mitigate the N losses from such grazing-based systems (Chapter 5), followed by final conclusions (Chapter 6).

1.6 References

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Chapter 2 | Nitrogen excretion and utilisation of dairy cows grazing temperate semi-natural grasslands [1]

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Abstract

Diets reliant on grazed, temperate herbage are prone to greater nitrogen (**N**) losses via urine than balanced stall-fed diets which poses a greater risk for N emissions. Measures for improving the N utilisation in grazing-based dairy cattle systems are predominantly investigated on homogenous clover-ryegrass pastures with high herbage yields and nutritional quality. In contrast, grazing-based systems reliant on less external inputs (e.g., synthetic fertilisers or concentrates) using semi-natural grassland as main feed source, such as in large parts of Central Europe, received less attention. The N utilisation and excretion of grazing cows in low-input dairy farms were, thus, investigated on nine commercial organic dairy farms in South Germany across one to four periods per farm. The dataset captured a diverse set of dairy production systems comprising 323 individual animal observations. A mean (± one SD) milk production, DM intake (**DMI**), and pasture DMI of 23.9 kg (± 5.35), 21.0 kg (± 3.21), and 11.3 kg/d (± 4.83), respectively, was determined. Feed intake was estimated using titanium dioxide and faecal CP concentration as markers of faecal excretion and diet digestibility, respectively. Milk N use efficiency (**MNE**; i.e., milk N secretion as share of N intake) averaged 24.7 g/100 g N intake (± 5.91), which is greater than observations in temperate, high-input grazing systems but lower than in cows receiving balanced diets in the barn. The MNE and another seven indicators of N utilisation and excretion displayed a wide range of values. The grazing management factors explaining this variation were, thus, identified via backward elimination. The supplementation strategy had the greatest potential for manipulating N utilisation and excretion of dairy cows. Increasing shares of fresh forages (i.e., meadow grass or clover-grass leys) as well as of hay in supplement DMI increased N utilisation (e.g., MNE) and decreased urinary N excretion (e.g., urinary N to creatinine ratio), while increasing shares of concentrates in supplement DMI are related to lower N losses via urine. At the same time, increases in total supplement DMI reduced N utilisation and increased urinary N excretion. Hence, full-time grazing combined with supplementation of fresh forage and hay in the barn is a viable option for low-input, grazing-based dairy operations with moderate levels of N losses.

2.1 Introduction

A major challenge in grazing-based dairy cattle feeding is the generally less efficient utilisation of dietary nitrogen (**N**) for milk production, leading to a greater risk for atmospheric and hydrospheric N emissions than with a balanced diet fed in the barn (Hoekstra et al., 2007; Huhtanen et al., 2015). Nitrogen utilisation for microbial and milk protein synthesis is predominately determined by N intake, rumen degradability of ingested CP, and dietary CP-to-energy ratio (e.g., Hoekstra et al., 2007; Li et al., 2013; Schuba et al., 2017). Grazed, temperate herbage contains high concentrations of readily degradable CP, but commonly has low concentrations of fermentable carbohydrates, which increases ammonium absorption from the rumen and thus urinary N excretion (Hoekstra et al., 2007). This high urinary N excretion increases the risk of nitrate leaching, in particular from urine spots on pastures, because available N exceeds plant nutrient requirements (Selbie et al., 2015). Faecal N, on the other hand, is present in a more stable form, decreasing the risk for environmental losses on pasture so that a greater faecal:urinary N ratio is desirable (Totty et al., 2013). The substantial spatial and seasonal heterogeneity in the availability and nutritional value of pasture herbage challenges the scope of compensating for the excessive rumen- degradable CP supply from pasture, for instance, by grazing management. It is, thus, necessary to identify grazing management factors, including paddock management factors (i.e., stocking density, herbage allowance and mass, timing of grazing, and nutritional quality and botanical composition of pasture herbage) and supple- mentation strategies, which effectively reduce urinary N excretion and increase N utilisation for microbial and milk protein synthesis.

The effects of such grazing management factors on N utilisation and urinary N excretion of grazing cows have been investigated mainly in high-input grazing systems using homogenous clover-ryegrass pastures with high biomass yields and nutritional quality of herbage (Hoekstra et al., 2007; Dodd et al., 2019). In contrast, grassland-dominated, low-input farming systems, including organic dairy farms, using semi-natural grassland as the main feed source, have received less attention in research so far (Akert et al., 2020). Semi-natural grasslands are multispecies, permanent grass- lands with no to little treatment with fertilisers, pesticides, or soil cultivation, and thus, at least in part, with lower yields and nutritional quality of herbage (Bruinenberg et al., 2003; Peeters et al., 2014). This type of grasslands can constitute the main feed resource in regions with low mountain ranges or in (pre-)alpine regions where less favourable soil and topographic conditions signify a high share of permanent grasslands. Moreover, in low-input, grassland-dominated farming systems, supplementation of grazing cows frequently consists of freshly cut meadow grass from permanent grasslands or of freshly harvested grass-clover mixtures originating from the farm’s cropland.

It was hypothesised that N utilisation in such dairy production systems is lower and urinary N excretion is greater than in high- input grazing or stall-fed systems, due to high herbage intakes and limited concentrate feeding, increasing the imbalance between ruminal N and fermentable carbohydrate supply (Akert et al., 2020; Correa-Luna et al., 2020). Further, the lower feed intake and performance level of dairy cows in such systems are typically related to a lower resource use efficiency – at least on the production level (e.g., conversion of ingested DM into milk) (Capper and Bauman, 2013). Yet, differences in N utilisation and excretion are also expected between different low-input grazing systems due to variation in grazing and supplementation intensity. For instance, milk N use efficiency (**MNE**) is likely lower in cows grazing full- time than in those that are grazing part-time and are supple- mented with more mature fresh forages with lower CP concentra- tions than pasture herbage (Akert et al., 2020).

Therefore, the present study aimed at (1) to quantifying the N excretion and utilisation of lactating cows grazing temperate, semi-natural grasslands in low-input dairy systems using eight N-related indicators, and (2) exploring the effect of various grazing management factors on N utilisation and excretion of cows within these systems. Preliminary results from the present study were previously published as abstracts (Perdana-Decker et al., 2023b, 2023c).

2.2 Materials and methods

2.2.1 Study farms and setup

The present study is based on a dataset collected on nine commercial, organically led dairy cattle farms located across five natural regions in Southwest Germany (i.e., Schwäbische Alb, Schwäbisches Keuper-Lias-Land, Donau-Iller-Lech-Platte, Voralpines Hügel- und Moorland, and Hochschwarzwald). Sampling was repeated in four sampling phases from May to October of 2019 and 2020. Phase 1 took place from May to July 2019, Phase 2 from August to October 2019, Phase 3 from May to July 2020, and Phase 4 from August to October 2020 (Table 2.1). Within each sampling phase, multiple farms were visited consecutively for examination periods of 11 d each. These examination periods consisted of 5 d of adaptation for feeding the external faecal marker titanium dioxide (**TiO_2**) followed by 6 d of sampling of faeces, milk, urine, standing pasture herbage on offer, and supplement feeds. Four farms were visited in every sampling phase, five farms only during the two phases of 2020, and one farm was only visited in Phase 4.

For animal-related data, 10–28 lactating dairy cows per farm and phase were selected for sampling depending on the herd size (Tables 2.1 and 2.2). In Phases 3 and 4, cows were, additionally, separated into two groups à 8–14 cows on three farms to investigate different supplementation strategies or differences between ani- mal breeds. Animals were chosen to obtain groups of similar mean days in milk (**DIM**) and mean parity per farm across all sampling phases. Hence, a total of n = 33 animal groups were sampled across both years.

Table 2.1 Details related to experimental farms and sampling across four sampling phases [1] on nine commercial, organic dairy cattle farms in South Germany using temperate semi-natural grasslands for grazing.

	General farm characteristics			Distribution of sampling periods		
Farm	Dairy herd size, n	Cow breed	Annual milk yield, kg/cow	Phase	Calendar week	Sampled cows, n
A	30	Simmental	5 698	1	26	19 *
				2	36	14 *
				3	23	18 *
				4	34	16 *
B	80	Holstein-Friesian	6 439	1	19	10
				2	34	9
				3	19	10
				4	32	10
C	40	Simmental	7 086	3	22	9
				4	38	9
D	100	Simmental	7 430	1	22	10
				2	33	10
				3	21	28 *
				4	37	23 *
E	70	Simmental, Brown Swiss	7 610	3	25	17 *
				4	35	17 *
F	40	Brown Swiss	5 224	1	22	9
				2	39	9
				3	26	10
				4	40	10
G	40	Brown Swiss, Holstein-Friesian	6 518	3	29	15
				4	37	14
H	40	Holstein-Friesian	8 566	3	23	9
				4	40	11
I	60	Holstein-Friesian	7 850	4	31	7

* Sampled cows further separated into two groups for investigating different supplementation treatments or differences between cow breeds.

[1] Phase 1: May to July 2019; Phase 2: August to October 2019; Phase 3: May to July 2020; Phase 4: August to October 2020.

Table 2.2. Performance and nutrient intake of lactating dairy cows grazing temperate semi-natural grasslands in South Germany, separated by dataset 1 and 2[1].

		Dataset 1						Dataset 2					
Item	Units	n	Mean	Median	SD	Min	Max	n	Mean	Median	SD	Min	Max
BW	kg	323	703	691	90.8	408	1 021	152	689	680	81.9	540	1 021
Parity	n	323	3.62	3.00	2.000	1.00	11.00	152	3.74	3.00	1.917	1.00	9.00
Milk yield	kg/d	323	23.9	23.8	5.35	9.7	39.8	152	23.2	23.0	5.53	9.7	37.2
Milk fat	g/100 g	323	3.93	3.86	0.544	2.37	6.04	152	3.97	3.92	0.529	3.02	5.50
Milk protein	g/100 g	323	3.26	3.23	0.305	2.54	4.29	152	3.26	3.23	0.335	2.54	4.29
DMI	kg/d	323	21.0	21.0	3.21	12.6	29.1	152	20.7	20.9	3.23	12.6	28.6
Pasture DMI	kg/d	323	11.3	10.7	4.83	0.6	23.3	152	10.5	9.8	4.58	0.6	23.2
N intake	g/d	323	506	504	105.8	294	811	152	514	515	111.1	296	811

Abbreviations: DMI = DM intake; Min = minimum; Max = maximum; N = nitrogen.

[1] Dataset 2 was a subsample of dataset 1 because it solely contained observations with urine spot samples.

Grazing management and study farms

Dairy cows grazed semi-natural, permanent grasslands with botanically diverse swards, fertilised solely with organic cow manure. The paddock management decisions (i.e., stocking density, daytime of pasture access, and choice of paddocks) recorded by the farm managers are summarised in Table 3. Grazing took place for 4–20 h/d during daytime (n = 169 cows), at night-time (n = 80 cows), or full-time (n = 74 cows). The 1–8 paddocks available to the lactating dairy cows per farm were either grazed continuously or rotated daily. Different combinations of concentrates (mainly cereals), fresh meadow grass, fresh clover-grass swards, grass hay, maize silage, and grass silage were supplemented in the barn. Daily supplementation of concentrate feeds ranged from 0 to 5.6 kg DM/- cow which translated to a concentrate inclusion of 0–244 g/kg milk. Total supplement feed (i.e., roughage plus concentrate feeds) ranged from 0 to 18.2 kg DM/cow.

Once per sampling period and farm, the standing pasture herbage on offer was sampled in three or six representative points per paddock, depending on paddock size. Per sampling point, the botanical composition of the vegetation was classified visually according to the share of grasses of the total fresh aboveground herbage biomass. Swards consisting of > 60% grasses were considered as grass-rich swards, whereas those with ≤ 45% grasses were classified as herb-rich. Swards with > 45% to ≤ 60% of grasses were considered balanced. Per sampling point, aboveground herbage biomass was manually harvested at 3 cm above the ground surface in a 1-m2-plot (0.5 m x 2.0 m), weighed fresh, and processed in the laboratory on the same day or the day after. Daily herbage biomass available to each cow (i.e., herbage allowance; kg DM/cow and d) was estimated by dividing herbage mass (kg DM/ha) by the stocking density on each paddock (n/ha). Mean herbage allowance across all grazed paddocks per farm and period was used for any further analysis.

Sampling and supplement feedstuffs

The individual amounts of concentrate intake via automatic feeders were recorded daily. To determine the amount of forage feedstuffs or partial mixed ration offered and refused by the entire herd of lactating cows, records of the scales built into the forage wagons were used, or the daily mass of offered and refused feed on the feeding banks was weighed manually. Samples of offered and refused supplement feed were collected (~200 g of fresh matter). Hay and concentrate samples were stored at room temperature, whereas samples of fresh forages and partial mixed rations were frozen immediately after collection (-20 °C).

Animal measurements

All animal procedures were conducted according to the guidelines of the German animal welfare act and were approved by the Institutional Animal Care and Use Committee of the University of Hohenheim. All animals were milked twice daily in the morning and afternoon or had access to an automatic milking system (n = 2 farms). They had free access to drinking water troughs, both in the free-stall barns and on pasture. Parity and DIM of individual animals at the time of sampling were retrieved from official milk reports by the regional association for milk inspection (LKV, Landesverband Baden-Württemberg für Leistungs- und Qualitätsprüfungen in der Tierzucht e.V.). Individual milk yield was measured and sampled once a day from Days 6–11, alternating between morning and afternoon. Milk samples (40 ml) were stored with 150 µL Bronysolv (ANA.LI.TIK Austria, Vienna, Austria) at 4 °C until analysis. The animal's BW was estimated once per period by measuring the heart girth with a tape calibrated for dual-purpose breeds (Animeter, Albert Kerbl GmbH, Buchbach, Germany).

The daily DM intake (**DMI**) of each cow per farm was estimated using a double-marker technique. For this, cows were orally dosed with 24–28 g/d of the external marker TiO_2 (Glindemann et al., 2009) for the entire examination period. After morning and evening milking on Days 6–11, faecal grab samples (~300 g fresh matter) were taken from the animals' rectum, frozen immediately after collection, and stored at -20 °C. On farms with two animal groups per period, faecal samples were taken only once daily, whereby sampling of each animal group alternated between morning and evening across the six sampling days.

Concomitant to faecal sampling, urine spot samples were collected on six farms from 3 to 10 cows per farm or animal group from Days 6–11 of every period. Urination was stimulated by gentle massage of the perineal area. Samples were collected in plastic buckets, homogenised, and strained to remove any solid impurities. Then, subsamples of ~200 ml were acidified with sulphuric acid (20% v/v) to reduce pH to < 3. Three aliquots of ~40 ml of the acidified urine samples were stored at -20 °C until analysis.

Laboratory analysis

Milk samples were analysed by the Milchprüfring Baden- Württemberg e.V. (Kirchheim/Teck, Germany) for their fat, protein, and lactose concentrations according to ASU L 01.00-78, 2018-06, as well as milk urea N (MUN) according to 05022100.QMD, 2011-03 (Milchprüfring Baden-Württemberg e.V., 2023). For statistical analyses, daily milk yield and composition were averaged per cow and period, whereby records were only considered if samples of one consecutive morning and evening milking were available.

After thawing, faecal samples were homogenised and pooled by cow and period using aliquots of ~15 g fresh matter of each daily sample. The pooled sample (~200 g of fresh matter) was weighed and then lyophilised for 72 h (LYO GT2 Basis, SRK Systemtechnik GmbH, Riedstadt, Germany). Similarly, urine samples were thawed, pooled by cow by taking 10 mL from each individual sample, and frozen until analysis. Samples of pasture herbage and offered as well as refused supplement feeds were dried at 45 °C for 72 h, except of samples including silages, which were lyophilised. After drying, all samples were weighed for DM determination, and ground through a 1-mm-sieve (Retsch SM 100, Retsch GmbH, Haan, Germany). Then, the entire samples of pasture herbage were pooled by paddock and period. Offered and refused feeds were each pooled by farm and period. For each pooled sample, a subsample of ~50 g dried sample material was taken for laboratory analyses.

All urine, faeces, and feed samples were analysed in duplicate using the official analytical methods in Germany (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, 2007). Samples of offered and refused supplement feeds, faeces, and herbage were analysed for DM and organic matter (**OM**) (method 3.1 and 8.1, respectively). Concentrations of N in samples of pasture herbage and of offered and refused supplement feed were determined by Dumas combustion (method 4.1.2) using a vario MAX CN analyser (Elementar Analysesysteme GmbH, Langenselbold, Germany). Offered feeds were also analysed for NDF (including residual ash) (method 6.5.1) by an Ankom200 Fiber Analyzer (Ankom Technology, Fairport, US) using sodium sulphite and heat-stable a-amylase. The Hohenheim Gas Test was used to estimate metabolisable energy (**ME**) concentrations of offered feedstuffs in triplicate in two independent runs following the procedures described by Menke and Steingass (1988). Specific equations were used for roughage (eq. 16e), concentrate (eq. 14b), and mixed feedstuffs (eq. 12f). Urine and faeces samples were analysed for N by Kjeldahl digestion (method 4.1.1; Vapotest 45 s, C. Gerhardt GmbH & Co. KG, Königswinter, Germany and behrostest K20L, Behr Labor-Technik GmbH, Düsseldorf, Germany). Faecal TiO_2 concentrations were determined according to Boguhn et al. (2009) with a modified digestion time of 4 h instead of 40 min to ensure maximum transparency of the digested solution. Concentrations of purine derivatives (**PD**; i.e. allantoin plus uric acid) and creatinine (**C**) in urine were analysed using high-performance reversed-phase liquid chromatography following the procedures by Balcells et al. (1992). The CP concentrations of feed and faecal samples were calculated by multiplying the N concentration by 6.25.

Calculation of intake and N excretion and utilisation parameters

More details on the determination of the DMI of supplements at the herd level and of the DMI of individual animals are described in Perdana-Decker et al. (2023a). In brief, the DMI of supplemental feed consumed in the barn (i.e., supplement DMI) was determined using the records of consumed concentrates by automatic feeding stations, plus the daily measurements of forage feedstuffs or partial mixed ration consumed by the entire herd of lactating cows divided by the number of animals per herd. Daily DMI was calculated from the apparent total tract digestibility of ingested OM based on faecal CP concentration (Lukas et al., 2005), daily faecal OM output as estimated from the daily TiO_2 dosage and its concentration in faeces (Glindemann et al., 2009), and from the approximated OM concentration of the total ration. Finally, the DMI of pasture herbage (i.e., pasture DMI) was calculated by subtracting supplement DMI from the total daily DMI of individual cows.

Individual N intake was calculated from the daily supplement DMI plus the animals' individual pasture DMI, each multiplied by the respective N concentrations. Milk N secretion was calculated from daily milk yield and milk N concentration. The latter was obtained by dividing the milk protein contents (g/kg milk) by 6.38 (Gesellschaft für Ernährungsphysiologie (GfE), 2023). Individual faecal N excretion was estimated using the faecal OM excretion by each cow multiplied by the respective N concentration in faecal OM. Urinary N excretion of individual cows was estimated as the difference between N intake and the sum of milk N secretion and faecal N excretion (all in g/d). Milk N secretion and faecal and urinary N excretions were calculated in absolute terms (g/d) and relative to the daily N intake of each animal (g/100 g N intake). In the following, milk N secretion in g/100 g N intake is referred to as MNE. The ratios between PD (mmol/L) and C (g/L) (i.e., PD:C ratio) and between PD and N (g/L) (i.e., PD:N ratio) in urine were considered as indicators for ruminal microbial CP synthesis and the efficiency of dietary N utilisation for ruminal microbial CP synthesis, respectively (Tas and Susenbeth, 2007). Finally, the N:C ratio in urine (both in g/L urine) was considered another indicator for daily urinary N excretion (Chizzotti et al., 2008).

2.2.2 Statistical analysis

The present study comprised a total of 325 individual animal observations. Two different datasets were used to analyse the relationship between 16 grazing management factors (Table 2.3) and the eight indicators of N utilisation and excretion (Table 2.4). Dataset 1, comprising all 325 animal-individual observations, was used to analyse three indicators of N utilisation, including milk N secretion, MNE, and MUN concentration, and two indicators of N excretion (relative faecal and urinary N excretions). Dataset 2 only comprised records of cows from which urine samples were collected (n = 152). Hence, this dataset was used to analyse two indicators of N utilisation (ratios of PD:C and PD:N in urine) and one indicator for urinary N excretion (N:C ratio). Both datasets were checked for outliers via their interquartile range, prior to statistical analyses. Two outliers were detected and excluded from dataset 1, because one observation was below and another observation above 1.5 times the interquartile range. For visual inspection of the correlation between grazing management and N-related indicators, a correlation matrix was plotted for each dataset using the R package *corrplot*.

A variable selection analysis was conducted to identify which grazing management factors were relevant for explaining the variation in N-related indicators. For each N-related indicator separately, backward elimination using a linear mixed-effects model was applied. The N-related indicator served as dependent variable, while the farm, sampling phase nested in farm, and cow nested in farm served as random effects. The random cow effect accounted for the instances when repeated measurements were taken of the same cow over several sampling phases. In total, 16 factors characterising supplementation strategy and paddock management were considered as candidate variables for each backward elimination analysis (Table 2.3), where the initial model was fitted using the R package *lme4*. Irrelevant grazing management variables were removed step-by-step based on P-values with a selection level of a = 0.1, using the R function step by the R package *lmerTest* (Kuznetsova et al., 2017).

After variable selection via backward elimination, the resulting reduced models were used to identify potential interaction effects. For this, seven predetermined interaction terms were added separately to the selected models. Per N-related indicator, the model with the lowest Akaike Information Criterium among the seven models containing interaction terms was reported, given that the addition of the interaction decreased the Akaike Information Criterium by > 5. To determine the proportion of the variation explained by the selected fixed effects and random effects, the final models' conditional and marginal R^2 were calculated using the *r.squaredGLMM* function by the R package *MuMIn* (Bartón, 2022). The conditional and marginal R^2 represent the variation explained by the total model and fixed effects, respectively. The difference between conditional and marginal R^2 indicates the share of variation explained by the random effects.

Table 2.3. Descriptive statistics of various grazing management factors determined for grazing-based dairy cattle production systems using temperate semi-natural grasslands in South Germany, separated by datasets 1 and 2[1].

		Dataset 1						Dataset 2					
Grazing management factors	Unit	n	Mean	Median	SD	Min	Max	n	Mean	Median	SD	Min	Max
DIM	d	323	146	145	65.0	12	412	152	151	158	61.3	27	288
Supplementation strategy													
Supplement DMI	kg/d	33	9.68	10.53	4.881	0.51	18.18	26	10.2	10.6	4.96	0.5	18.2
Fresh forage	g/100 g supplement DMI	33	29.3	0.0	34.09	0.0	100.0	26	36.2	37.4	36.20	0.0	100.0
Grass hay	g/100 g supplement DMI	33	17.0	5.1	22.10	0.0	100.0	26	11.8	2.3	16.48	0.0	57.8
Silage[2]	g/100 g supplement DMI	33	23.9	0.0	33.51	0.0	100.0	26	25.5	0.0	35.48	0.0	100.0
Concentrates[3]	g/100 g supplement DMI	33	23.9	28.3	22.39	0.0	100.0	26	14.1	4.5	17.39	0.0	59.1
CP of supplements	g/100 g DM	33	14.2	13.1	4.37	8.7	28.5	26	15.7	14.2	5.37	8.7	28.5
NDF of supplements	g/100 g DM	33	37.8	38.0	8.54	17.2	53.5	26	38.8	38.3	9.11	20.0	53.5
ME of supplements	MJ/kg DM	33	10.1	10.1	0.89	7.3	12.2	26	9.93	10.04	0.999	7.35	11.64
Paddock management													
CP of pasture	g/100 g DM	33	16.7	16.7	2.93	11.0	22.7	26	17.0	16.7	3.36	11.0	22.7
NDF of pasture	g/100 g DM	33	45.2	44.6	3.75	38.0	52.0	26	45.6	46.0	4.32	38.0	52.0
ME of pasture	MJ/kg DM	33	9.32	9.23	0.624	8.11	10.37	26	9.27	9.23	0.634	8.11	10.26
Stocking density	cows/ha	33	17.2	13.2	10.83	5.0	43.7	26	21.4	20.0	12.28	5.0	43.7
Paddock size	ha	33	3.76	3.47	2.204	0.92	8.17	26	3.33	3.11	2.254	0.92	8.17
Herbage allowance	kg DM/cow per day	33	29.3	16.1	24.89	6.7	94.8	26	22.5	14.2	20.88	6.7	73.2
Herbage mass	kg DM/ha	33	380	315	283.7	84	1 558	26	340	292	210.0	108	1 113
Grazing time	h/d	33	10.7	10.0	5.33	3.5	20.0	26	10.1	8.0	4.82	3.5	20.0
Botanical composition*													
Grass-rich		118	--	--	--	--	--	59	--	--	--	--	--
Balanced		107	--	--	--	--	--	42	--	--	--	--	--
Herb-rich		98	--	--	--	--	--	51	--	--	--	--	--
Daytime of pasture access*													
Daytime		169	--	--	--	--	--	91	--	--	--	--	--
Night-time		80	--	--	--	--	--	35	--	--	--	--	--
Full-time		74	--	--	--	--	--	26	--	--	--	--	--

Abbreviations: DIM = days in milk; DMI = DM intake; ME = metabolisable energy.

[1] Dataset 2 was a subsample of dataset 1, solely containing records of cows from which urine spot samples were collected.

[2] Silage: grass or maize silage.

[3] Concentrates: different combinations of oat, barley, maize, wheat bran, wheat, triticale, and partly protein concentrates such as peas.

*Categorial factors.

Table 2.4. Descriptive statistics of indicators of nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany, separated by datasets 1 and 2[1].

		Dataset 1						Dataset 2					
N-related indicators	Unit	n	Mean	Median	SD	Min	Max	n	Mean	Median	SD	Min	Max
N utilisation indicators													
Milk N secretion	g/d	323	121	121	24.5	58	190	152	117	116	24.3	58	176
MNE	g/100 g N intake	323	24.7	24.5	5.91	11.4	39.5	152	23.6	22.4	6.02	11.4	39.2
MUN	mg/dL	323	10.1	9.2	4.27	3.3	27.0	152	10.7	9.9	4.57	4.0	27.0
PD:C	mmol/g	--	--	--	--	--	--	152	28.2	27.3	5.41	19.7	47.8
PD:N	mmol/g	--	--	--	--	--	--	152	3.01	2.86	1.069	1.21	7.56
N excretion indicators													
Faecal N excretion	g/d	323	131	130	23.4	67	184	152	124	123	21.7	67	172
Urinary N excretion	g/d	323	254	248	93.1	79	529	152	273	277	99.5	80	529
Total N excretion	g/d	323	385	381	101.0	182	697	152	397	393	106.9	186	697
Faecal N excretion	g/100 g N intake	323	26.5	26.3	4.96	15.2	37.2	152	24.9	23.7	4.97	15.2	35.2
Urinary N excretion	g/100 g N intake	323	48.9	47.8	9.77	25.6	71.3	152	51.6	52.3	10.07	25.6	71.3
N:C	g/g	--	--	--	--	--	--	152	10.6	9.9	4.42	2.7	25.7

Abbreviations: MNE = milk N use efficiency; MUN = milk urea N; N:C = ratio between urinary N and urinary creatinine concentrations; PD:C = ratio between urinary purine derivates and urinary creatinine concentrations; PD:N = ratio between urinary purine derivatives and urinary N concentrations.

[1] Dataset 2 was a subsample of dataset 1, solely containing records of cows from which urine spot samples were collected.

Further, to evaluate the model selection stability of the above- mentioned backward elimination analysis, a bootstrap approach was applied (Heinze et al., 2018). Per dependent factor, the backward elimination process was repeated on 1 000 bootstrap resamples drawn using the function bootstrap by the R package *sjstats* (Lüdecke, 2018). Consequently, the model inclusion frequency for each potential fixed effect, and the frequency of positive and negative signs of the resulting regression coefficients were calculated using the R package *bootStepAIC* (Rizopoulos, 2022) with slight adaptations to the linear mixed-effects models. All statistical analyses were conducted using R Version 4.2.0 (R Core Team, 2022).

2.3 Results

2.3.1 Nitrogen utilisation of lactating dairy cows grazing on semi-natural grassland

The two datasets captured a diverse set of organic dairy production systems using multispecies, permanent grassland for grazing, comprising 323 animal-individual observations (Tables 2.2 and 2.3). This diversity in grazing systems resulted in a wide range of N intake (294–811 g/d), total N excretion (182–697 g/d), and MNE values (11–39 g/100 g N intake) (Table 4). On average (± SD), the ingested N was predominantly excreted via urine (48.9 g/100 g N intake ± 9.77), and to a smaller extend via faeces (26.4 g/100 g N intake ± 4.96). In contrast, MNE averaged 24.7 g/100 g N intake (± 5.91) across all farms, years, and periods. Dataset 2 was a representative subsample of dataset 1 with a similar mean milk production, pasture DMI, and MNE of 23.2 kg/d (± 5.53), 10.5 kg/d (± 4.58), and 23.6 g/100 g N intake (± 6.02), respectively, compared to the values of dataset 1 (23.9 kg/d ± 5.35, 11.3 kg/d ± 4.83, and 24.7 g/100 g N intake ± 5.91).

Daily milk yield correlated moderately with the animals' DIM (r = -0.55), their MNE (r = 0.56), and relative urinary N excretion (g/100 g N intake; r = -0.41), as well as with several grazing management factors such as stocking density (r = -0.48) or share of fresh forage in supplement DMI (r = -0.37; Fig. 2.1). The pasture DMI correlated strongly with supplement DMI (r = -0.78) and daily grazing time (r = 0.62), and moderately with the CP concentration of pasture herbage (r = -0.37). Only a marginal correlation between pasture DMI and paddock management factors was found, such as with daily herbage allowance on pasture (r = 0.14) or stocking density (r = -0.12). Furthermore, stocking density correlated with the shares of concentrate (r = -0.66) and fresh forage (r = 0.69) of supplement DMI. However, no correlation was found between the stocking density and the supplement DMI (r = -0.09). There was a strong correlation between MUN concentration and the urinary N:C (r = 0.87) or PD:N ratios (r = -0.81), and between the urinary N excretion as a share of N intake and the N:C ratio (r = 0.62; Fig. 2.2).

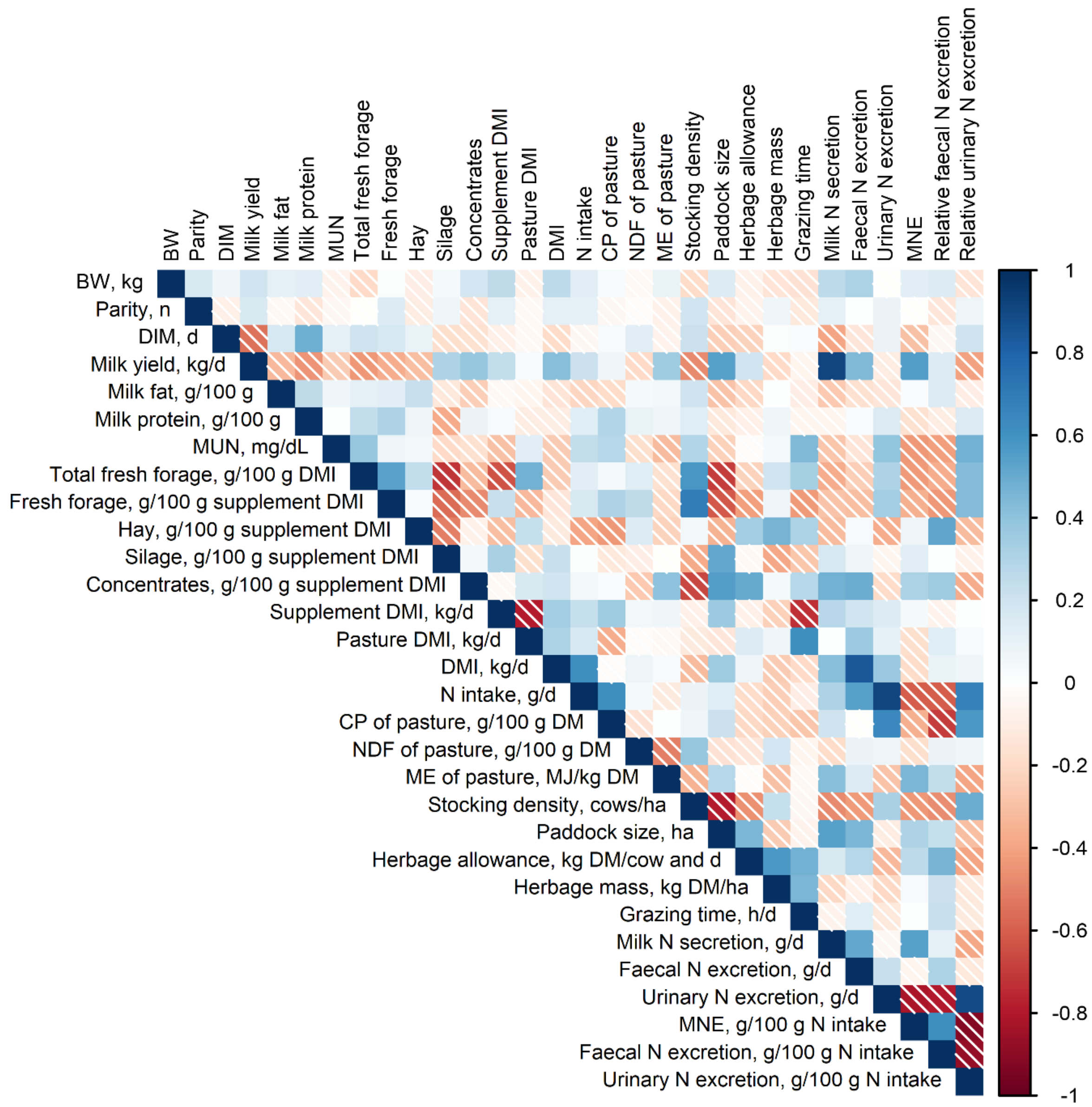

Fig. 2.1. Correlation matrix for dataset 1 (n = 323) with various variables related to milk production, grazing management, and nitrogen (N) utilisation and excretion of lactating dairy cows grazing temperate semi-natural grasslands in South Germany (DIM: days in milk; DMI: DM intake; ME: metabolisable energy; MNE: milk N use efficiency; MUN: milk urea N).

2.3.2 Results of bootstrapping procedures

The animals' DIM was selected in 100% of the bootstrapping resamples to predict milk N secretion, MNE, and relative urinary N excretion, and their regression coefficients indicated an increase in N utilisation and a decrease in urinary N excretion with decreasing DIM (Tables 2.5 and 6). For all other N-related indicators, DIM was selected with a low frequency (<60%). The supplement DMI and shares of different supplement feedstuffs were frequently (>80%) selected for the majority of N-related indicators, except the share of concentrates in supplement DMI. Increasing daily supplement DMI reduced MNE and increased relative urinary N excretion, MUN concentration, and urinary N:C ratio. The share of fresh forage or hay of supplement DMI was frequently included for multiple N-related indicators (i.e., MNE, relative urinary N excretion, N:C

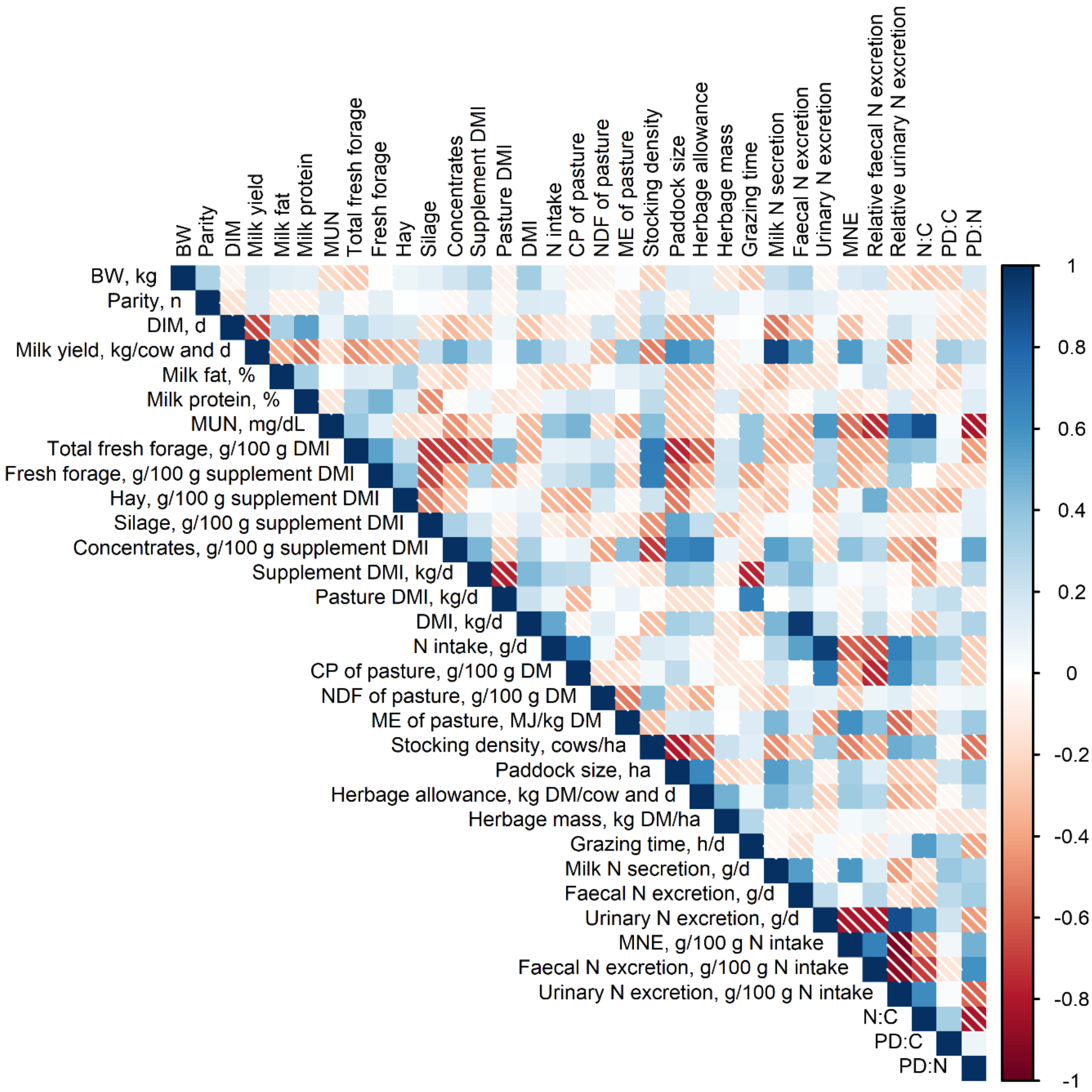

Fig. 2.2. Correlation matrix for dataset 2 (n = 152; i.e., subsample of dataset 1 solely containing records of cows from which urine spot samples were collected) with various variables related to milk production, grazing management, and nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany (DIM = days in milk; DMI = DM intake; ME = metabolisable energy; MNE = milk N use efficiency; MUN = milk urea N; N:C = ratio between urinary N and urinary creatinine concentrations; PD:C = ratio between urinary purine derivates and urinary creatinine concentrations; PD:N = ratio between urinary purine derivatives and urinary N concentrations).

ratio, and MUN). Their respective coefficient signs indicated an increase in N utilisation and a decrease in urinary N excretion with increasing shares of forage and hay in supplement DMI. The MUN concentration, however, increased with increasing shares of fresh forage or hay in supplement DMI, indicating a negative relation between N utilisation and forage and hay supplementation. The share of concentrates in supplement DMI was solely selected with a high frequency (>80%) for the N:C ratio, which decreased with increasing concentrate proportion in supplement DMI.

The ME concentration was included with a selection frequency >85% for all N-related indicators except the PD:C ratio. Greater ME concentrations in pasture herbage increased milk N secretion, MNE, relative faecal N excretion, and the PD:N ratio, and reduced the relative urinary N excretion, MUN concentration, and N:C ratio. The NDF concentration of pasture herbage was positively related to faecal N excretion as share of N intake and PD:N ratio, and negatively related to relative urinary N excretion and MUN concentration. The CP concentration of pasture herbage was selected with a frequency >80% for MNE, relative faecal N excretion, MUN concentration, and the N:C ratio. The respective coefficient signs indicated a decrease in N utilisation and an increase in urinary N excretion with increasing CP concentration of pasture herbage.

None of the paddock management factors had a consistent effect across multiple N-related indicators. Stocking density, day- time of pasture access, and daily grazing time were chosen for several indicators with a frequency >80%, but the distribution of their regression coefficient signs was inconsistent or sometimes even contradictory. Regarding the daytime of pasture access, for instance, full-time and night-time grazing were related to a lower urinary PD:N ratio (i.e., lower efficiency of dietary N use for microbial CP synthesis in the rumen) than daytime grazing. In contrast, MNE was greater and MUN concentration lower when animals grazed full-time than only during daytime.

Table 2.5. Inclusion frequencies and frequencies of positive and negative coefficient signs of selected mixed regression models using backward elimination in M = 1 000 bootstrap resamples to identify significant predictor variables for indicators of nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany, using dataset 1 (n = 323).

		Milk N secretion, g/d			MNE, g/100 g N intake			Faecal N excretion, g/100g N intake			Urinary N excretion, g/100g N intake			MUN, mg/dL		
			Sign, %			Sign, %			Sign, %			Sign, %			Sign, %	
Predictor variable	Unit	Selection, %	+	-	Selection, %	+	-	Selection, %	+	-	Selection, %	+	-	Selection, %	+	-
DIM	d	100	0	100	100	0	100	14	38	62	100	100	0	30	22	78/
Supplement DMI	kg/d	95	100	0	100	0	100	22	38	62	93	98	2	93	99	1
Total fresh forage	g/100 g DMI	81	100	0	100	0	100	100	0	100	100	100	0	36	16	84
Fresh forage	g/100 g supplement DMI	77	1	99	100	100	0	50	92	8	100	0	100	91	99	1
Hay	g/100 g supplement DMI	59	3	97	91	100	0	69	48	52	88	0	100	93	99	1
Concentrates	g/100 g supplement DMI	55	93	7	66	1	99	45	56	44	57	99	1	79	95	5
CP of pasture	g/100 g DM	50	84	16	84	0	100	88	0	100	66	99	1	99	100	0
NDF of pasture	g/100 g DM	75	98	2	53	91	9	91	100	0	92	0	100	95	0	100
ME of pasture	MJ/kg DM	92	100	0	100	100	0	85	100	0	100	0	100	100	0	100
Botanical class (basis: Balanced)		62			52			71			85			92		
Grass-rich			65	35		87	13		41	59		0	100		71	29
Herb-rich			89	11		28	72		0	100		96	4		0	100
Stocking density	n/ha	57	2	98	53	24	76	48	15	85	94	100	0	92	3	97
Paddock size	ha	44	55	45	64	88	12	17	1	99	42	85	15	84	2	98
Herbage allowance	kg DM/cow and d	75	0	100	73	1	99	59	100	0	49	100	0	68	3	97
Herbage mass	kg DM/ha	53	91	9	27	82	18	43	0	100	5	34	66	55	62	38
Daytime of grazing (basis: Daytime)		71			100			63			100			93		
Full-time			96	4		94	6		75	25		69	31		1	99
Night-time			84	16		6	94		2	98		100	0		1	99
Grazing duration	h/d	52	32	68	41	12	88	37	92	8	69	0	100	99	100	0

Abbreviations: DIM = days in milk; DMI = DM intake; ME = metabolisable energy; MNE = milk N use efficiency; MUN = milk urea nitrogen.

Table 2.6. Inclusion frequencies and frequencies of positive and negative coefficient signs of selected mixed regression models using backward elimination in M = 1 000 bootstrap resamples to identify significant predictor variables for indicators of nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany, using dataset 2, which included indicators based on urine spot sampling (n = 152).

		N:C, g/g			PD:C, mmol/g			PD:N,mmol/g		
			Sign, %			Sign, %			Sign, %	
Predictor variable	Unit	Selection, %	+	-	Selection, %	+	-	Selection, %	+	-
DIM	d	41	43	57	54	21	79	60	35	65
Supplement DMI	kg/d	93	96	4	49	51	49	57	33	67
Total fresh forage	g/100 g DMI	47	25	75	48	71	29	63	58	42
Fresh forage	g/100 g supplement DM	53	18	82	56	16	84	56	37	63
Grass hay	g/100 g supplement DM	39	13	87	58	8	92	56	28	72
Concentrates	g/100 g supplement DM	86	1	99	54	93	7	37	89	11
CP of pasture	g/100 g DM	92	100	0	63	9	91	38	31	69
NDF of pasture	g/100 g DM	75	6	94	64	40	60	88	97	3
ME of pasture	MJ/kg DM	89	1	99	50	89	11	81	97	3
Botanical class (Basis: Balanced)		83			99			62		
Grass-rich			45	55		92	8		70	30
Herb-rich			9	91		99	1		71	29
Stocking density	n/ha	51	28	72	86	98	2	77	30	70
Paddock size	ha	53	25	75	78	90	10	73	35	65
Herbage allowance	kg DM/cow and d	77	4	96	62	9	91	63	66	34
Herbage mass	kg DM/ha	68	85	15	48	69	31	66	22	78
Daytime of grazing (Basis: Daytime)		65			98			91		
Full-time			23	77		100	0		39	61
Night-time			30	70		98	2		23	77
Grazing duration	h/d	89	98	2	85	1	99	50	34	66

Abbreviations: DIM = days in milk; DMI = DM intake; ME = metabolisable energy; N:C = ratio between urinary N to urinary creatinine concentrations; PD:C = ratio between urinary purine derivates to urinary creatinine concentrations; PD:N = ratio between urinary purine derivatives to urinary N concentrations.

2.3.3 Results of backward elimination

The range of the conditional R^2 across all final models selected by backward elimination (0.74–0.99, Table 2.7) shows that the predictor variables selected prior to statistical analyses explained most of the variation in the outcome parameters. The random effects explained 5–26% of the variation in the indicators of N utilisation and excretion. Selected fixed effects accounted for the greatest share of variation explained by the selected models with a marginal R^2 ranging from 0.40–0.89.

Table 2.7. Partitioning of the R^2 [1] for the linear mixed models determined via backward elimination to identify significant predictor variables for indicators of nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany.

Partitioning of R^2	Milk N secretion	MNE	Faecal N excretion	Urinary N excretion	MUN	N:C	PD:C	PD:N
Marginal R^2	0.58	0.67	0.89	0.85	0.74	0.89	0.53	0.82
Conditional R^2	0.77	0.74	0.99	0.90	0.95	0.93	0.76	0.97
Random R^2	0.19	0.07	0.09	0.05	0.21	0.04	0.23	0.15

Abbreviations: MNE = milk N use efficiency; MUN = milk urea N; N:C = ratio between urinary N to urinary creatinine concentrations; PD:C = ratio between urinary purine derivates to urinary creatinine concentrations; PD:N = ratio between urinary purine derivatives to urinary N concentration.

[1] R^2: partitioned into conditional R^2 i.e., variation explained by total statistical model, marginal R^2 i.e., variation explained by fixed effects, and random R^2 i.e., variation explained by random effects.

Variable selection by the backward elimination, and the signs of their regression coefficients matched the results of the bootstrapping procedure for the majority of N-related indicators (Tables 2.8 and 2.9). Regression coefficients of the models selected by backward elimination indicated a strong effect of full-time grazing compared to daytime grazing on milk N secretion, MUN concentration, and PD:C ratio. Full-time grazing reduced MUN concentration by 31 mg/dL and N:C ratio by 18 g/g, and increased PD:C ratio by 29 mmol/g compared to daytime grazing, indicating greater N utilisation when cows grazed full-time. Likewise, the urinary PD:N ratio was greater for full-time than daytime grazing, which contradicted the negative effect of full-time grazing on the urinary PD:N ratio determined by the bootstrapping procedure.

One interaction term was added to each of the mixed models for milk N secretion, relative urinary N excretion, N:C ratio, and PD:N ratio, based on the rule that its addition decreased the models' Akaike Information Criterium by >5, indicating a better fit for the model. The added interaction terms increased the marginal R^2 for the N:C and PD:N ratios by 0.07 and 0.25, respectively.

Table 2.8. Results of backward elimination with linear mixed models to identify significant predictor variables for indicators of nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany, using dataset 1 (n = 323).

		Regression coefficients and significances[1]									
Predictor variables	Units	Milk N secretion, g/d		MNE, g/100 g N intake		Faecal N excretion, g/100 g N intake		Urinary N excretion, g/100 g N intake		MUN, mg/dL	
Intercept		-49.86		18.75		14.38		71.32		4.23	
DIM	d	-0.10	***	-0.02	***	--		0.02	***	--	
Supplement DMI	kg/d	3.23	***	-1.65	***	--		1.78	***	0.61	***
Total fresh forage	g/100 g DMI	0.52	**	-0.49	***	-0.11	***	0.62	***	--	
Fresh forage	g/100 g supplement DMI	-0.17	NS	0.22	***	0.05	***	-0.28	***	0.06	***
Grass hay	g/100 g supplement DMI	-0.12	NS	0.04	***	0.04	***	-0.05	*	0.06	***
Concentrates	g/100 g supplement DMI	0.93	**	-0.06	***	0.03	**	-4.52	**	0.03	***
CP of pasture	g/100 g DM	0.19	NS	--		-0.94	****	0.90	***	1.00	***
NDF of pasture	g/100 g DM	1.04	*	0.29	***	0.30	*	-0.60	***	-0.21	***
ME of pasture	MJ/kg DM	6.64	*	4.59	***	1.96	*	-7.45	***	-2.11	***
Botanical class (Basis: Balanced)		--			***	--			*		***
Grass-rich				1.61				-2.91		0.39	
Herb-rich				-0.50				0.61		-3.38	
Stocking density	n/ha	-0.61	.	-01.7	***	-0.14	**	0.31	***	-0.56	***
Paddock size	ha	--		--		---		--		-1.09	***
Herbage allowance	kg DM/cow and d	-0.23	**	--		--		--		-0.03	**
Herbage mass	kg DM/ha	--		--		--		--		--	
Daytime of grazing (Basis: daytime)			NS		***	--			*		***
Full-time		13.47		2.39				-4.25		-30.52	
Night-time		-1.49		-2.23				1.66		-5.89	
Grazing duration	h/d	1.54	NS	--		--		--		3.05	***
Grazing duration * concentrates	h/d * g/100 g supplement DMI	-0.04	**	--		--		--		--	
Concentrates * hay	g/100 g supplement DMI * g/100 g supplement DMI	--		--		--		-0.003	**	--	

Abbreviations: DIM = days in milk; DMI = DM intake; ME = metabolisable energy; MNE = milk N use efficiency; MUN = milk urea N.

[1] Significance codes: '***': P-value ≤ 0.001, '**': P-value ≤ 0.01, '*': P-value ≤ 0.05, NS: not significant.

Table 2.9. Results of backward elimination with linear mixed models to identify significant predictor variables for indicators of nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany, using dataset 2, which included indicators based on urine spot sampling (n = 152).

		Regression coefficients and significances[1]					
Predictor variable	Unit	N:C, g/g		PD:C, mmol/g		PD:N, mmol/g	
Intercept		30.06		-15.43		-5.81	
DIM	d	--		-0.01	*	0.00	**
Supplement DMI	kg/d	1.02	***	0.74	**	-0.12	***
Total fresh forage	g/100 g DMI	--		0.18	**	--	
Fresh forage	g/100 g supplement DMI	-0.03	*	-0.15	***	0.00	*
Grass hay	g/100 g supplement DMI	--		-0.10	***	0.02	***
Concentrates	g/100 g supplement DMI	-0.14	***	--		0.04	***
CP of pasture	g/100 g DM	0.27	**	--		--	
NDF of pasture	g/100 g DM	-0.44	***	0.32	***	0.11	***
ME of pasture	MJ/kg DM	-2.93	***	2.22	***	0.75	***
Botanical class (Basis: Balanced)			***		***		***
Grass-rich		-1.06		3.44		0.54	
Herb-rich		-3.55		5.95		0.65	
Stocking density	n/ha	-0.14	*	0.29	**	--	
Paddock size	ha	--		--		--	
Herbage allowance	kg DM/cow and d	-0.17	***	--		--	
Herbage mass	kg DM/ha	0.01	***	--		--	
Daytime of grazing (Basis: Daytime)			***		***		***
Full-time		-18.14		29.14		2.37	
Night-time		-5.31		7.47		0.44	
Grazing duration	h/d	2.55	***	-1.97	***	-0.31	***
Concentrates * fresh forage	g/100 g supplement DMI * g/100 g supplement DMI	-0.03	***	--			
Concentrates * hay	g/100 g supplement DMI * g/100 g supplement DMI	--		--		0.00	***

Abbreviations: DIM = days in milk; DMI = DM intake; ME = metabolisable energy; N:C = ratio between urinary N to urinary creatinine concentrations; PD:C = ratio between urinary purine derivates to urinary creatinine concentrations; PD:N = ratio between urinary purine derivatives to urinary N concentrations.

[1] Significance codes: '***': P-value ≤ 0.001, '**': P-value ≤ 0.01, '*': P-value ≤ 0.05, NS: not significant.

2.4 Discussion

2.4.1 Limitations of dataset

The present study evaluated the N utilisation and excretion of dairy cows grazing semi-natural grasslands Southwest Germany under commercial on-farm conditions. This study region was chosen, because it was representative for regions where permanent, multispecies grasslands constitute the main feed resource which is often the case in semi-mountainous regions in Central Europe (Barrachina et al., 2015). Although only a few farms were included in the present study, they belong to the main types of dairy farms in the study region as determined by Velasco et al. (2021): six farms were grassland-based dairy farms with a great share of their agricultural land being grassland (> 61.9%) with relatively low (n = 3) or high annual precipitation (n = 3), three of the study farms represented mixed farming systems with a considerable proportion of their land used for cropping (> 32.8%) with variable annual precipitation, and one farm was characteristic of the larger mixed farming systems in the region with a greater grassland and crop- ping area than the other farm types.

Correlations between several grazing management factors were detected in the present study, such as between the stocking density and the share of concentrates of supplement DMI, which were not necessarily of a causal nature. These correlations should be recognised when interpreting the present study's findings, despite their non-causality as they could not be prevented in our on-farm approach. Nevertheless, compared to controlled experiments on research stations testing one or few treatments, such on-farm research allows for analyses of multiple interactions between diverse factors, and thus an understanding of the true effects of grazing management across the diverse production conditions in low-input dairy farms. This approach was, therefore, deliberately chosen to investigate the practical implications of adaptations in grazing management on N utilisation and excretion in low-input, grazing-based dairy farms.

Urinary N excretion was estimated as the difference between the daily N intake and the sum of the daily milk N secretion and faecal N excretion of cows, because urine volume could not be measured. These estimates may be subject to measurement errors in determining N intake and faecal N excretion. The N intake, for instance, was calculated from supplement and pasture DMI and the respective N concentrations of these feedstuffs. The supplement DMI was determined on herd level, which did not capture the variation in N intake from supplement feedstuffs between individual cows. A bias in N intake from pasture herbage is also possible, because pasture herbage samples could not fully reflect the nutritive value of ingested pasture herbage due to the cows' selective grazing behaviour (Schneider et al., 2011). The N:C ratio in urine spot samples, however, served as an independent, measured indicator for daily urinary N excretion and correlated moderately (r = 0.62) with estimated urinary N excretion as proportion of daily N intake. Moreover, the ratios of N:C and PD:N in urine correlated strongly with MUN concentrations (r = 0.87 and -0.81, respectively), and the latter is considered a reliable indicator for MNE and urinary N excretion in dairy cows (Spek et al., 2013; Huhtanen et al., 2015). The correlations between the different kinds of independently measured N-related indicators, thus, illustrate the robustness and suitability of the chosen N-related indicators to reflect intra-animal variation in N utilisation and excretion of dairy cows.

2.4.2 Nitrogen utilisation and excretion of lactating dairy cows grazing temperate, semi-natural grasslands

It was hypothesised that the limited supplementation with energy-rich concentrates coupled with the supplementation of freshly cut meadow grass or grass-clover mixtures would increase the imbalance between the supply of dietary CP and energy, and thus, reduce N utilisation and increase N excretion in the investigated grazing systems when compared to high-input grazing systems. This hypothesis, however, was not confirmed. Mean MNE (± one SD) of dairy cows averaged 24.7 g/100 g N intake (± 5.91) in the present study. This MNE is similar to or even greater than the reported MNE for grazing dairy cows in North Germany (Löw et al., 2020: 22–25 g/100 g N intake) or for Irish dairy cows grazing high-quality pastures (Doran et al., 2022: 12–25 g/100 g N intake). Urinary N excretion of cows (48.9 g/100 g N intake; ± 9.77) was lower than reported for dairy cattle grazing high-quality ryegrass-clover pastures in Ireland (Doran et al., 2022: 56.8–57.8 g/100 g N intake) or New Zealand (Totty et al., 2013: 64.3–74.0 g/ 100 g N intake), also indicating lower urinary N losses in the low- input grazing systems of the present study. In temperate high- input grazing systems, dairy cows use high-quality pastures with CP concentrations of up to 30 g/100 g DM that provide an excess of rumen-degradable CP and metabolisable protein relative to the requirements of rumen microbes and their host animals (Waghorn and Clark, 2004), which largely explains the low N utilisation in such grazing systems (Totty et al., 2013). In contrast, cows in the present study grazed multispecies, permanent grass-lands with a lower CP concentration of herbage across the entire grazing season (16.7 g/100 g DM ± 2.93). Hence, the lower N intake in the present study (506 g/d ± 105.8) compared to that of grazing cows in New Zealand (Totty et al., 2013: 551–610 g/d) or Ireland (Doran et al., 2022: 546 and 568 g/d) to some degree explains the greater N utilisation and lower urinary N excretion observed here than reported for high-input grazing systems (Castillo et al., 2000).

However, MNE was lower than that measured in solely stall-fed dairy cattle determined in meta-analyses conducted by Spek et al. (2013) for Northwest European dairy farms (27 g/100 g N intake) and by Schuba et al. (2017) using a global dataset (29 g/100 g N intake). Nevertheless, the lower mean MUN concentration in the present dataset (10.1 mg/dL ± 4.23) compared to values determined by Spek et al. (2013: 12.5 mg/dL ± 5.07) also indicate that relative urea N losses may not be substantially greater in low- input grazing systems than in stall-based feeding systems.

The present dataset further showed a large range in the N-related indicators despite the strong focus on organic dairy farms located in South Germany using temperate semi-natural grasslands for grazing. For instance, MUN concentrations averaged per farm, period, and animal group ranged between 5.0 and 22.5 mg/dL. The lower observed MNE and greater urinary N excretion compared to observations from stall-fed dairy cattle systems and the large range in N-related indicators highlight that, on the one hand, there were periods within farms with a substantially greater risk for N losses through leaching and volatilisation on pastures than under confinement conditions. On the other hand, among the investigated grazing strategies, there were some which achieved levels of N excretion and MNE comparable with those of stall-fed feeding-systems. The management factors explaining these differences were, thus, analysed via a bootstrapping procedure and backward elimination.

2.4.3 Effect of performance level

The MNE and urinary N excretion were calculated from daily milk N secretion. Hence, milk yield was not considered in the boot- strapping analysis and backward elimination for any of the eight indicators of N utilisation and excretion to avoid artifactual correlations. However, there is a close link between nutrient use efficiency and performance level, as seen in the moderate correlation between milk yield and MNE (r = 0.56). Therefore, DIM was included to correct the effect of milk yield on the indica- tors of N utilisation and excretions. As expected, DIM was selected with high frequency for all indicators related to milk yield (i.e., milk N secretion, MNE, relative urinary N excretion) but with low frequency for indicators which were independently determined from milk performance (i.e., ratios of N:C, PD:N, PD:C), suggesting that milk yield played a minor role in explaining variation in microbial CP synthesis and total urinary N excretion. Lastly, it cannot be determined whether DIM significantly affected milk N secretion, MNE, and relative urinary N excretion, because DIM was representative of the animal's productivity or because DIM correlated with milk yield (i.e., represented an artifactual effect).

2.4.4 Random effect of sampling phase

Data collection was repeated twice per year and farm to capture different years and pasture conditions at different times of the year, which were considered to differ with regard to availability, botanical composition, and nutritional quality of pasture herbage and forage supplements

(Waghorn and Clark, 2004). During the backward elimination procedure, seasonal and annual differences that were not captured by selected grazing management factors were considered using the random effect of the sampling phase. This random effect only played a role in the mixed models for the relative faecal and urinary N excretions (i.e., no variance estimates were provided for the remaining N-related indicators). How- ever, solely R^2 values of 0.06 and 0.16 were attributable to the random effects in the backward selected models for relative urinary and faecal N excretion, respectively. Hence, most variation in relative N excretion was explained by the selected grazing management factors, such as the nutritional quality of pasture herbage, as demonstrated by the marginal R^2 of 0.84 and 0.83 for relative urinary and faecal N excretion, respectively.

2.4.5 Effect of the nutritional quality of pasture herbage

Increases in ME and NDF concentrations of pasture herbage were related to increased relative faecal N excretion and MNE, and reduced relative urinary N excretion. The positive effect of ME on N utilisation and simultaneous reduction of urinary N excretion was expected from the more synchronised CP-to-energy ratio in pasture herbage leading to a lower ruminal N balance (Castillo et al., 2000). The CP concentration of pasture herbage, on the other hand, was chosen with high frequency for MUN concentration and urinary N:C ratio, eliciting that it was a key driver for excessive N intake, leading to inefficient ruminal N utilisation and increased milk and urinary urea N excretion (Hoekstra et al., 2007). The nutritional quality of pasture herbage thus had a major influence on the chosen N-related indicators, although supplement DMI had a substantial share of the total DMI (46 g/100 g DMI) of dairy cows grazing semi-natural grasslands.

2.4.6 Effect of the amount and type of supplementation

Results of backward elimination and bootstrapping indicated that the type and amount of supplementation were decisive for N utilisation and excretion. Nitrogen utilisation increased (e.g., MNE) and daily relative urinary N excretion decreased with increasing proportion of fresh forage or grass hay of supplement DMI. At the same time, increases in total supplement DMI had the opposite effect (i.e., reduced N utilisation and increased urinary N excretion). Hence, increasing shares of forages and/or hay in supplement DMI solely increased N utilisation at moderate levels of supplement DMI (i.e., if it did not substitute pasture DMI substantially). These findings support the hypothesis that substitution of full-time grazing with part-time grazing combined with moderate supplementation with more mature forages lower in CP concentration (14.2 g/100 g DM ± 4.37) than young, grazed herbage (16.7 g/100 g DM ± 2.93) would increase dietary N utilisation and thus decrease urinary N excretion of grazing dairy cows.

Concentrate supplementation mainly consisted of energy-rich ingredients (i.e., cereal grains), with mean ME and CP concentrations of 12.1 MJ/kg DM (± 0.67) and 11.7 g/100 g DM (± 2.29), respectively. It was expected that such supplementation with energy-rich concentrates would increase N utilisation and decrease urinary N excretion of grazing cows by supplying readily fermentable carbohydrates in addition to the CP excess from pasture herbage for more efficient microbial CP synthesis. The share of concentrates in supplement DMI was solely chosen for the N-related indicators determined in urine (i.e., dataset 2), and in interaction with the hay and fresh forage share in supplements. Daily urinary N excretion (i.e., N:C) declined and dietary N utilisation for rumen microbial CP synthesis (i.e., PD:C and PD:N) increased with increasing concentrate share of supplement DMI. Consequently, increases in concentrate share in supplement DMI improved the CP-to- energy ratio (i.e., ruminal N balance). However, the improved ruminal N balance did not translate into greater N utilisation for milk protein synthesis (i.e., greater MNE), likely because utilisable protein was above the animal’s requirements. The negative inter- action coefficients indicated that the concentrate effect was diminished by increasing shares of fresh forage or hay in supplements.

2.4.7 Effect of paddock management factors

In general, paddock management factors were chosen with the lowest frequency in the bootstrapping analysis, and factors chosen with higher frequency showed an inconsistent distribution of their regression coefficient signs. The herbage allowance and stocking density play an important role in determining pasture DMI in high-input grazing systems (e.g., McCarthy et al., 2011; Pérez- Prieto and Delagarde, 2013). In the present study, neither herbage allowance (r = -0.09) nor stocking density (r = -0.09) correlated notably with pasture DMI, and they did not explain the variation in the observed N-related indicators. The low correlation between these paddock management variables and pasture DMI may be attributed to the substantial supplementation in the barn, substituting pasture DMI and lowering the influence of changes in pad- dock management on pasture DMI, and, therefore, N utilisation and excretion.

Several indicators demonstrated a greater N utilisation and lower urinary N excretion when animals grazed full-time com- pared to grazing during the day. This is in contrast to our expectation that N utilisation would be greater with part-time compared to full-time grazing but supports the earlier finding that N utilisation increased, and urinary N excretion decreased with a reduction in supplement DMI (e.g., as a consequence of longer daily access to pasture). In the present study, cows with full-time access to pasture were still supplemented in the barn after each milking time (3.9 kg DM/d), mainly with concentrates (41 g/100 g supplement DMI) and grass hay (28 g/100 g supplement DMI). Apart from the supplementation, no other management factor explained the greater N utilisation and lower N excretion of cows grazing full- time. For instance, they received less concentrates (2.2 kg DM/d) compared to animals grazing during the day (3.0 kg DM/d), had a similar milk yield (24.1 kg/d) than cows with pasture access during daytime (24.4 kg/d), and the CP concentration of their diet (15.1 g/100 g DM) did not differ from that of diets of the daytime group (15.7 g/100 g DM). Although the distribution of cows between the groups grazing during the day (n = 169), night (n = 80), and full-time (n = 74) was unbalanced, these findings indicate that there were farms making great use of their available pasture resource by full-time grazing and moderate supplementation in the barn without excessive urinary N excretion or hampering milk protein yield.

Finally, our results indicate that individual paddock management factors such as botanical composition, herbage allowance, or daytime of grazing, which have been shown to improve availability and nutritional values of pasture herbage, or feed intake, performance, and nutrient use of grazing livestock in controlled experiments, may not have any effect under practical farming conditions. Instead, they may only effectively influence forage intake and use as well as N utilisation and excretion of grazing cows when combined with other grazing management factors (e.g., supple- mentation strategy).

2.5 Conclusion

The N utilisation and excretion by lactating dairy cows vary greatly across diverse organic dairy farms in South Germany using temperate semi-natural grasslands for grazing. On average, observed MNE is greater than in high-input grazing systems but lower than in stall-fed cows receiving balanced diets. For the investigated farming conditions, the supplementation strategy has the greatest potential for manipulating N utilisation and excretion. Increasing shares of fresh forages as well as of hay in supplement DMI increases N utilisation and decreases urinary N excretion, while increasing shares of concentrates in supplement DMI are related to lower N losses via urine. Hence, full-time grazing combined with moderate supplementation in the barn is a viable option for low-input, grazing-based dairy operations with moder- ate levels of N losses. Yet, nutritional value of pasture herbage is a key driver for milk protein production and N excretions even at substantial supplementation, emphasising the need for real-time applications to quantify the nutritional quality of pasture herbage to enable adequate supplementation in the barn. The present study was based on on-farm trials which allowed for the analysis of interactions between diverse factors, and thus an understanding of the true effects of grazing management across the diverse pro- duction conditions in low-input dairy farms.

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2.9 Supplementary Material

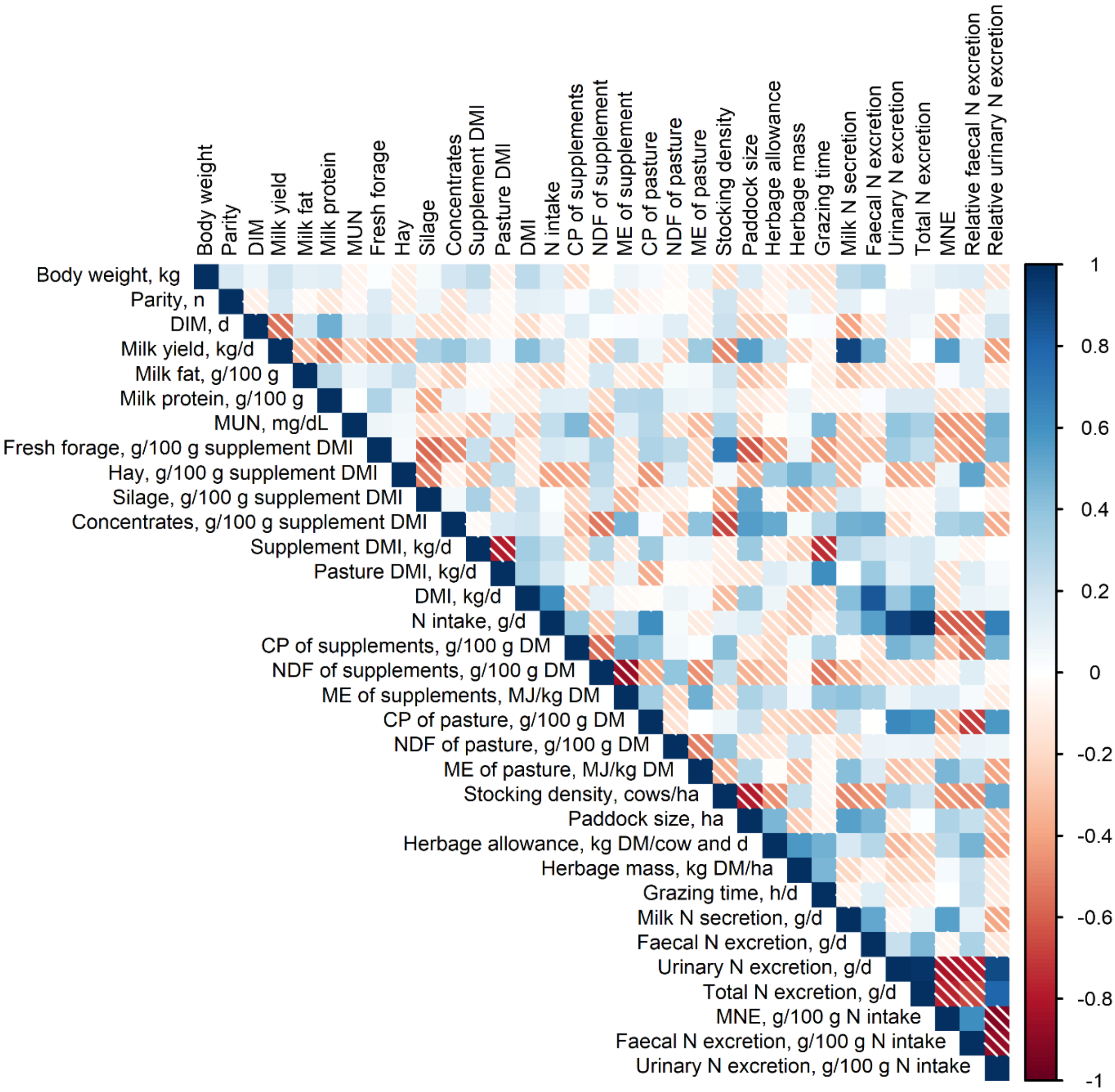

Figure S2.1. Correlation matrix for dataset 1 (n = 323) with various variables related to milk production, grazing management, and nitrogen (N) utilisation and excretion of lactating dairy cows grazing temperate semi-natural grasslands in South Germany (DIM: days in milk; DMI: DM intake; ME: metabolisable energy; MNE: milk N use efficiency; MUN: milk urea N).

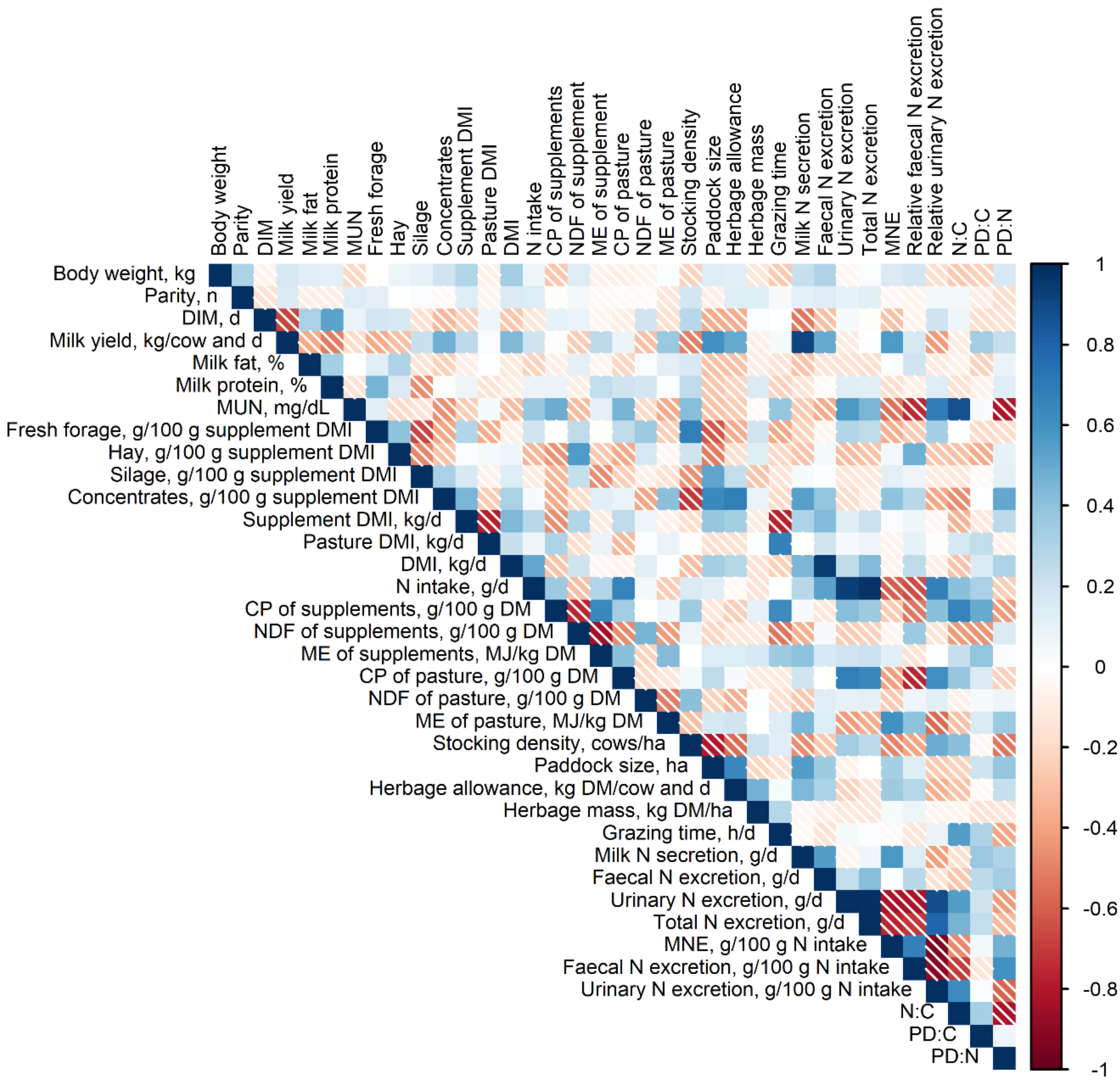

Figure S2.2. Correlation matrix for dataset 2 (n = 152; i.e., subsample of dataset 1 solely containing records of cows from which urine spot samples were collected) with various variables related to milk production, grazing management, and nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural in South Germany (DIM = days in milk; DMI = DM intake; ME = metabolisable energy; MNE = milk N use efficiency; MUN = milk urea N; N:C = ratio between urinary N and urinary creatinine concentrations; PD:C = ratio between urinary purine derivates and urinary creatinine concentrations; PD:N = ratio between urinary purine derivatives and urinary N concentrations).

Chapter 3 | On-farm evaluation of models to predict herbage intake of dairy cows grazing temperate semi-natural grasslands [2]

[2] This chapter is published as:

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Abstract

The objective of the present on-farm study was to evaluate the adequacy of existing models in predicting the pasture herbage DM intake (PDMI) of lactating dairy cows grazing semi-natural grasslands. The prediction adequacy of 13 empirical and semi-mechanistic models, which were predominantly developed to represent stall-fed cows or cows grazing high-quality pastures, were evaluated using the mean bias, relative prediction error (RPE), and partitioning of mean square error of prediction, where models with an RPE 20% were considered adequate. The reference dataset comprised n = 233 individual animal observations from nine commercial farms in South Germany with a mean milk production, DM intake, and PDMI (arithmetic means ± one SD) of 24 kg/d, (±5.6), 21 kg/d (±3.2), and 12 kg/d (±5.1), respectively. Despite their adaptation to grazing conditions, the behaviour-based and semi-mechanistic grazingbased models had the lowest prediction adequacy among the evaluated models. Their underlying empirical equations likely did not fit the grazing and production conditions of low-input farms using seminatural grasslands for grazing. The semi-mechanistic stall-based model Mertens II with slight modifications achieved the highest and a satisfactory modelling performance (RPE = 13.4%) when evaluated based on the mean observed PDMI, i.e., averaged across animals per farm and period (n = 28). It also allowed for the adequate prediction of PDMI on individual cows (RPE = 18.5%) that were fed < 4.8 kg DM of supplement feed per day. Nevertheless, when used to predict PDMI of individual animals receiving a high supplementation level, the model Mertens II also did not meet the threshold for an acceptable adequacy (RPE = 24.7%). It was concluded that this lack of prediction adequacy for animals receiving greater levels of supplementation was due to a lack of modelling precision, which mainly could be related to interanimal and methodological limitations such as the lack of individually measured supplement feed intake for some cows. The latter limitation is a trade-off of the on-farm research approach of the present study, which was chosen to represent the range in feed intake of dairy cows across the diverse low-input farming systems using semi-natural grasslands for grazing.

3.1 Introduction

In contrast to improved or temporary grasslands, semi-natural grasslands can be defined as lower-yielding, multi-species permanent grasslands, with at least in part, lower nutritive value of herbage due to no or little treatment with fertilisers and pesticides, and reduced soil cultivation (Bruinenberg et al., 2003; Peeters et al., 2014). In regions with low mountain ranges, such as in great parts of Southern Germany, Austria, and Switzerland, semi-natural grasslands can constitute an import feed component for dairy cows (Akert et al., 2020). Accurate measurements of the pasture herbage DM intake (**PDMI**) of lactating dairy cows are essential to optimise grazing management and supplemental feeding (Tedeschi et al., 2019). For research purposes, a combination of external and internal markers to quantify the PDMI of cows is commonly used. This method, however, is labour- and cost-intensive, and invasive due to the need for daily faecal samples to quantify marker excretions (Hellwing et al., 2015). Alternatively, mathematical models have been developed to predict feed intake of cows from animal, feed, and management characteristics. Such models can be applied for research but could also be integrated into decision-support tools for grazing and feeding management (Tedeschi et al., 2019).

Empirical models, based on regression equations using animal characteristics and productive parameters i.e., animal growth and milk yield, as input factors, are among the most widely applied intake models, but are criticised for being overfitted to the production conditions of the underlying empirical dataset (Tedeschi et al., 2019). Alternatively, mechanistic and semi-mechanistic models attempt to simulate the underlying processes regulating the voluntary feed intake of animals and are thus more applicable to a wider set of farming conditions (Pittroff and Kothmann, 2001). The majority of such mechanistic models rely on the principle that voluntary feed intake of cows is either determined by (1) the physical intake capacity governed by dietary fill-effect and rumen volume, or by (2) the physiological demand for feed intake, foremostly regulated by the animal's energy requirements (Pittroff and Kothmann, 2001). The underlying processes during grazing are, however, more complex than under stall-feeding conditions, because additional variation in feeding behaviour and energy expenditures need to be considered (Gregorini et al., 2015). Therefore, grazing-specific models with semi-mechanistic (e.g., e-cow; Baudracco et al., 2012) or empirical approaches using behavioural data to predict PDMI (e.g., Rombach et al., 2019) have been developed.

Most of these models were developed and their prediction adequacy evaluated under high-input grazing conditions using improved, homogenous ryegrass-clover swards with high biomass yields for grazing (e.g., Baudracco et al., 2012; Roca-Fernández and González-Rodríguez, 2018). These conditions differ greatly from the production conditions on low-input farms using semi-natural grasslands for grazing, which, so far, have received less attention in research despite their common use for dairy production in grassland-dominated regions of Central Europe. It was hypothesised that existing models cannot adequately predict PDMI of dairy cows grazing semi-natural grasslands, due to the differences in yield, botanical composition, and nutritional value of the grassland vegetation, common animal breeds, and their milk yield and composition, as well as the herd, feeding, and grazing management factors between low-input and high-input dairy farming. The objective of the present study was, thus, to evaluate the adequacy (judged on the level of precision and accuracy; Tedeschi, 2006) of various existing models for estimating the PDMI of individual and groups of cows grazing semi-natural grasslands. For this, we chose an on-farm research approach to assure the transferability of our results to the wide variation in feed intake of grazing dairy cows across the diverse low-input farming systems using semi-natural grasslands for grazing. Preliminary results from the present study were previously published in abstract form (Perdana-Decker et al., 2021, 2022).

3.2 Materials and methods

3.2.1 Model selection

Literature research was conducted to identify existing models to predict daily feed intake of lactating cows on pasture. In total, 13 prediction models with four different approaches to estimate PDMI were selected (Supplementary Table S3.1). (1) Three behaviour-based models using chewing behaviour, animal characteristics, and milk performance to predict PDMI (**Rombach** (Rombach *et al.*, 2019: model GA7); **Greenwood** (Greenwood *et al.*, 2017); **Oudshoorn** (Oudshoorn *et al.*, 2013: model BT11T)), and (2) two semi-mechanistic models specifically developed to predict PDMI of grazing cows, called semi-mechanistic pasture-based models thereafter, using pasture characteristics, supplementation, and milk performance parameters (**GrazeSim** (Vazquez and Smith, 2001: modelPIsup); **e-cow** (Baudracco *et al.*, 2012: equation 5 for a ryegrass-based pasture)). Additionally, models based on data from stall-fed animals were selected which predict total DM intake: (3) six empirical (**AFRC** (Vadiveloo and Holmes, 1979: model 1); **Cornell** (Fox *et al.*, 2004); **De Souza** (de Souza *et al.*, 2019); **Gruber** (Deutsche Landwirtschaftgesellschaft, 2006: equation 1); **NRC** (National Research Council (NRC), 2001); **Sauvant** (Sauvant *et al.*, 2014)), and (4) two semi-mechanistic models (**Conrad** (Conrad *et al.*, 1964; Kahn and Spedding, 1984); **Mertens I** (Mertens, 1987)). The latter eight empirical and semi-mechanistic stall-based models were used to predict PDMI indirectly by subtracting measured DM intake of supplemental feed (**DMIsupp**) from the models' predictions of total DM intake.

The Rombach model was slightly modified by using the DMIsupp as an input variable instead of corn silage intake and by using a constant value for post-grazing herbage mass i.e., the mean observed value of the model's development dataset (222 kg DM/ha), instead of a measured value. For the Cornell model, two different DM intake equations were applied (Fox *et al.*, 2004): (1) one for lactating dual-purpose cows used for Simmental cows (Table 11: equation 9) and (2) one for lactating dairy cows, which was applied for the remaining breeds (Table 11: equation 8). The Mertens I model was modified assuming that grazing cows had a higher physical intake capacity, due to their high allowance to pasture herbage and forage supplements (Baudracco *et al.*, 2012). Hence, a physical i.e., NDF, intake capacity of 1.65 % of the animals' BW (Vazquez and Smith, 2001) was assumed for the model **Mertens II**, instead of the 1.30 % of BW in the original Mertens

I model. The model e-cow is a combination of models predicting milk yield, BW change, and PDMI. For the present study, instead of using e-cow's predictions of the animal's milk yield and BW change, the actual records of milk yield were used as an input factor to the PDMI module, and no changes in BW were assumed. For the calculation of metabolisable energy (**ME**) requirements and the model NRC, the energy-corrected and fat-corrected milk yields were calculated using equations (1) and (2) (Gesellschaft für Ernährungsphysiologie (GfE), 2001 and NRC, 2001):

$$ECM = (0.39 \times fat + 0.24 \times protein + 0.17 \times lactose + 0.1) \times DMY \div 3.28 \quad (1)$$

$$FCM = 0.4 \times DMY + 15 \times fat \times DMY \div 100 \quad (2)$$

where ECM is the energy-corrected milk yield (kg/d), fat the fat concentration in milk (g/100 g milk), protein the protein concentration in milk (g/100 g milk), lactose the lactose concentration in milk (g/100 g milk), DMY the milk yield (kg/d), and FCM the fat-corrected milk yield (kg/d). The models GrazeSim, e-cow, Mertens I, and Mertens II included the ME requirements for maintenance and milk production as input variable. For these models, an updated version of the German feeding recommendation system for dairy cows and heifers was applied to determine the daily ME requirements of cows, assuming that animals neither gained nor lost BW (eq. 3) (GfE, 2001; Gruber *et al.*, 2021):

$$ME_{req} = 0.65 \times BW\text{^}0.75 + 1.37 \times ECM \times 3.28 \quad (3)$$

where ME_{req} are the ME requirements for maintenance and milk production (MJ/d), BW^0.75 is the metabolic BW (kg^0.75), and ECM (kg/d) was calculated using equation (1). Furthermore, energy expenditure of grazing animals for locomotion was considered. For this, additional daily ME requirements based on horizontal and vertical walking distance were added (2.6 J/kg BW and m horizontal walking distance and 28 J/kg BW and m vertical walking distance; Heard *et al.*, 2004).

3.2.2 Reference data collection

Study farms and setup

The reference dataset for model evaluation was gathered on nine commercial organic dairy farms in South Germany in 2019 and 2020. Four farms were visited in both years, whereas five farms were only visited in 2020. Per year, every farm except one was visited for two examination periods to cover spring (May to July) and summer (August to October). Every examination period lasted for 11 d with an adaptation period from Days 1 to 5 and a sampling period for faeces, milk, pasture herbage, and supplement feeds from Days 6 to 11. Depending on herd size, 10 to 15 lactating dairy cows per farm and period were selected. Per farm, animals were chosen to obtain groups of cows with similar days in milk and number of parities across all periods. On two farms, two groups à 8 to 14 cows per period were used for testing supplementation treatments.

Animals grazed semi-natural grasslands with mean ME and CP concentrations of 9.3 MJ/kg DM and 16.1 g/100 g DM, respectively, across all sampling periods (Table 3.1). On five farms, the botanical composition of fresh aboveground herbage biomass of grazed paddocks was assessed visually once per examination period. For these paddocks, mean proportions of grasses, clover, and other forbs were 50.8, 20.4, and 28.8 % of the entire aboveground herbage mass, respectively. Grazing took place for 4 to 20 h/d, during the day, at night, or full-time. The 1 to 8 paddocks per farm were either grazed continuously or rotated daily for the total herd of lactating dairy cows. Different combinations of concentrates, fresh forage of meadows or grass-clover leys, grass hay, grass silage, and maize silage were supplemented in the barn (Supplementary Table S3.2). Daily supplementation with concentrate feeds ranged from 0 to 5.6 kg DM and total supplementation (i.e., forage and concentrate feeds) between 0.5 and 18.2 kg DM/d.

Table 3.1. Chemical composition (g/100 g DM), organic matter (OM) digestibility, and metabolisable energy (ME) of semi-natural grasslands grazed by lactating dairy cows on nine commercial organic farms in South Germany. Arithmetic means and one SD in parentheses.

Year	Season [1]	OM	CP	NDF	ADF	ME, MJ/kg DM	$DOM_{in\ vitro}$, g/100 g OM
2019	Spring	87.8 (2.74)	15.7 (0.30)	42.5 (2.63)	24.3 (1.58)	9.1 (0.46)	67.7 (2.99)
2019	Summer	88.5 (0.56)	19.8 (2.14)	43.0 (3.35)	24.2 (1.52)	9.1 (0.36)	69.8 (1.49)
2020	Spring	91.1 (0.57)	14.8 (2.03)	45.6 (2.81)	22.1 (2.73)	9.8 (0.51)	71.4 (2.68)
2020	Summer	89.7 (0.99)	16.2 (2.50)	46.0 (4.14)	23.7 (2.65)	8.9 (0.45)	68.4 (3.99)

Abbreviations: $DOM_{in\ vitro}$ = apparent total tract digestibility of OM determined by *in vitro* incubation.
[1] Season: spring = May to July, summer = August to October.

The final evaluation dataset comprised 233 observations averaged per farm, period, and animal from a total of 176 animals (i.e., 44 animals were repeatedly sampled during 2 to 4 examination periods). The 233 observations were taken from 22 primiparous and 211 multiparous cows (Table 3.2) and different breeds (Simmental, n = 156; Brown Swiss, n = 48; Holstein-Friesian, n = 70; and cross-bred cows, n = 7). Mean (± one SD) milk production was 24 kg/d (± 5.6) and mean observed DM intake and PDMI were 21 kg/d (± 3.2) and 12 kg/d (± 5.1), respectively. On seven farms, animals were milked twice per day in the morning and afternoon, whereas automatic milking systems were used on two farms. All animals had free access to water troughs, both, in the free-stall barns and on pasture.

Table 3.2. Arithmetic mean, one SD, minimum, and maximum of grazing conditions, and animal and feed intake variables of lactating dairy cows grazing semi-natural grasslands on nine commercial organic dairy farms in South Germany.

Variables	n	Mean	SD	Minimum	Maximum
Grazing time, h/d	28	11.5	5.27	3.5	20.0
Herbage mass, kg DM/ha	28	348.0	184.82	83.2	822.5
Herbage allowance, kg DM/cow and d	28	30.9	26.14	6.7	94.8
Days in milk, n	233	142.4	64.02	16.0	411.5
Parity, n	233	3.7	2.01	1.0	9.0
BW, kg/cow	233	700.0	82.77	408.0	1000.0
Milk yield, kg/d	233	23.9	5.57	9.7	39.8
Milk fat, g/kg milk	233	39.2	5.52	23.7	60.4
Milk protein, g/kg milk	233	32.1	2.77	25.9	42.9
PDMI, kg/d	233	12.3	5.09	0.0	28.7
DMIsupp, kg/d	233	8.8	5.03	0.5	24.3

Abbreviations: DMIsupp = DM intake of supplemental feed; PDMI = pasture herbage DM intake.

Data and sample collection

Ambient air temperature and relative air humidity were recorded during the sampling period in a 1 h-interval (HOBO® U23 Pro v2 External Temperature/Relative Humidity Data Logger, Onset Computer Corporation, Bourne, United States). Body weight of individual cows was estimated once per period by measuring the heart girth with a tape calibrated for common cattle breeds (Animeter, Albert Kerbl GmbH, Buchbach, Germany). Additionally, days in milk and parity of individual animals at the time of sampling were retrieved from milk reports by the Regional Association for Performance and Quality Inspection in Animal Breeding of Baden-Württemberg (LKV-Baden-Württemberg). Individual milk yield was measured, and individual milk samples taken once daily from Days 6 to 11 alternating between morning and afternoon milking. For this, mobile milk meters provided by the LKV-Baden-Württemberg (HI, Tru-Test Datamars, Auckland, New Zealand) were used, or the farms' own in-parlour electronic milk meters or mobile milk meters (MK5, Waikato Milking Systems, New Zealand). Milk samples (40 ml) were conserved with 150 µL Bronysolv (ANA.LI.TIK Austria, Vienna, Austria) and stored at 4°C for later analysis.

A double-marker technique was used to determine daily DM intake of each cow, using faecal CP concentration as internal marker to estimate the apparent total tract digestibility of diet organic matter (OM) (Lukas et al., 2005), and titanium dioxide (**TiO_2**) as external marker to determine daily faecal output (Glindemann et al., 2009). For this, each cow orally received 24 to 28 g TiO_2 daily, depending on the expected DM intake and faeces excretion level, across the entire 11-d examination period. The marker was administered twice daily in two equal dosages after morning and evening milking. On one farm, animals received apples filled with the marker. The animals of five farms received the TiO_2 mixed into ~ 250 g fresh matter of concentrate feed per dosage. On the remaining three farms, the marker was fed via two daily doses à ~ 265 g fresh matter of pelleted concentrate mixed with TiO_2 (Ökokuh 164 Mais, Raiffeisen Kraftfutterwerk, Kehl, Germany). After morning and evening milking on Days 6 to 11, faecal grab samples (~ 300 g fresh matter) were taken from the animals' rectum, frozen immediately after collection, and stored at -20 °C. On farms with two supplementation groups per period, faecal samples of individual animals of each group were taken only once daily, alternating between morning and evening.

The amount of concentrate feed consumed by each cow at automatic feeding stations was automatically recorded. If cows were additionally supplemented with forages or partial mixed rations, the amount of each feed consumed by the entire herd or supplementation group of lactating cows was measured daily throughout the sampling period. For this, scales built into the forage wagons were used, whenever available, to determine the amount of feed offered to all dairy cows, or the offered feed mass on the feeding banks were weighed manually. To do so, feed mass in different segments (~ 3 m) of the feeding bank was weighed at each feeding, and the weight extrapolated to the total length of the bank to estimate total offered feed mass. The total amount of daily feed refusals of the herd or treatment group were weighed. Samples of 200 g fresh matter of offered and refused feeds were collected and weighed daily. Additionally, one sample of the pelleted concentrate mixture with TiO_2 was collected per farm and period. Samples of fresh forage and partial mixed rations were frozen immediately after collection (-20 °C), whereas samples of grass hay and concentrate feeds were stored at room temperature until analysis.

Grazing management decisions, such as timing, frequency, duration of daily pasture access, as well as stocking densities were recorded by the farm managers throughout the grazing period. Once per sampling period, the herbaceous vegetation on grazed paddocks was sampled in three or six representative points per paddock, depending on paddock size.

Hence, 9 ± 5.7 pasture herbage samples were taken per farm and period. At each sampling point, herbage mass was manually harvested using electronic shears at an average cutting height of 3 cm above ground surface to resemble grazing depth in a 1-m2-plot (0.5 m x 2.0 m) and weighed. The entire harvested herbage per sampling point was dried for 72 h at 45 °C, weighed, and its DM concentration determined. The pasture herbage available to each cow (i.e., herbage allowance, kg DM/cow and d) was estimated by dividing the mean aboveground herbage mass (kg DM/ha) by the stocking density on each paddock (n/ha). The mean herbage allowance across all grazed paddocks per farm and sampling period was used as model input.

Laboratory analysis

At the end of each period, all milk samples were analysed for fat, protein, and lactose by Milchprüfring Baden-Württemberg e.V. (Kirchheim/Teck, Germany) according to ASU L 01.00-78, 2018-06 (Milchprüfring Baden-Württemberg e.V., 2021). Results on milk yield and composition were averaged per cow and period. For this, records of milk yield and composition were only considered, if records of morning and evening samples of consecutive days were available. After thawing, faeces samples of each cow and period were homogenised, and aliquots of ~ 15 g fresh matter per sample were taken and pooled by cow and period. The pooled sample (~ 200 g fresh matter) was lyophilised for 72 h (LYO GT2 Basis, SRK Systemtechnik GmbH, Riedstadt, Germany). Samples of pasture herbage, offered feeds, and refused feeds were dried at 45 °C for 72 h, except of feed samples including silages that were lyophilised. Dried samples of faeces, pasture herbage, and offered and refused feeds were weighed, then ground to pass through a 1-mm-sieve (Retsch SM 100, Retsch GmbH, Haan, Germany). After grinding, the whole samples of pasture herbage were pooled by paddock and period, and samples of offered and refused feed were each composited by farm and period. All faeces, pasture herbage, and feed samples were analysed in duplicate using the official analytical methods in Germany (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, 2007). All samples were analysed for DM and crude ash (methods 3.1 and 8.1, respectively). Concentrations of nitrogen in samples of pasture herbage, offered feed, and feed refusals were determined by Dumas combustion (method 4.1.2) using the Vario Max CN (Elementar Analysesysteme GmbH, Langenselbold, Germany). In faecal samples, nitrogen concentration was determined via Kjeldahl digestion (method 4.1.1; Vapotest 45s, C. Gerhardt GmbH & Co. KG, Königswinter, Germany and behrotest K20L, Behr Labor-Technik GmbH, Düsseldorf, Germany). Subsequently, CP concentrations were calculated as nitrogen concentration x 6.25. The samples of herbage and offered feed were additionally analysed for NDF and ADF (methods 6.5.1 and 6.5.2, respectively) using an Ankom200 Fiber Analyzer (Ankom Technology, Fairport, US) and for crude lipid by ether extraction (method 5.1.1A) using a Soxtec System (HT 1043 Extraction Unit, Foss Tecator, Hillerød, Denmark). The Hohenheim Gas Test was applied to determine digestible OM (g/100 g OM) and ME concentrations of herbage and offered feedstuffs in triplicate in two independent runs, according to the procedures and feedstuffs-specific equations described by Menke and Steingass (1988). Concentration of net energy for lactation in offered feed and pasture herbage was estimated from the ME concentration using an updated coefficient for lactation efficiency ($k = 0.73$; Gruber et al., 2021). Apparent total tract DM digestibility of herbage and offered feed was estimated from ADF and nitrogen concentrations in feed using equation 2 by Oddy et al. (1983).

The TiO_2 concentration in faeces and pelleted concentrate mixtures with TiO_2 was determined in duplicate using the slightly modified method by Boguhn et al. (2009).

Chewing and locomotion behaviour

Ten of the experimental cows per period and farm were equipped with a sensor system to monitor their chewing behaviour, which combines a noseband-pressure sensor with a triaxial accelerometer (RumiWatch®, ITIN + HOCH, Liestal, Switzerland). On five farms, five of these animals were additionally equipped with Global Positioning System collars (VERTEX Lite Collars, Vectronics Aerospace, Berlin, Germany) to determine their walking distances during pasture access. The animals were equipped with the sensors 1.5 d prior to the 6-d-sampling period to allow for adaptation to the sensors. The chewing sensors recorded at a frequency of 10 Hz, whereas the Global Positioning System collars registered the Universal Transverse Mercator coordinates and the altitude of the cows' location every 5 min. These registered Universal Transverse Mercator coordinates were then used to calculate the horizontal walking distance during pasture access using the Euclidean Distance with equation (4). The vertical walking distance was calculated from the recorded altitude (eq. 5):

$$Horizontal\ walking\ distance = \sum_{i=1}^{n} \sqrt{(Y_{i+1} - Y_i)^2 + (X_{i+1} - X_i)^2} \qquad (4)$$

$$Vertical\ walking\ distance = \sum_{i=1}^{n} |A_{i+1} - A_i| \qquad (5)$$

where horizontal walking distance was in m/d, Yi is the y coordinate of the cow's location at time i, Xi is the x coordinate of the cow's location at time i, vertical walking distance was in m/d, and Ai is the altitude of the cow's location at time i in m. For calculating the ME requirements, the mean horizontal and vertical walking distances averaged per farm and period were used for each cow of the respective farm and period. For animals of farms where the Global Positioning System collars were not applied, the mean horizontal and vertical walking distances across all sampling periods and farms with Global Positioning System records were used. Using the RumiWatch Converter V0.7.3.36 (ITIN + HOCH, Liestal, Switzerland), jaw movement observations were converted to the total number of eat chews per day (i.e., mastication and prehension bites), bite rate (i.e., eat chews per minute of eating time), daily eating time on pasture, and daily number of prehension bites (i.e., only eat bites excluding mastication) in a 1-h resolution. Observations of these chewing variables were averaged for each animal per period and farm, but only mean values based on a minimum of four complete sampling days per animal were considered as model input.

Calculation of pasture herbage intake

Individual daily DM intake was calculated from the apparent total tract digestibility of ingested OM (DOM_{CP}, g/100 g OM) and daily faecal OM output. The DOM_{CP} was estimated using the CP concentration in faecal samples using the Gumpenstein/Hohenheim coefficient (Lukas et al., 2005). Faecal OM output (kg/d) was calculated by dividing the TiO_2 dosage (g/d) by the concentration of TiO_2 in faeces (g/kg OM). For the three farms, on which TiO_2 was administered via pelleted concentrate mixtures, analysed TiO_2 concentration in concentrate pellets and daily pellet intake was used to calculate TiO_2 dosage. Total OM intake was determined by dividing faecal OM output by [(1 – DOM_{CP}) / 100]. Finally, DM intake was estimated by dividing OM intake by dietary OM concentration (kg/kg). The latter was estimated based on the ratio between PDMI and DMIsupp, forecasted using the ME requirements issued by the GfE (2001).

In case there were no records of individual DMIsupp from sole concentrate supplementation by feeding stations, it was determined proportionally to the animal's individual eating time in the barn using equations (6) and (7):

$$DMIsupp_i = \frac{eat_i}{eat_{sampled}} \times DMIsupp_{sampled} \quad (6)$$

$$DMIsupp_{sampled} = \frac{DMIsupp_{herd}}{n_{sampled} \div n_{herd}} \quad (7)$$

where $DMIsupp_i$ is the DM intake of supplemental feed of cow i (kg/d), eat_i the eating time in barn of cow i (min/d), eatsampled the sum of the time spent eating in barn across the cows with eati records per farm and period (min/d), $DMIsupp_{sampled}$ the sum of DMIsupp across the cows with eati records per farm and period (kg DM/d), $DMIsupp_{herd}$ the difference between the offered and refused DM by the total herd (kg DM/d), nsampled the number of cows with eati records per period and farm, and nherd the total number of cows per herd, period, and farm. Finally, individual PDMI was calculated by subtracting the DMIsupp of each cow from its DM intake. Thus, individual PDMI could solely be calculated when complete behavioural records or measured individual DMIsupp values were available for the animal.

3.2.3 Statistical analysis

Evaluation of the models' prediction adequacy

Mean observations per farm and period of 296 animals were collected; however, only 233 were used for model evaluation. Sixty-three observations were excluded, because of missing records of chewing behaviour, due to technical issues with the sensors, or if animals refused to ingest daily TiO_2 dosages. Additionally, only 220 observations were used for evaluating the behaviour-based models Rombach and Greenwood, because these observations were missing the chewing behaviour data as input variables, whereas individual DMIsupp values were based on feed station measurements. For the evaluation of the model Oudshoorn, even less observations were considered (n = 208). In this case, the chewing behaviour sensors failed to record the prehension bites.

All following statistical analyses were conducted in R Version 4.2.0 (R Core Team, 2022). Mean bias (**MB**), mean square error of prediction (**MSEP**), and relative prediction error (**RPE**) were calculated to evaluate the models' prediction adequacy (i.e., accuracy and precision) (Tedeschi, 2006). The MB, MSEP, and RPE were calculated for both, the PDMI based on individual observations (n = 233, 220, or 208) and on mean basis, i.e., comparing the observed versus predicted PDMI averaged per farm, period, and supplementation group (n = 28). Equation (8) was used to calculate the MB (Tedeschi, 2006):

$$MB = \sum_{i}^{n} \frac{(Obs_i - Pred_i)}{n} \quad (8)$$

where MB is the mean bias per model, n the number of observations, Obs_i the observed PDMI of animal i (kg/d), and $Pred_i$ the predicted PDMI of animal i (kg/d). A negative MB indicates an overprediction, a positive MB an underprediction of PDMI, and a MB ≤ 10 % acceptable modelling accuracy (Yungblut *et al.*, 1981). The MSEP was used to decompose the source of prediction error and was therefore calculated as the sum of three error terms (ep. 9), representing the mean prediction bias, line bias, and random variation (Fuentes-Pila *et al.*, 1996):

$$MSEP = (\overline{obs} - \overline{pred})^2 + s_{obs}^2 (1 - b)^2 + s_{pred}^2 (1 - R^2) \quad (9)$$

where MSEP is the mean square error of prediction per model, $\overline{obs}$ the mean observed PDMI (obs, kg/d), $\overline{pred}$ the mean predicted PDMI (pred, kg/d), s^2_{obs} the variance of obs, s^2_{pred} the variance of pred, b the slope of the regression between obs and pred, and R^2 the coefficient of determination of the regression between obs and pred. Whilst a large proportion of line bias (i.e., $s^2_{obs}(1 - b)^2$) is a sign for major systematic errors in the model structure and therefore not desired, a large proportion of random variation (i.e., $s^2_{pred}(1 - R^2)$) is an indicator for the model's high predictive adequacy where the greatest share of error is caused by variation in the observed data (Fuentes-Pila *et al.*, 1996). The RPE (%) was calculated as the root of the MSEP divided by the mean observed PDMI. It was classified according to Fuentes-Pila *et al.* (1996), who assumed that the prediction adequacy is satisfactory with an RPE < 10 %, acceptable between 10 % and 20 %, and not acceptable with an RPE > 20 %.

Evaluation of the models' adequacy by animal subgroups

It was hypothesised that existing models were not suitable to predict PDMI of animals grazing on semi-natural grassland due to their inability to reflect the variable grazing and production factors under such conditions. Hence, the RPE was calculated separately for animal subsets differing in grazing or production levels. For this, the grazing factors daytime of pasture access, herbage allowance, stocking density, and DMIsupp, were considered and the production factors DM intake of concentrates, energy-corrected milk yield, feed use efficiency (kg energy-corrected milk/kg DM intake), and dietary ME and CP concentrations. For the quantitative factors, subgroups were formed by dividing animals into three groups of equal size per factor.

Analysis of residuals

To identify variables that explain the difference between observed and predicted values (i.e., the residuals), the most adequate models (i.e., with the lowest RPE) among the evaluated models were further examined using Bland-Altman plots, mixed-effects models, and stepwise regression. The Bland-Altman plots were used to visually assess the agreement between the observed and predicted PDMI and created with the R package *ggplot2* (Wickham, 2016). For this, the residuals were plotted against the means of the observed and predicted values. Additionally, the 95%-limits of agreement were calculated as ± 1.96 x the SD of the residuals to quantify the residuals' spread (i.e., the models' precision; Bland and Altman, 1999).

Linear mixed-effects models were used to analyse the relation between the residuals of the most adequate prediction models and their respective input variables. The residuals were considered as the dependent variable, while the input variables of the respective prediction models were considered as independent variables. The animal was used as a random effect. Variables were considered significant at $P \leq 0.05$. For this analysis, the *lme4* package was deployed (Bates et al., 2015). The marginal and conditional R^2 was calculated for each mixed model to determine the share of variation in residuals explained by the input variables (marginal R^2) and by the random effects (conditional R^2 minus marginal R^2). Mixed model and stepwise analyses were executed using three different variants of the residuals as dependent variable, with (1) absolute values (i.e., as difference between observed and predicted PDMI, kg DM/d), (2) relative values (i.e., the absolute value in percent of the respective observed PDMI (%)), or (3) relative values without negative signs (%).

Stepwise regression was used to analyse the relation between the residuals and potential independent predictors (i.e., the remaining variables excluding the model input factors). Forward, backward, and bi-directional elimination were applied to identify possible independent predictors of the residuals among the following candidate variables: grazing season (early or late), animal breed, parity, days in milk, milk yield, fat concentration and protein concentration prior to the examination period, feed use efficiency, DM intake of fresh forage, silage, hay, and concentrate, DOM_{CP}, grazing system (i.e., continuous, rotational, or short-grass grazing), length of daily pasture access, daytime of pasture access (i.e., night-time, day-time, full-time), herbage allowance, stocking density, stocking rate, herbage mass, ambient air temperature and relative air humidity, daily eating time, daily rumination time, daily eating time during grazing, daily rumination time during grazing, daily number of rumination chews, and daily number of rumination chews during grazing. Variables were entered and removed based on P values, using the functions *ols_step_forward_p*, *ols_step_backward_p*, and *ols_step_both_p* by the R package *olsrr* (Hebbali, 2020). The resulting models after stepwise regression were checked for variable inflation factors (i.e., multicollinearity) using the *VIF* function of the R package *regclass* (Petrie, 2020). Variables with a variable inflation factor ≥ 10 were removed successively from the statistical model until no variable with a variable inflation factor ≥ 10 remained. Finally, among the three resulting models from forward, backward, and bi-directional elimination, the model with the greatest R^2 and lowest Bayesian information criterion was reported in the present study. The relative importance of the predictor variables selected by stepwise regression (i.e., their individual contribution to the R^2 of the selected linear model) was quantified using the method by Lindeman et al. (1980) using the R function lmg by the package relaimpo (Grömping, 2006).

3.3 Results

3.3.1 Evaluation of the models' prediction adequacy

Based on the evaluation of individual animal observations, the MB was lowest for the stall-based models NRC, Mertens II, and De Souza with -0.1, 0.3, and -0.5 kg DM/d, respectively (Table 3.3). The behaviour-based models resulted in the greatest MB. The model Oudshoorn overestimated daily PDMI by 3.1 kg DM, whereas models Greenwood and Rombach underestimated daily PDMI by on average 2.6 and 3.3 kg DM, respectively. The models e-cow and GrazeSim overestimated PDMI by 3.2 and 1.0 kg DM/d, respectively. None of the fourteen evaluated models reached an RPE < 20 %, indicating that there was no model which predicted the PDMI with an acceptable adequacy when evaluated based on observed data from 208 to 233 individual animals (Table 3.3). The RPE for the behaviour-based as well as semi-mechanistic grazing-based models ranged from 41.9 to 52.0 %, and the proportion of their MSEP attributable to the mean plus line bias ranged from 37.1 to 48.1 % of total MSEP. The lowest RPE, and therefore the highest adequacy, was achieved by the semi-mechanistic stall-based model Mertens II (24.7 %), closely followed by the empirical model NRC (25.3 %) and the semi-mechanistic model Conrad (25.5 %). For these three models, the greatest proportion of MSEP was the random error, ranging from 84.0 to 90.2 % of total MSEP. When evaluated based on means per farm, period and supplementation group (n =

28), these three models and the original Mertens I model achieved an acceptable prediction adequacy (Table 3.4) with lowest RPE with 13.4 % for Mertens II. The evaluation on mean-basis did not substantially improve the prediction adequacy of the remaining models.

Table 3.3. Statistical evaluation of models to predict the individual pasture herbage DM intake (PDMI) of lactating dairy cows grazing semi-natural grasslands.

Models	n	MB, kg DM/d	MB, % [1]	MSEP, kg DM^2/d	Partitioning of MSEP, % MB	Line bias	Random error	RPE, %
Behaviour-based [2]								
Rombach	220	3.3	27.6	27.1	40.9	7.2	51.9	43.2
Greenwood	220	2.6	21.9	25.5	27.4	13.5	59.1	41.9
Oudshoorn	208	-3.1	-25.6	26.6	36.0	1.2	62.9	42.6
Empirical stall-based [3]								
AFRC	233	5.2	42.6	35.6	77.5	0.7	21.8	48.5
Cornell	233	1.8	14.4	13.4	23.3	13.8	62.9	29.7
De Souza	233	-0.5	-4.4	16.6	1.8	32.1	66.1	33.1
Gruber	233	3.3	27.0	19.6	56.2	1.7	42.1	36.0
NRC	233	-0.1	-0.4	9.7	0.0	9.8	90.2	25.3
Sauvant	233	3.1	24.9	22.2	42.2	7.2	50.6	38.3
Semi-mechanistic stall-based [4]								
Conrad	233	-1.1	-8.8	9.8	12.0	4.0	84.0	25.5
Mertens I	233	1.3	10.6	11.2	15.1	7.4	77.5	27.2
Mertens II	233	0.3	2.5	9.2	1.0	11.1	87.9	24.7
Semi-mechanistic pasture-based [5]								
e-cow	233	-3.2	-25.6	31.0	32.0	8.5	59.5	45.3
GrazeSim	233	-1.0	-8.1	40.9	2.4	40.1	57.5	52.0

Abbreviations: MB = mean bias; MSEP = mean square error of prediction; RPE = relative prediction error, % of observed mean PDMI.

[1] MB, % of mean observed PDMI.

[2] Rombach (Rombach *et al.*, 2019), Greenwood (Greenwood *et al.*, 2017), Oudshoorn (Oudshoorn *et al.*, 2013).

[3] AFRC (Vadiveloo and Holmes, 1979), Cornell (Fox *et al.*, 2004), De Souza (de Souza *et al.*, 2019), Gruber (Deutsche Landwirtschafts-Gesellschaft, 2006), NRC (National Research Council, 2001), Sauvant (Sauvant *et al.*, 2014).

[4] Conrad (Conrad *et al.*, 1964; Kahn and Spedding, 1984), Mertens I (Mertens, 1987), Mertens II: modified Mertens I considering a higher intake capacity (Baudracco *et al.*, 2012).

[5] e-cow (Baudracco *et al.*, 2012), GrazeSim (Vazquez and Smith, 2001).

Table 3.4. Statistical evaluation of models to predict the pasture herbage DM intake (PDMI) of lactating dairy cows grazing semi-natural grasslands based on the mean values per farm, period and supplementation group (n = 28). Only models with an RPE < 20 % are reported.

Models	MB, kg DM/d	MB, % [1]	MSEP, kg DM^2/d	Partitioning of MSEP, % MB	Line bias	Random error	RPE, %
NRC [2]	-0.1	-1.2	4.0	0.5	13.3	86.2	16.9
Conrad [3]	-1.2	-9.9	3.9	36.1	1.8	62.1	16.5
Mertens I [4]	1.3	10.5	5.4	29.4	9.2	61.3	19.4
Mertens II [5]	0.2	2.1	2.6	2.4	15.7	81.9	13.4

Abbreviations: MB = mean bias; MSEP = mean square error of prediction; RPE = relative prediction error, % of observed PDMI.

[1] MB, % of observed mean PDMI.

[2] NRC (National Research Council, 2001).

[3] Conrad (Conrad *et al.*, 1964; Kahn and Spedding, 1984).

[4] Mertens I (Mertens, 1987).

[5] Mertens II: modified Mertens I considering a higher intake capacity (Baudracco *et al.*, 2012).

3.3.2 Evaluation for different animal subgroups

To evaluate the prediction adequacy of the models based on different levels of grazing (Table 3.5) and production factors (Table 3.6), the RPE was calculated separately for different subgroups of animals. Across all evaluated models, the RPE was lower in subgroups with full-time pasture access (i.e., 20 h/d) as compared to day-time (5-9 h/d) or night-time (9-12 h/d) access, and with DMIsupp < 4.8 kg/d as compared to the subgroups with medium and high DMIsupp, i.e., ≥ 4.8 kg/d. The RPE of the full-time access and low DMIsupp subgroups was below the threshold of 20 %, for the models Cornell, NRC, Conrad, Mertens I, and Mertens II indicating an acceptable prediction accuracy for individual cow observations. Predictions by the models Conrad for animals with a feed use efficiency < 1.2 also had an RPE < 20 %. For the majority of models, the RPE was also lower for the subgroups on pastures with medium and high herbage allowance i.e., ≥ 14.3 as compared to a low herbage allowance < 14.3 kg DM/d and for Brown Swiss and Holstein-Friesian cows as compared to Simmental cows. There was no substantial difference in the RPE between different levels of energy-corrected milk yield or dietary CP concentrations.

Table 3.5. Relative prediction error (RPE, % of observed pasture herbage DM intake (PDMI)) of models to predict PDMI for different subgroups of lactating dairy cows grazing semi-natural grasslands, separated by different levels of grazing factors.

	Daytime of pasture access [1]			Herbage allowance [2]			Stocking density [3]			DMIsupp [4]		
Models	Day-time	Full-time	Night-time	low	medium	High	low	medium	high	low	medium	high
Behaviour-based [5]												
Rombach	53.4	26.2	48.0	47.7	40.4	38.6	45.4	37.9	44.0	32.6	43.1	63.6
Greenwood	44.6	21.7	54.4	47.2	43.7	29.6	32.6	42.3	46.0	29.4	48.7	53.2
Oudshoorn	63.4	24.0	37.6	53.4	29.8	42.7	52.3	36.7	36.2	21.4	34.1	90.9
Empirical stall-based [6]												
AFRC	61.4	29.1	56.8	60.6	42.8	42.6	47.2	45.0	53.6	32.3	53.0	77.8
Cornell	42.4	16.7 *	31.2	33.5	25.3	30.2	36.1	26.7	26.0	17.5 *	30.7	54.9
De Souza	36.8	29.1	32.8	36.7	26.8	35.2	37.0	31.6	30.7	28.0	29.2	49.5
Gruber	42.5	19.1 *	46.6	44.5	33.0	30.9	34.7	33.4	40.2	22.7	40.8	58.1
NRC	32.9	17.0 *	27.1	30.7	23.7	21.7	25.2	26.4	23.8	18.3 *	25.8	40.4
Sauvant	52.4	23.0	41.2	42.0	33.8	38.8	45.5	30.8	39.0	24.1	38.7	69.4
Semi-mechanistic stall-based [7]												
Conrad	35.6	16.8 *	25.2	31.5	25.4	19.6 *	25.0	27.2	23.6	17.4 *	28.9	38.2
Mertens I	33.4	17.2 *	32.1	34.5	24.5	23.1	25.8	25.5	30.6	18.6 *	30.8	41.1
Mertens II	32.2	16.9 *	26.1	30.7	22.0	21.8	24.0	25.8	23.9	18.5 *	26.4	35.9
Semi-mechanistic, pasture-based [8]												
GrazeSim	71.8	31.1	55.3	54.2	45.6	54.8	60.1	50.6	44.1	33.6	50.9	94.5
E-cow	64.2	36.2	34.4	45.5	24.5	58.2	58.3	40.3	34.8	29.3	35.6	92.4

Abbreviations: DMIsupp = DM intake supplement feed.

[1] Daytime: pasture access during day-time for 5-9 h/d (day-time), during the night for 9-12 h/d (night-time), or for full day except for milking for 20 h/d (full-time).

[2] Herbage allowance: low (< 14.3), medium (14.3 – 29.1), and high allowance (> 29.1 kg DM/cow and d).

[3] Stocking density: low (< 10.9), medium (10.9 – 20.2), and high level (> 20.2 cows/ha).

[4] DMIsupp: low (< 4.8), medium (4.8 – 11.1), and high level (> 11.1 kg DM/d).

[5] Rombach (Rombach *et al.*, 2019), Greenwood (Greenwood *et al.*, 2017), Oudshoorn (Oudshoorn *et al.*, 2013).

[6] AFRC (Vadiveloo and Holmes, 1979), Cornell (Fox *et al.*, 2004), De Souza (de Souza *et al.*, 2019), Gruber (Deutsche Landwirtschafts-Gesellschaft, 2006), NRC (National Research Council, 2001), Sauvant (Sauvant *et al.*, 2014).

[7] Conrad (Conrad *et al.*, 1964; Kahn and Spedding, 1984), Mertens I (Mertens, 1987), Mertens II: modified Mertens I considering a higher intake capacity (Baudracco *et al.*, 2012).

[8] e-cow (Baudracco *et al.*, 2012), GrazeSim (Vazquez and Smith, 2001).

* Groups with an RPE < 20 %, i.e., acceptable prediction accuracy, are indicated by an asterisk.

Table 3.6. Relative prediction error (RPE, % of observed pasture herbage DM intake (PDMI)) of models to predict PDMI for different subgroups of lactating dairy cows grazing semi-natural grasslands, separated by different levels of production factors.

	Concentrate DMI [1]			ECM before trial [2]			Feed use efficiency [3]			ME [4]			CP [5]			Breed [6]		
Models	low	medium	high	low	medium	high	low	medium	high	low	medium	high	low	medium	high	Brown	HF	Simm
Behaviour-based [7]																		
Rombach	39.2	37.5	52.2	41.4	41.5	43.5	49.8	39.6	31.4	43.0	39.7	42.8	38.2	46.6	39.7	28.0	40.2	48.7
Greenwood	50.8	33.6	38.7	43.2	41.5	35.2	50.3	33.9	32.2	44.1	43.5	32.9	41.8	42.6	37.1	34.5	28.8	51.0
Oudshoorn	36.5	30.9	61.1	40.7	41.4	39.6	31.5	42.2	53.1	30.8	45.6	50.3	41.2	40.1	44.6	41.1	34.9	48.1
Empirical stall-based [8]																		
AFRC	56.9	40.2	50.2	51.8	51.7	41.3	58.6	45.0	33.7	44.8	59.7	41.2	52.7	43.8	49.1	48.1	37.9	56.4
Cornell	27.9	22.4	41.3	27.5	29.9	30.1	33.4	28.6	24.9	24.3	34.5	31.7	31.5	30.5	26.5	21.0	22.5	37.5
De Souza	26.5	31.7	40.6	30.3	37.0	29.5	26.7	32.2	41.3	32.9	33.7	32.3	34.5	33.1	31.5	32.9	33.8	32.6
Gruber	41.8	30.1	37.6	37.3	38.8	29.9	45.8	31.1	22.9	36.2	42.9	27.5	39.7	33.5	34.7	27.1	29.3	43.7
NRC	23.4	21.9	31.7	25.5	25.8	21.9	25.5	18.8 *	30.9	24.0	25.5	26.3	26.0	23.9	26.1	18.2 *	23.4	29.3
Sauvant	36.7	31.9	48.8	36.4	38.8	37.7	42.1	38.3	31.8	34.2	46.0	35.7	44.3	35.4	34.5	30.2	30.7	46.4
Semi-mechanistic stall-based [9]																		
Conrad	23.9	21.0	33.2	24.6	26.7	23.2	19.6 *	17.8 *	37.7	22.2	26.0	28.8	26.6	24.9	24.9	18.5 *	23.1	29.9
Mertens I	29.1	24.0	29.5	30.7	28.0	22.5	32.4	22.4	24.0	27.2	30.1	24.0	29.7	22.9	29.4	23.5	24.8	30.9
Mertens II	25.2	21.4	28.7	26.3	24.7	21.8	26.8	18.8	27.5	23.9	26.4	23.8	24.8	23.1	26.4	21.3	21.6	28.4
Semi-mechanistic, pasture-based [10]																		
GrazeSim	51.0	38.2	71.3	44.7	46.9	52.3	47.5	48.3	61.1	45.1	61.8	50.9	58.3	53.6	41.2	31.5	36.3	67.5
E-cow	34.1	36.2	66.5	35.7	48.5	43.8	28.8	50.0	56.7	34.1	56.4	47.6	51.5	40.4	43.7	46.1	33.3	52.6

Abbreviations: DMI = DM intake; ECM = energy-corrected milk yield; ME = metabolisable energy.
[1] Concentrate DMI: low (< 0.5), medium (0.5 – 2.8), and high level (> 2.8 kg DM/d).
[2] ECM before trial: low (< 22.1), medium (22.1 – 27.1 kg), and high level (> 27.1 kg/d).
[3] Feed use efficiency: low (< 1.0), medium (1.0 – 1.2), and high level (> 1.2 kg ECM/kg DMI).
[4] ME: low (< 9.2), medium (9.2 – 10.0), and high level (> 10.0 MJ/kg DM).
[5] CP: low (< 14.3), medium (14.3 – 15.7), and high level (> 15.7 MJ/kg DM).
[6] Breed: Brown = Brown Swiss, HF = Holstein-Friesian, Simm = Simmental.
[7] Rombach (Rombach *et al.*, 2019), Greenwood (Greenwood *et al.*, 2017), Oudshoorn (Oudshoorn *et al.*, 2013).
[8] AFRC (Vadiveloo and Holmes, 1979), Cornell (Fox *et al.*, 2004), De Souza (de Souza *et al.*, 2019), Gruber (Deutsche Landwirtschafts-Gesellschaft, 2006), NRC (National Research Council, 2001), Sauvant (Sauvant *et al.*, 2014).
[9] Conrad (Conrad *et al.*, 1964; Kahn and Spedding, 1984), Mertens I (Mertens, 1987), Mertens II: modified Mertens I considering a higher intake capacity (Baudracco *et al.*, 2012).
[10] e-cow (Baudracco *et al.*, 2012), GrazeSim (Vazquez and Smith, 2001).
* Groups with an RPE < 20 %, i.e., acceptable prediction accuracy, are indicated by an asterisk.

3.3.3 Analysis of residuals

The Bland-Altman graphs (Fig. 3.1) plot the residuals of the models NRC, Conrad, and Mertens II against the mean of the observed and predicted PDMI. The plots for the models NRC and Mertens II illustrate a uniform scatter of residuals around the zero-line, which resulted in a MB close to zero for these two models. The lower 95%-limits of agreement were located at -6.1, -6.9, and -5.6 and the upper 95%-limits at 6.1, 4.7, and 6.3 kg DM/d for the models NRC, Conrad, and Mertens II, respectively. This indicates a uniform, but wide distribution for 95 % of the models' residuals.

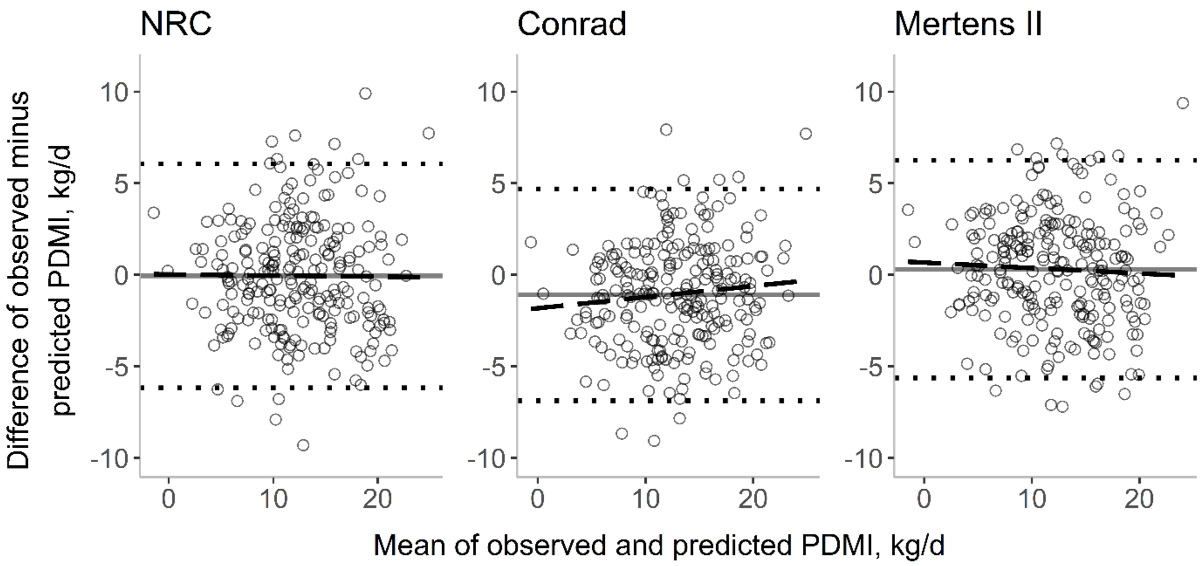

Fig. 3.1. Bland-Altman plots showing the relationship between the residuals and the mean of observed and predicted pasture herbage DM intake (PDMI) for the models NRC, Conrad, and Mertens II evaluated with a dataset with lactating dairy cows grazing semi-natural grasslands on organic dairy farms (n = 233). The dashed line corresponds to the simple linear regression line between residuals and mean of observed and predicted PDMI; the dotted lines indicate the lower and upper 95%-limits of agreement; the solid line indicates the mean bias between observed and predicted PDMI.

The residuals for the models with the lowest RPE i.e., NRC, Conrad, and Mertens II, were analysed using mixed effects models, and stepwise regression. The results of the mixed models using the input variables of the intake models as fixed effects and the relative residuals with positive and negative signs as dependent variable are summarised in Supplementary Table S3.3. The DMIsupp was the only significant input factor for the relative residuals of all three models. The marginal R^2 of the respective mixed models, however, indicated that the DMIsupp effect was negligible. For the relative residuals only 3 to 5 % of the variation were explained by the fixed effects of the mixed models. In case of the absolute residuals, this share was greater (marginal R^2 between 14 to 23 %).

Stepwise regression was used to identify potential predictors among a pool of independent grazing and production factors for explaining the relative residuals of the models NRC, Mertens II and Conrad (Supplementary Table S3.4). Backward selection rendered greater adjusted R^2 values and lower Bayesian information criterion values than forward or bi-directional selection for both analyses, based on relative residuals including or excluding negative signs. The adjusted R^2 values of the backward-selected models ranged between 0.03 and 0.05 (Table 3.7). When stepwise regression was performed for predicting the absolute residuals, the greatest adjusted R^2 and lowest Bayesian information criterion values were achieved by forward selection. For these forward-selected models, the adjusted R^2 ranged between 0.73 and 0.77. The predictor with the greatest relative importance (between 67.9 and 71.9 %) among the retained variables was feed use efficiency. It was related to absolute residuals of the models NRC, Conrad, and Mertens II by a decrease of 12.6, 12.7, and 12.5 kg PDMI/d per unit increase in feed use efficiency (kg energy-corrected milk yield/kg DM intake), respectively.

Table 3.7. Adjusted R^2 of linear regression models identified via stepwise regression to identify independent predictors for the residuals of pasture herbage DM intake (PDMI, i.e., observed – predicted PDMI) estimated by the models NRC [1], Conrad [2], and Mertens II [3] for lactating dairy cows grazing semi-natural grasslands.

Models	Absolute residuals	Relative residuals	Relative residuals without negative signs
NRC	0.77	0.05	0.04
Conrad	0.73	0.03	0.05
Mertens II	0.76	0.05	0.05

[1] NRC (National Research Council, 2001).
[2] Conrad (Conrad *et al.*, 1964; Kahn and Spedding, 1984).
[3] Mertens II: modified Mertens I considering a higher intake capacity (Mertens, 1987; Baudracco *et al.*, 2012).

3.4 Discussion

The reference dataset represents the wide range of grazing and production factors found on commercial farms in South Germany using semi-natural grasslands for grazing, which differed substantially from the commonly studied temperate grazing systems with regards to their supplementation strategy and pasture conditions (Bargo et al., 2003; Pérez-Prieto and Delagarde, 2012). Cows in the present study were predominantly supplemented with forages (mean forage to concentrate ratio of DMIsupp: 71:29, on DM basis), such as grass hay, grass silage, or freshly cut forages. The type and combination of forage and concentrate supplements, however, differed greatly between farms, which is typical of the diversity of farm structures and management systems found in this region (Velasco et al., 2021). Further, a comparably low herbage mass (348 kg DM/ha) was measured on the grazed semi-natural grasslands of the observed farms. In contrast, herbage mass values between 1 800 to 3 700 kg DM/ha were identified in a meta-analysis characterising intensive strip-grazing systems with minimal supplementation (i.e., < 1 kg DM/d; Pérez-Prieto and Delagarde, 2012).

3.4.1 Limitations of reference dataset

The aim of the present study was to evaluate the adequacy of existing models to estimate the PDMI of individual and groups of dairy cows grazing semi-natural grasslands, using the above-described on-farm reference dataset. As it is inherently challenging to measure the actual feed intake of individual grazing animals (Hellwing et al., 2015), a certain error in the reference PDMI values is expected due to (1) the chosen methodology to determine DM intake and (2) the method used to estimate individual DMIsupp.

The DM intake was determined by double-marker technique using TiO_2 as external and faecal CP as internal marker. There are limitations to the TiO_2 methodology related to total marker recovery and the use of faecal spot samples (de Souza et al., 2015; Guinguina et al., 2019). Yet, errors related to these limitations were minimised by several measures. Firstly, the TiO_2 was administered via concentrate mixtures and its complete ingestion was assured by feeding each animal an individual dosage, and by observing the animal until the total mixture was consumed. Thus, nine total cow observations were cleared from the dataset due to reluctance of animals to ingest the entire marker dosages. Secondly, faecal spot sampling started from Day 6 after five days of TiO_2 dosing to assure that the marker mixed homogenously with digesta and thus a total faecal recovery of the TiO_2 marker. Moreover, to minimise diurnal variation in faecal TiO_2 excretion and to be able to collect representative samples, TiO_2 was administered and faecal samples taken twice daily (Glindemann et al., 2009). The DOM_{CP} of diets ingested by individual animals was estimated from faecal CP concentrations (Lukas et al., 2005) to account for selective foraging behaviour of cows on pasture and to capture possible interactive effects between different diet ingredients (Bruinenberg et al., 2003; Schneider et al., 2011). This equation was recently re-evaluated by Lukas et al. (2021), who confirmed its adequacy for determining the DOM_{CP} of diets ingested by lactating cows grazing intensive as well as extensive pastures and with a moderate to high concentrate supplementation (3.2 – 7.7 kg DM/d). Overall, the chosen approach to determine the DM intake of individual cows resulted in realistic DM intake values, despite the mentioned limitations. The observed mean DM intake of 21.1 kg/d was comparable to values (20.7 kg/d) observed for lactating Simmental cows with a milk yield of 23.0 kg/d with a forage-dominant diet in a Swiss organic dairy farm (Leiber et al., 2015). Similarly, a mean DM intake of 21.6 kg/d was observed by Haselmann et al. (2020) in organic lactating Holstein-Friesian cows with an intake of 18.1 kg forage DM/d and 3.5 kg concentrate DM per day.

For 178 of 233 individual cow observations, individual DMIsupp was not measured, but calculated using measured DMIsupp on herd-level and the individual animal's eating time in barn. The mean time spent eating (560 ± 72.1 min/d) was comparable to values observed for grazing

Holstein-Friesian cows with concentrate supplementation (527 min/d; Dohme-Meier et al., 2014) and for grazing Simmental cows receiving ryegrass or sainfoin pellets in barn (555 min/d; Kapp-Bitter et al., 2021). Eating time might not be the most adequate parameter for direct estimation of feed intake of dairy cows due to the substantial variation in eating rate between individual animals (Leiber et al., 2016). In view of the on-farm approach of the present study, this approach was the most sensitive alternative to capture the variation in individual DMIsupp of the observed cows, although it does represent a limitation to the validity of the reference dataset. On-farm experiments may face methodological limitations but make use of the variation in target variables across farms which enables a greater transferability of the present findings to the diversity of practical grazing conditions.

3.4.2 Prediction adequacy across models

The models NRC, Conrad, and Mertens II resulted in the lowest RPE among the evaluated models. When evaluated based on the mean observed PDMI, their RPE values were comparable with the RPE of previous studies evaluating the adequacy of PDMI predictions by the model e-cow using weekly PDMI means for different Holstein-Friesian strains (9.1 %; Baudracco et al., 2012) and the grazing-based model GrazeIn using PDMI values on paddock level (10.0 %; Roca-Fernández and González-Rodríguez, 2018). In case of the models Conrad and Mertens II, their acceptable prediction accuracy may be due to the semi-mechanistic nature of these models, which made them less prone to be overfitted to specific production conditions (Tedeschi et al., 2019). Among these two models, Mertens II was even more adequate with a lower MB and RPE than the Conrad model, both, when evaluated on individual cow basis or for animal groups. There were two options available to adapt Mertens II to the reference dataset: (1) by assuming a greater NDF intake capacity for animals used to forage-dominant diets and (2) by using an updated equation of the German recommendations for ME requirements of dairy cows. This adaptability and its semi-mechanistic nature made Mertens II the most promising model to predict the PDMI for cows grazing on semi-natural grasslands. The prediction adequacy of the model NRC for individual animals was comparable with the modelling performance of the Mertens II model, despite the empirical nature of the NRC model, which is likely related to the substantial size of its reference dataset (n = 17 087 weekly cow observations), and the moderate performance level of cows in the NRC reference data (Rayburn and Fox, 1993: mean fat-corrected milk yield = 26.8 kg/d) that was similar to the mean milk yield in the present study.

None of the remaining models were nearly as adequate as the models NRC, Conrad, Mertens I or Mertens II, neither when evaluated based on individual cow observations nor when predicting the mean PDMI of different animal groups. A greater prediction adequacy was expected for the

Gruber model, because it was developed from a wide dataset with cows from Germany, Switzerland, and Austria and uses breed-specific coefficients for the same breeds as covered in the present study (i.e., for Holstein-Friesian, Simmental, and Brown Swiss). The Gruber model, however, relies on empirical equations, primarily based on the observed correlation between the DM intake and lactation stage of stall-based dairy cows, which likely overfitted the model for the stall-based diet and controlled production conditions of their dataset, while ignoring additional energy expenditures, for instance, due to stress or grazing activity (Dohme-Meier et al., 2014). Similarly, the De Souza model was developed using data of stall-fed Holstein-Frisian cows with a mean milk energy production of 29.8 Mcal/d, which substantially exceeded the mean milk energy yield in the present study (17.4 Mcal/d), implying considerable differences in the ME use efficiency between the cows of the De Souza reference dataset and the cows of the present study (Gruber et al., 2021). The empirical nature of the Gruber and De Souza models and the outlined fit of their equations to their underlying datasets are exemplary for explaining the poor modelling adequacy of empirical models for production conditions other than the conditions of the model development dataset.

Despite their adaptation to grazing conditions, the behaviour- and semi-mechanistic grazing-based models had the lowest prediction adequacy among the evaluated models. This was likely linked to (1) differences in sward characteristics and (2) herbage availability between the observed grazing conditions and those considered for model development. Firstly, Boval and Sauvant (2019, 2021) summarised that PDMI was determined by the interaction of sward characteristics, such as sward height and herbage mass, with the cows' ingestive behaviour during grazing. Their meta-analyses, for instance, revealed positive relations between sward height (cm) and intake rate (mg DM intake/kg BW and min) and sward height and bite mass (g/eat bite). Considering the lower herbage availability observed in the present study due to the low sward height (mean compressed sward height = 4.1 ± 1.44 cm), the predictive models likely overpredicted PDMI for cows grazing on low-yielding semi-natural grasslands, because they assumed a greater intake rate (e.g., Greenwood) or bite mass (e.g., Oudshoorn) than was attainable under the present grazing conditions. Further, the relationship between PDMI and herbage availability is considerably affected by the cutting height of the sampled herbage due to differences in pasture bulk density across the sward's strata (Pérez-Prieto and Delagarde, 2012). The e-cow model, for instance, uses an empirical relationship between PDMI and herbage biomass 4 cm aboveground. However, pasture herbage was harvested at 3 cm aboveground in the present study, which may have also contributed to the prediction bias. Another possible explanation for the low prediction adequacy of the behaviour-based models was related to the sensor-technology used. For the development of the two behaviour-based models, Oudshoorn and Greenwood, chewing behaviour of cows was measured by different sensors (accelerometer), for which different algorithms for the classifications of mastication, prehension, rumination, and eating chews or bites were used as compared to those used for determining the chewing behaviour in the present study (RumiWatch Converter V0.7.3.36). These sensor-related differences and the differences in the herbage characteristics between the observed grassland conditions and those considered for the models' development are potential reasons for the low prediction adequacy of the behaviour- and semi-mechanistic grazing-based models.

3.4.3 Prediction adequacy on individual animal basis

Prediction adequacy was lowest in Simmental cows

The RPE for different subgroups distinguished by grazing and production factors revealed substantial differences in the prediction adequacy between different breeds, levels of DMIsupp, and animals with different timing and duration of pasture access. The PDMI predictions for Holstein-Friesian and Brown Swiss cows were more adequate than for Simmental cows across most models. The differences in prediction adequacy between breeds could, however, not be attributed to core systematic factors by the statistical analyses. According to the residual analysis, none of the considered production management and behaviour variables, which may differ between Simmental cows and other breeds, such as milk yield, BW, or chewing behaviour, were sources for systematic prediction biases. There was also no difference in the RPE of subgroups differentiated by feed use efficiency. Prendeville et al. (2010) and Sæther et al. (2006) reported differences in grazing behaviour and activity between different dairy breeds with potential influences on nutrient intake and intake rate on pasture. Such differences in grazing behaviour cannot be fully excluded, and might, at least partly, explain the differences in prediction adequacy between breeds. Moreover, lower prediction adequacy in Simmental cows can also be related to random variation in the predicted PDMI values. One source of random variation might be the measured BW. Body weight is an input variable for estimating energy requirements for maintenance and locomotion, and, hence, for several PDMI prediction models (Smith et al., 2021). Variation in and mean of individual BW observations (710 ± 72.4 kg) are, however, comparable with values observed in Austrian Simmental dairy cows by Köck et al. (2018: 731 ± 85 kg) demonstrating that the BW of Simmental cows was adequately captured by the measuring tape. Thus, the lower prediction adequacy cannot be fully explained by systematic or random variation but should be kept in mind as this dual-purpose breed is common within organic and small-scale dairy farming, as seen in Germany, Austria, or Switzerland.

Prediction adequacy was greatest with low supplementation level and high daily pasture access

The RPE differed between animal subgroups with pasture access at different daytime or with different levels of DMIsupp. The models Cornell, Conrad, NRC, and Mertens I and II were able to predict the PDMI of individual cows with an acceptable adequacy, when animals had access to pasture at day and night (i.e., full-time), or when their DMIsupp was < 4.8 kg/d. Both factors were interrelated. Full-time grazing was only performed in periods when pasture herbage mass per paddock was high, which concurred with a low supplementation level. Possible reasons for differences in the adequacy of the estimation models depending on the DMIsupp level may have been (1) the methodological approach for calculating DMIsupp, or (2) differences in the grazing behaviour depending on the supplementation level or types of supplements. For 55 of the 233 observations, there was no need to estimate the individual DMIsupp via the individual eating time in barn, because measurements from feeding stations were available, or because no supplement feed was offered. All these observations belonged to the subgroup with a DMIsupp < 4.8 kg/d. Hence, the lower RPE for this subgroup may be, at least in part, associated to the lower error in DMIsupp values. Nevertheless, the greater RPE with high than with low DMIsupp may also be related to observations by Wright et al. (2016), who detected a greater between-cow variation in eating time on pasture and in barn with greater supplementation in barn. These inter-animal variations in eating behaviour potentially increased variation in individual PDMI, and thus RPE in the subgroups with a DMIsupp ≥ 4.8 kg/d.

Lack of precision affected prediction adequacy more than lacking accuracy

The models NRC, Conrad, and Mertens II achieved a satisfactory prediction accuracy for PDMI of individual cows with a MB ≤ 10 %. However, the Bland-Altman plots showed a wide distribution of the models' residuals around the MB i.e., a lack of precision (Montenij et al., 2016), indicating that the models' substantial MSEP i.e., prediction bias, was rather owing to a lack of precision than to low accuracy. The high share of random error in total MSEP, which constituted 84 to 90 % of the MSEP of the models NRC, Conrad, and Mertens II, is another indication for the lack of precision. The random variation was likely related to inter-animal variation or methodological limitations. In this line, RPE of these three models greatly declined when they were evaluated using the mean PDMI per treatment group to 11.4, 16.5, and 16.9 % for the models Mertens II, Conrad, and NRC, respectively, which removed the natural and measurement-related variance between individual animals. The remaining components of the MSEP showed that only 10 to 16 % of the prediction bias was related to structural deficiencies in the models. Mixed model and stepwise analysis were used to identify variables that possibly explain this systematic bias. When the absolute residuals were used as outcome variable for these analyses, factors were selected, such as the daily milk yield or feed use efficiency, that were correlated to DM intake and PDMI. The latter variables, in turn, were related to the absolute residuals. The relative residuals, with and without negative signs, thus, represented a more sensitive basis for identifying potential bias factors independent of the level of the PDMI. The marginal R^2 of the mixed models (< 0.01) and the adjusted R^2 of the stepwise regression models (≤ 0.05) revealed that the selected significant fixed effects were of negligible importance for explaining the variation in the relative residuals of the models NRC, Conrad, and Mertens II. The remaining systematic bias of 10 to 16 % could, thus, not be explained further by the methodology and considered predictor variables chosen for the analysis of the residuals.

Random error due to natural variation between individual cows

The relation between feed intake and productive performance i.e., feed use efficiency, is well known to greatly vary between individual animals despite similar diets, feed intake and performance levels, as well as husbandry and environmental conditions. This is not only attributable to differences in physical or physiological parameters, but also to individual differences in energy losses and retention (Guinguina et al., 2020; Martin et al., 2021), factors that may not be accounted for by mathematical intake models but add a source of natural variation that hampers the models' precision. For instance, Gregorini et al. (2015) and Lahart et al. (2020) found differences in the residual feed intake (as an indicator for feed use efficiency) between individual cows. The authors of both studies related this to between-cow differences in grazing behaviour. Hence, a negative correlation (Spearman's rank correlation: -0.24) between residual feed intake and grazing meal duration and a positive relation between number of grazing meals and residual feed intake (0.42) in the study of Lahart et al. (2020) indicated that more efficient beef cattle appear to have fewer, but longer grazing meals. Further, the ranking of animals according to their residual feed intake was not constant but varied depending on whether the cows received a stall-based or a grazing-based diet (Lawrence et al., 2012; Lahart et al., 2020), implying diet-induced differences in the inter-animal variation in residual feed intake. Finally, selective grazing behaviour determines nutrient intake of grazing cows and could be another reason for between-cow variation in feed use efficiency. Schneider et al. (2011) compared the digestible OM of dairy cows receiving clipped herbage from grazed pastures to cows grazing on the same paddock. Although both groups were offered the same herbage, digestible OM values determined with the same methodology differed between cow groups, indicating differences in selective feeding behaviour between the grazing and stall-fed cows. Differences in residual feed intake, selective feeding behaviour, and thus in nutrient intake between and even within animals are sources of natural variation which might at least partly explain the lack of precision in PDMI predictions observed in the present study.

3.4.4 Outlook to improve herbage intake models

The Mertens II model appeared to be the most promising amongst all tested models for predicting PDMI of lactating dairy cows grazing semi-natural grasslands. It achieved the highest modelling performance when evaluated based on the mean observed PDMI and allowed for adequate prediction of PDMI of individual cows with low DMIsupp level. Nevertheless, when used to predict PDMI of individual animals of other subgroups, this model also did not meet the threshold for an acceptable adequacy. It was concluded that the low prediction adequacy was predominantly related to a lack of precision, and hence, either to the model's inability to capture inter-animal variation in PDMI or to random variation pertaining to the present study's methodological limitations. Possible options for improving the precision and thus adequacy of the PDMI predictions by Mertens II, or possibly other PDMI models, could be the addition of further or alternative explanatory variables, the integration of sensor data, and/or the use of more sophisticated approaches in data analysis. Firstly, there might be merit in attempting to capture the influence of the amount of DMIsupp on PDMI by adding respective variables to the model. Secondly, the use of behavioural, sensor-based data allows for integration of measured, individualised data into prediction models in addition to, for instance, performance characteristics which are already part of the Mertens II model. This could help in accounting for inter-animal differences in feeding behaviour and thus PDMI. Nevertheless, the behaviour-based models evaluated in the present study could neither accurately nor precisely predict PDMI. This is likely since feeding behaviour and feed use efficiency vary between individual cows despite similar PDMI due to herd- and management-specific differences in the learned and socially driven behaviours of lactating dairy cows (Illius et al., 2004; Moreno García et al., 2020) and due to natural variation between cows (Guinguina et al., 2020). The consideration of behaviour-based data could be combined with new approaches in data analysis. For instance, the inclusion of non-linear components in existing PDMI models such as Mertens could account for the fact that the coefficients for the relationship between the number of bites and the PDMI are not constant but vary depending on animal traits, such as BW (Boval and Sauvant, 2019), or forage availability (Boval and Sauvant, 2021). However, the solution to increase the precision and accuracy of PDMI models should not be the development of even more complex mechanistic models (Tedeschi et al., 2019), but rather the adaptation of the existing models to better capture inter-animal variation in PDMI, e.g., using the individual or a combination of the aforementioned options. Alternatively or additionally, machine learning could be used to capture the inter-animal variation in feeding behaviour and efficiency by combining available data from sensor-based behavioural observations and milk performance records to adapt PDMI models for individual animals (Ellis et al., 2020).

3.5 Conclusions

Among the models evaluated in the present on-farm study, the slightly modified semi-mechanistic stall-based model by Mertens (1987) allowed for most adequate and acceptable prediction of the mean PDMI of groups and even of individual lactating dairy cows grazing temperate, semi-natural grasslands full-time and receiving limited amounts of supplemental feeds. Instead, existing empirical, behaviour-based, and semi-mechanistic grazing-based models cannot predict PDMI of lactating dairy cows with acceptable accuracy and precision. The model Mertens II can be applied for groups of cows across a diverse range of practical farms using semi-natural grasslands for grazing, but lack of precision hampers the adequate prediction of PDMI of individual cows by the model Mertens II, likely owing to pronounced inter-animal variance in PDMI of individual animals as well as methodological challenges such the determination of supplement feed intake of individual animals. Possible options to improve the precision of semi-mechanistic prediction models are the addition of further or alternative explanatory variables, the integration of sensor data, and/or the use of more sophisticated approaches in data analysis. Such improved models may allow for accurate and precise prediction of intake of individual dairy cows and thus for an optimised use of semi-natural grasslands in Central Europe for grazing-based milk production.

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3.8 Supplementary material

Table S3.1. Input variables of the 13 models to predict pasture herbage DM intake (PDMI) of dairy cows grazing temperate semi-natural grasslands.

	Behaviour-based			Empirical stall-based						Semi-mechanistic stall-based		Semi-mechanistic pasture-based	
Models [1]	Rombach	Greenwood	Oudshoorn	AFRC	Cornell	De Souza	Gruber	NRC	Sauvant	Conrad	Mertens	e-cow	Graze-sim
Animal characteristics													
Body condition score						x							
Breed					x		x						
BW, kg/cow	x			x	x	x	x	x	x	x	x	x	x
Days in milk, n						x	x						
ME or NEL requirements, MJ/d						x					x	x	x
Parity, n	x				x		x						
Week of lactation, n								x	x		x	x	x
Feed characteristics													
DMD, g/100 g DM										x			
Concentrate DMI, kg/d	x			x			x						
ME or NEL, MJ/kg DM					x		x				x	x	x
NDF, g/100 g DM						x							
CP, g/100 g DM	x												
Herbage allowance, kg DM/cow and d						x					x	x	x
DMIsupp, kg/d	x											x	
Milk production													
Milk yield, kg/d	x			x		x	x	x		x	x	x	x
Milk fat, lactose, protein, g/100 g milk	x					x		x		x	x	x	x
Chewing behaviour													
Bite rate, n/min	x												
Eating time on pasture, min/d		x											
Prehension chews on pasture, n/d			x										

Abbreviations: DMD = DM digestibility; DMI = DM intake; DMIsupp = DM intake of supplemental feed; ME = metabolisable energy; NEL = net energy for lactation.

[1] Rombach (Rombach *et al.*, 2019), Greenwood (Greenwood *et al.*, 2017), Oudshoorn (Oudshoorn *et al.*, 2013), AFRC (Vadiveloo and Holmes, 1979), Cornell (Fox *et al.*, 2004), De Souza (de Souza *et al.*, 2019), Gruber (Deutsche Landwirtschafts-Gesellschaft, 2006), NRC (National Research Council, 2001), Sauvant (Sauvant *et al.*, 2014), Conrad (Conrad *et al.*, 1964; Kahn and Spedding, 1984), Mertens I (Mertens, 1987), Mertens II: modified Mertens I considering a higher intake capacity (Baudracco *et al.*, 2012), e-cow (Baudracco *et al.*, 2012), GrazeSim (Vazquez and Smith, 2001).

Table S3.2. Overview of grazing and farm management of nine commercial organic dairy cattle farms in South Germany during each examination period (n = 28 supplementation treatment groups).

						Diet composition, % of total DMI					
Treatment group	Farm	Year	Season [1]	Sampled cows, n	Cow breed	Pasture herbage	Grass hay	Fresh forage [2]	PMR/ Silage [3]	Concen-trates [4]	Time of daily pasture access
1	A	2019	Spring	10	Simmental	79	6	10	0	5	20:00 - 6:00
2	A	2019	Spring	10	Simmental	85	10	0	0	5	20:00 - 06:00
3	A	2019	Summer	8	Simmental	58	22	18	0	2	08:00 - 16:00
4	A	2019	Summer	9	Simmental	49	49	0	0	2	20:00 - 06:00
5	A	2020	Spring	9	Simmental	50	27	21	0	2	07:00 - 17:00
6	A	2020	Spring	9	Simmental	54	17	27	0	2	19:00 - 05:00 and 07:00 -17:00
7	A	2020	Summer	8	Simmental	68	17	14	0	1	19:00 - 05:00 and 07:00 -17:00
8	A	2020	Summer	8	Simmental	63	19	16	0	2	19:00 - 05:00
9	B	2019	Spring	10	Holstein-Friesian	81	0	0	19	0	09:00 - 16:00
10	B	2019	Summer	10	Holstein-Friesian	97	0	0	0	3	09:00 - 11:30
11	B	2020	Spring	10	Holstein-Friesian	97	0	0	0	3	09:00 - 16:00 and 18:00 - 06:00
12	B	2020	Summer	10	Holstein-Friesian	42	0	0	56	2	09:00 - 16:00
13	C	2020	Spring	10	Simmental	44	0	3	38	15	09:00 - 16:00
14	C	2020	Summer	10	Simmental	24	53	13	0	10	09:00 - 16:00

15	D	2019	Spring	10	Simmental	58	0	2	15	25	08:00 - 16:00
16	D	2019	Summer	10	Simmental	22	8	0	46	24	08:00 - 16:00
17	D	2020	Spring	14	Simmental	47	0	0	32	21	18:00 - 06:00
18	D	2020	Spring	14	Simmental	51	0	0	29	20	08:00 - 16:00
19	D	2020	Summer	12	Simmental	32	0	0	50	18	07:00 - 17:00 and 19:00 - 05:00
20	D	2020	Summer	12	Simmental	34	0	0	48	18	07:00 - 17:00 and 19:00 - 05:00
21	E	2019	Summer	10	Brown Swiss	46	47	7	0	0	07:00 - 17:00 and 19:00 - 05:00
22	E	2020	Spring	10	Brown Swiss	46	47	7	0	0	07:00 - 17:00 and 19:00 - 05:00
23	E	2020	Summer	10	Brown Swiss	26	59	15	0	0	07:00 - 17:00 and 19:00 - 05:00
24	F	2020	Spring	15	Brown Swiss, crossbred cows	84	0	8	0	8	07:00 - 17:00 and 19:00 - 05:00
25	F	2020	Summer	14	Brown Swiss, crossbred cows	84	0	8	0	8	07:00 - 17:00 and 19:00 - 05:00
26	G	2020	Spring	13	Red-Holstein	83	0	0	0	17	07:00 - 17:00 and 19:00 - 05:00
27	G	2020	Summer	12	Red-Holstein	31	0	5	43	21	07:00 - 17:00 and 19:00 - 05:00
28	H	2020	Spring	9	Holstein-Friesian, Red-Holstein	50	0	36	0	14	07:00 - 17:00 and 19:00 - 05:00

Abbreviations: DMI = DM intake; PMR = partial mixed ration.

[1] Season: spring = May to July, summer = August to October.

[2] Fresh forage: freshly cut forage from meadow or grass-clover leys.

[3] Silage: grass or maize silage.

[4] Diets with a concentrate share ≤ 5 % of DMI solely contained concentrates for titanium dioxide administration.

Table S3.3. Effect of input variables of the models NRC, Conrad and Mertens II [1] on their relative residuals of pasture herbage DM intake (PDMI, i.e., observed – predicted PDMI as % of observed PDMI) predicted for lactating dairy cows grazing semi-natural grasslands, analysed with animal as random variable.

	Estimate, % PDMI [3]			SE, % PDMI [3]			*P* value [4]		
Input variables [2]	NRC	Conrad	Mertens II	NRC	Conrad	Mertens II	NRC	Conrad	Mertens II
Intercept	-477.4	-967.0	-1481.8	769.0	8652.4	22293.0			
Milk yield, kg/d [5]	4.6	-38.9	186.0	19.5	79.7	165.2	0.81	0.63	0.26
BW, kg	1.0	-5.8	6.3	1.0	4.7	8.4	0.36	0.22	0.46
DMIsupp, kg/d	-38.3	202.5	-413.5	16.3	76.0	156.1	0.02	0.01	0.01
Week of lactation, n	-2.0	--	--	10.6	--	--	0.85	--	--
DMD of ration, g/100 g DM	--	63.5	--	--	122.8	--	--	0.61	--
Distance walked, km/d	--	--	-1365.2	--	--	830.2	--	--	0.10
ME of pasture, MJ/kg DM	--	--	-261.1	--	--	1449.5	--	--	0.86
ME of supplements, MJ/kg DM	--	--	-511.2	--	--	1529.5	--	--	0.74
NDF of pasture, g/100 g DM	--	--	249.4	--	--	249.4	--	--	0.44
NDF of supplements, g/100 g DM	--	--	-186.8	--	--	186.8	--	--	0.83

Abbreviations: DMD = DM digestibility; DMIsupp = DM intake of supplemental feed; ME = metabolisable energy.

[1] NRC (NRC, 2001), Conrad (Conrad *et al.*, 1964; Kahn and Spedding, 1984), Mertens II: modified Mertens I considering a higher intake capacity (Baudracco *et al.*, 2012; Mertens, 1987).

[2] Input variables marked with '--' were not included in the statistical model.

[3] Estimate and SE in % of observed PDMI.

[4] Not significant at $P \geq 0.05$ and no trend to differ at $P \geq 0.1$.

[5] For NRC and Conrad: Fat-corrected milk yield; for Mertens II: Energy-corrected milk yield.

Table S3.4. Results of stepwise regression with backward elimination: Effect of independent variables on the relative residuals of pasture DM intake (PDMI, i.e., observed – predicted PDMI, % of observed PDMI) estimated by the models NRC, Conrad and Mertens II [1] for lactating dairy cows grazing semi-natural grasslands.

	Estimate, % PDMI [3]			SE, % PDMI [3]			Relative importance, %			*P* value [4]		
Input variables [2]	NRC	Conrad	Mertens II	NRC	Conrad	Mertens II	NRC	Conrad	Mertens II	NRC	Conrad	Mertens II
Intercept	-722.7	28516.9	-4929.0	3558.5	21043.3	36543.7						
Parity, n	81.2	-467.5	398.5	48.8	229.5	398.5	15.8	12.6	12.8	0.18	0.14	0.15
Milk protein before trials, g/100 g	334.9	-1564.2	2712.5	356.1	1685.5	2927.0	5.8	3.5	3.6	0.44	0.53	0.53
Concentrate DMI, kg/d	-207.7	1515.4	-2566.0	73.0	433.8	753.3	44.3	34.8	34.6	0.03	0.03	0.04
DOM_{CP}, g/100 g DM	91.0	-474.9	806.5	42.7	243.9	753.6	21.3	13.3	13.2	0.10	0.06	0.06
Stocking density, n/ha	-22.3	119.0	-199.1	13.0	69.8	121.3	11.4	7.9	7.8	0.09	0.04	0.04
Eating time, min/d	--	17.1	-28.9	--	8.9	15.4	--	5.9	5.7	--	0.37	0.40
Rumination time, min/d	--	12.0	-20.3	--	9.9	17.3	--	5.6	5.5	--	0.28	0.30
Eating time on pasture, min/d	--	-8.2	14.2	--	5.4	9.4	--	6.7	6.9	--	0.15	0.15
Rumination chews on pasture, n/min	-2.0	-77.4	135.6	18.9	100.1	173.9	1.4	1.5	1.5	0.92	0.44	0.44
Type of supplementation (basis: Conserved) [5]							--	8.3	8.4	--	0.44	0.45
Fresh forage	--	1251.1	-2113.2	--	1302.6	2262.1						
No supplementation	--	4298.2	-7373.0	--	2305.5	4003.7						

Abbreviations: DMI = DM intake; DOM_{CP} = apparent total tract digestibility of organic matter, determined from faecal CP concentration.
[1] NRC (NRC, 2001), Conrad (Conrad *et al.*, 1964; Kahn and Spedding, 1984), Mertens II: modified Mertens I considering a higher intake capacity (Baudracco *et al.*, 2012; Mertens, 1987).
[2] Input variables marked with '--' were not included in statistical model.
[3] Estimate and SE in % of observed PDMI.
[4] Not significant at $P \geq 0.05$ and no trend to differ at $P \geq 0.1$.
[5] Type of supplementation: conserved = concentrates, grass silage, corn silage, grass hay; fresh forage = freshly cut meadow grass or grass-clover ley.

Chapter 4 | Adaptation of the LIVestock SIMulator (LIVSIM) to model nitrogen excretion of dairy herds grazing temperate, semi-natural grasslands

4.1 Introduction

European permanent grasslands provide several ecosystem services such as the supply of nutrients for livestock, carbon sequestration, or serving as habitat for pollinators (Schils et al., 2022). The ability of permanent grasslands to provide these ecosystem services, however, has declined during the past decades, owing to an increasingly intensified use (e.g., via greater fertilisation rates) (Schils et al., 2022). It is therefore necessary to support production systems, such as grazing-based, low-input dairy farms, which use permanent grasslands at lower intensity (i.e., with lower N fertilisation and soil cultivation intensity). One challenge in grazing-based systems, however, is that dairy cattle kept on pasture tend to use dietary nitrogen (**N**) less efficiently than cattle fed with balanced diets in the barn. Grazing-based feeding can, thus, increase the risk of N emissions to the atmosphere and hydrosphere, mainly via losses of N from urinary N excretions (Hoekstra et al., 2007).

Studies increasingly apply process-based models to evaluate the efficacy of strategies to increase N utilisation of farming systems as an alternative to time-consuming experimental approaches (Christie et al., 2014). Process-based models enable researchers to simplify the representation of pasture-based livestock systems and simulate the interactions between non-linearly interacting biotic and abiotic components of the whole system (Reed et al., 2015). As such, the effect of different grazing management strategies (e.g., supplement feeding) under different pasture conditions (e.g., pastures with different nutritional value) can be evaluated simultaneously. The ability to capture such interaction is a key requirement for reflecting the diverse grazing and farming systems which can be found across low-input grazing systems. Modelling grazing-based systems further requires a dynamic approach to capture the seasonally and spatially variable resource availability in grassland production (Snow et al., 2014). Developing a new simulation model is equally as time-consuming as experimental approaches. Thus, adapting existing models to the production system of interest has been deemed a more viable option.

Different process-based models exist to simulate grazing dairy cows (e.g., Molly: Gregorini et al., 2016) or farms within a grazing context (e.g., APSIM: Holzworth et al., 2018, and Orfee: Mosnier et al., 2017). However, they commonly require inputs which are not routinely available for low-input grazing systems e.g., due to their interaction with elaborated grassland models to predict pasture growth and nutritional value (e.g., APSIM), or the integration of intricate rumen sub-modules (e.g., Molly). Adapting a model developed for (sub-)tropical systems represents a suitable alternative since they are generally developed for data constrained systems (Bateki, 2020). One model developed to simulate cattle systems within data constrained situations where code was additionally available within the working group, is the LIVestock SIMulator (**LIVSIM**). The LIVSIM is a dynamic (monthly time-step) herd model that simulates the productive and reproductive performance of ruminants based on feed and animal genetic resources available in smallholder systems in Africa (Rufino et al., 2009). The model also predicts the associated nutrient excretion (e.g., N excretion) and greenhouse gas emissions from different animal groups (i.e., calves, heifers, and cows).

The LIVSIM was further chosen as suitable candidate model to be adapted for low-input grazing systems in temperate regions, because (1) it is semi-mechanistic in nature, and (2) because its modules were largely developed based on data and feeding recommendation systems from temperate dairy cattle systems (Bateki and Dickhoefer, 2020). It was, thus, hypothesised that the underlying biological processes underlying milk production, body weight (**BW**) change, and N excretion will be similar across production systems, where changes are predominantly required for individual coefficients and equations within the model to adapt it for temperate grassland-based ruminant systems.

Therefore, the present study aimed at (i) adapting the LIVSIM to simulate the performance and N excretion of dairy herds in low-input production systems as a response to changes in grazing conditions and management under various local farming conditions(ii) assessing the accuracy and sensitivity of predictions from the adapted LIVSIM; and (iii) using scenarios to evaluate the model's capability to simulate the influence of different supplementation strategies varying in types of feedstuffs and inclusion level on animal performance and N excretion.

4.2 Materials and methods

The modified LIVSIM from Bateki and Dickhoefer (2020) was used as the starting point for the present study, and is referred to **LIVSIM-mod**, thereafter. In the following, (1) the model adaptations, (2) reference datasets used for model assessment, and (3) the three methods used for model assessment (i.e., model evaluation, sensitivity analysis, and scenario analysis) are described in detail.

4.2.1 Adaptations to the LIVestock SIMulator (LIVSIM)

Given the modular nature of LIVSIM, the adaptations were made in key processes responsible for N use and animal production. Hence, the following modules were adapted by adopting new coefficients or by exchanging single equations or total modules to reflect the targeted production conditions: metabolisable energy (**ME**) requirements, lactation, dry matter intake (**DMI**), N excretion, and herd management. The adapted LIVSIM is, hereafter, referred to as (**LIVSIMtemp**).

4.2.1.1 Metabolisable energy requirements module

The LIVSIM-mod estimated the ME requirements (MJ/animal and d) for maintenance, lactation, activity, gestation, and protein and fat deposition (i.e., growth) following the factorial, semi-mechanistic approach of the German feeding recommendations (GfE, 2001). This factorial approach was maintained in LIVSIMtemp but with changes in the ME requirements for maintenance (eq. 1) and lactation (eq. 2 and 3) which followed the findings of Gruber et al. (2021), who suggested updated coefficients based on an extensive dataset gathered on German, Austrian, and Swiss dairy research farms:

$$MEreq\ for\ maintenance\ =\ 0.65 * BW^{0.75} \tag{1}$$

$$MEreq\ for\ lactation = potential\ milk\ yield * Milk\ ME \tag{2}$$

$$Milk\ ME = \frac{(0.39 * milk\ fat + 0.24 * milk\ protein + 0.17 * milk\ lactose + 0.1)}{k_l} \tag{3}$$

where MEreq are the ME requirements (MJ/animal and d); $BW^{0.75}$ the individual metabolic body weight (kg) of each cow; potential milk yield in kg/cow and d; milk fat, protein and lactose are in g/100 g milk; Milk ME the ME requirements in MJ/kg milk; and k_l the efficiency of ME use for lactation (k_l = 0.73).

4.2.1.2 Lactation module

For predicting the potential milk yield (kg/cow and d), the lactation curve used for the tropical LIVSIM model from Mulindwa et al. (2011) was exchanged by lactation curves fitted to primiparous and multiparous dairy cows in temperate regions as shown in equations (4; primiparous) and (5; multiparous) (INRA, 2019: equations 17.1 and 17.2, respectively).

$$PotMY_{prim} = PeakMY_{prim} \times \left(-0.55 + 1.66 \times e^{-0.0065 \times WL} - 0.72 \times e^{-0.44 \times WL} - 0.69 \times e^{-0.16(45 - WG)}\right) \tag{4}$$

$$PotMY_{mult} = PeakMY_{mult} \times \left(-0.83 + 1.92 \times e^{-0.0083 \times WL} - 0.74 \times e^{-0.88 \times WL} - 0.50 \times e^{-0.12(45 - WG)}\right) \tag{5}$$

where PotMYprim and PotMYmult are the potential milk yield (kg/cow and d) of primiparous and multiparous cows, respectively; PeakMYprim and PeakMYprim the peak milk yield (kg/animal and d) of primiparous and multiparous cows, respectively; and WL and WG are the weeks of lactation and gestation, respectively.

4.2.1.3 Dry matter intake module

In the LIVSIM-mod, voluntary DMI is predicted using a semi-mechanistic model based on Mertens (1987), which was adjusted for stall-fed cattle in the (Sub-)Tropics by Bateki and Dickhoefer (2019; model M4). For cattle grazing temperate, semi-natural grasslands, the same DMI model of Mertens (1987) was adopted, however, with slight modifications according to results of a study by Perdana-Decker et al. (2023). Accordingly, it was assumed that total DMI (kg/animal and d) is either limited by the offered feed mass (for restricted feeding situations), by the animal's energy requirements (i.e., physiologically regulated DMI), or by its intake capacity (i.e., physically regulated DMI). The total DMI was further partitioned into DMI from (1) concentrates, (2) forages offered in barn, and (3) pasture herbage. The concentrate DMI equals to the total amount of concentrates offered in barn. The DMI of forages in barn corresponds to the lowest of either the offered forages in barn, or the estimates of physiologically (eq. 6) or physically (eq. 7 and 8) regulated DMI of forages in barn:

$$Physiologically\ regulated\ forage\ DMI = \frac{MEreq - concentrate\ ME\ intake}{forage\ ME} \tag{6}$$

$$Physically\ regulated\ forage\ DMI = \frac{NDF\ intake\ capacity - concentrate\ NDF\ intake}{forage\ NDF} \tag{7}$$

$$NDF\ intake\ capacity = 0.0165 \times BW \tag{8}$$

where DMI is in kg/animal and d; MEreq the total ME requirements (MJ/animal and d); forage ME the weighted average ME concentration across the various forage types offered in barn (MJ/kg DM); concentrate ME intake calculated as the product of the ME concentration of concentrates multiplied by the amount of concentrates offered in barn (MJ/animal and d); NDF intake capacity the capacity for neutral detergent fibre (**NDF**) intake (kg/animal and d); concentrate NDF intake calculated as the product of the NDF concentration of concentrates multiplied by the amount of concentrates offered in barn (kg/animal and d); and BW the individual BW of each cow (kg) derived from the growth module.

It was stipulated that forages offered in barn are more easily accessible to the animal and fed in limited amounts. Therefore, it was assumed that total forage DMI (i.e., of forages in barn and pasture herbage) is first met by forages in barn, and that the remaining capacity for forage DMI is covered by pasture herbage. Accordingly, the DMI of pasture herbage is calculated based on the estimated DMI of forage in barn and concentrate DMI using equations 9 and 10:

$$Physiologically\ regulated\ pasture\ DMI = \frac{MEreq - forage\ ME\ intake - concentrate\ ME\ intake}{pasture\ ME} \quad (9)$$

$$Physically\ regulated\ \ pasture\ DMI = \frac{NDF\ intake\ capacity - forage\ NDF\ intake - concentrate\ NDF\ intake}{pasture\ NDF} \quad (10)$$

where forage ME intake is the intake of ME from DMI of forages in barn (derived from estimated DMI of forages in barn and its ME concentration) (MJ/animal and d); concentrate ME intake calculated as the product of the ME concentration of concentrates multiplied by the amount of concentrates offered in barn (MJ/animal and d); forage NDF intake the intake of NDF from DMI of forages in barn (derived from estimated DMI of forages in barn and its NDF concentration); concentrate NDF intake calculated as the product of the NDF concentration of concentrates multiplied by the amount of concentrates offered in barn (kg/animal and d); and pasture NDF the NDF concentration in pasture herbage (g/kg DM).

4.2.1.4 Nitrogen excretion module

In the LIVSIM-mod, the N excretions in urine and faeces (g/animal and d) are calculated as the difference between the predicted N intake, and the predicted N retained via BW gain, lactation, and conception according to the utilisable CP requirements following the feeding recommendations by GfE (2001). This approach was changed to the INRA (2019) feeding recommendations for ruminants in the present study. The model which was considered for adoption in LIVSIMtemp adheres to the description of the INRA model (model I) outlined by (Salazar-Cubillas et al., 2024: Table 4). In a preliminary evaluation, the predictions of urinary and faecal N excretion by model I and by slightly modified versions thereof were evaluated based on the dataset described in Chapter 2 (n = 33 treatments with n = 323 animals). An amended version of model I with two modifications was finally adopted in LIVSIMtemp (Table 4.1). The first modification involved a shift in the approach for calculating fermented organic matter (a precursor for estimating microbial protein synthesis). Instead of an empirical equation (INRA, 2019: equation 26.7), a semi-mechanistic approach was adopted in LIVSIMtemp (INRA, 2019: equation 3.34). In the latter, fermented organic matter is not determined as a function of organic matter digestibility but calculated by subtracting from digested organic matter all the dietary fractions (i.e., starch, protein, NDF, fatty acids) that are digested postruminally. The second modification was exchanging equation 17.22 by equation 17.7 by INRA (2019) to estimate the use efficiency of metabolisable protein.

Table 4.1. Main fractions and inputs required for estimating urinary and faecal nitrogen (N) excretion using the modified LIVestock SIMulator for temperate, low-input dairy production systems (LIVSIMtemp). The model structure follows the INRA (2019) feeding recommendations as outlined in detail in Salazar-Cubillas et al. (2024; model I), with two modifications.

Urinary N excretion (g/animal and d) = $(1 - P_{eff}) \times MP/6.25 + EUN + N_{recycled} + MNA + 0.47 \times N_{bal}$

Faecal N excretion (g/animal and d) = $BEDN + UDN + WSN + 4.30 \times DMI$

Fraction	Description	Required inputs or fractions
BEDN	Microbial and endogenous debris N (g/d)	- MCP (g/d)
DMI	DM intake (kg/d)	- See 2.1.3
EUN	Endogenous urinary N (g/d)	- Body weight, kg
FOM	Dietary fermentable OM concentration (g/kg DM)	- OMd (g/100 g OM) - Dietary OM concentration (g/kg DM) - Protein, starch, NDF, and fatty acids truly digestible at the intestine (g/kg DM)
MCP	Duodenal microbial CP flow (g/d)	- FOM (g/kg DM) - Proportion of concentrate in the diet (g DM/g DM)
MNA	Microbial nucleic acids (g/d)	- MCP (g/d)
MP	Metabolisable protein supply (g/d)	- RUP (g/kg DM) - MCP (g/d) - True intestinal digestibility of RUP, (g/100 g RUP)
MP_{eff}	Use efficiency of metabolisable protein (g/g)	- MP (g/d) - DMI (kg/d) - Milk yield (kg/d) - Milk protein concentration (g/100 g milk)
N_{bal}	N balance (g/d)	- DMI (kg/d) - Dietary ME concentration (MJ/kg DM) - ME requirements for lactation (MJ/d) - ME requirements for maintenance (MJ/d)
$N_{recycled}$	N recycled excreted in urine	- Rumen N balance (g/d)
OMd	Dietary OM digestibility (g/100 g OM)	- Dietary CP concentration (g/kg DM)
UDN	Undigested dietary N (g/d)	- RUP (g/kg DM) - DMI (kg/d)
WSN	Water-soluble N (g/d)	- Non-digestible NDF (g/kg DM) - DMI (kg/d)

CP = crude protein; DM = dry matter; ME = metabolisable energy; NDF = neutral detergent fibre; OM = organic matter; RUP = rumen-undegradable CP.

4.2.1.5 Breed- and herd management-specific adaptations

Breed-specific model coefficients for BW at birth, age-dependent minimum BW for reproduction, age-dependent minimum and maximum BW, maximum average daily BW gain, as well as potential peak milk yield, and milk composition were used to represent Simmental, Brown Swiss, and Holstein-Friesian cattle breeds (Table 4.2). Further, breed-specific protein and energy content in BW gain are determined through interpolation between the animal's age and the corresponding values for protein and energy in 1 kilogram of BW gain (Table 4.3).

Table 4.2. Sources for breed-specific coefficients integrated in the modified LIVestock SIMulator for temperate, low-input dairy production systems (LIVSIMtemp) to represent Simmental, Brown Swiss, and Holstein-Friesian dairy cows.

Breed-specific coefficients	Source
Body weight at birth, kg	KTBL, 2015
Age-dependent minimum body weight at first conception, kg	Eilers, 2004; Anacker et al., 2009; Reiter et al., 2014
Age-dependent minimum and maximum body weight, kg	Coffey et al., 2006; Gappmaier et al., 2021
Maximum average daily body weight gain, kg/d	Albertí et al., 2008
Potential peak milk yield, kg/d	Jeretina et al., 2013; Glatz-Hoppe et al., 2019
Milk composition, g/100 g milk	Regional Association for Performance and Quality Inspection in Animal Breeding of Baden-Württemberg (LKV-Baden-Württemberg): annual reports from 2012 to 2022 across all registered members of the association; combined with INRA (2019, eq. 17.3) to retrieve lactation-stage dependent milk fat concentrations.

To account for differences in herd management between temperate and tropical dairy farms, the age-related calving rate, maximum length of calving interval, maximum length of lactation, weaning age, and age-dependent mortality were adapted using the mean value of the respective parameters observed across 27 practical dairy farms interviewed by Velasco et al. (2021). Further, in the original LIVSIM, probability of conception was determined by the animal’s body condition and increased with increasing postpartum length. In LIVSIMtemp, the average days open were added to consider the influence of the farmer’s individual breeding and insemination strategy on the probability of conception.

Table 4.3. Protein and energy content in body weight (BW) gain of Simmental (SIM), Holstein Friesian (HF), and Brown Swiss (BS) cattle[1] integrated in the modified LIVestock SIMulator for temperate, low-input dairy production systems (LIVSIMtemp).

Age (yr)	Average daily BW gain (g/d)		BW (kg)		Protein content (g/kg BW gain)	Energy content (MJ/kg BW gain)
	SIM	HF/BS	SIM	HF/BS		
0.25	800	750	120	130	158	6.6
0.75	700	700	268	270	146	10.8
1.58	650	650	516	480	140	16.0
3.00	140	130	750	590	138	17.0
4.50	0	0	750	640	138	17.0
20.0	0	0	750	640	138	17.0

[1] For animals with a BW < 550 kg: estimated from BW and daily BW (GfE, 2001: Table 1.5.1). The age- and breed-specific daily BW gain and BW were computed in accordance with the data by Gappmaier et al. (2021). For animals with a BW > 550 kg and a daily BW gain < 500 g/d: fixed protein and energy contents (GfE, 2001; page 22, 23, and 36).

4.2.2 Model Assessment

The LIVSIMtemp was assessed (1) by evaluating the accuracy of LIVSIMtemp outputs based on comparisons with observed data, (2) by comparing outputs for different feeding scenarios to test the model's plausibility, and (3) by a sensitivity analysis. For these three types of assessments, a reference dataset comprising data from two on-farm studies was used, which is described in the following.

4.2.2.1 Reference herd data

The reference data for model assessment was gathered during interviews (2018, n = 27 farms) and during feeding trials (2019 and 2020, n = 9), which are described in more detail by Velasco et al. (2021), Perdana-Decker et al. (2023), and in Chapter 2. The studies involved commercial organic dairy farms located across five natural regions in Southwest Germany (Schwäbische Alb, Schwäbisches Keuper-Lias-Land, Donau-Iller-Lech-Platte, Voralpines Hügel- und Moorland, Hochschwarzwald) covering diverse agroecological conditions.

In the first study, semi-quantitative interviews were used to gather information on size and structure of dairy herds, animal feeding across the year, and grazing management. The trial dataset comprised one to four trial periods (à 6 days) for each of the nine farms. Dry matter intake, N intake, milk yield, and milk composition were measured daily during the six consecutive sampling days per trial period. The faecal N excretion was calculated from estimated faecal DM output multiplied by its N concentration, whereas urinary N excretion was estimated as the difference between N intake and the milk N secretion and faecal N excretion. The interviews and trials were complemented with collecting farm-individual milk reports by the Regional Association for Performance and Quality Inspection in Animal Breeding of Baden-Württemberg (LKV-Baden-Württemberg). The reports contained information on milk yield and composition of individual animals, and on the herd composition on a monthly and annual basis. For the 27 interviewed farms, the report for the year 2017 was used, for the nine trial farms, the reports covered the years 2017 to 2020.

Reference herds for comparison of observed vs. simulated outputs

From the trial data (2019 and 2020), data from two opposing farms were selected for model evaluation (Table 4.4). The two farms differed in their natural region, farm type (according to Velasco et al., 2021), breed, and supplementation strategy. The first farm was located in a natural region dominated by grassland and characterised by low annual precipitation levels (Schwäbische Alb) (**GRASS**). The GRASS herd kept Simmental cattle, and mainly supplemented grazing cows with grass hay and fresh meadow-grass forage. The second farm was located in a natural region characterised by grassland-based and arable farming and greater annual precipitation (Voralpines Hügel- und Moorland), favouring mixed-farming systems (**MIXED**). The MIXED farm kept Brown Swiss cattle that were mainly supplemented with forage from leguminous leys. On both farms, dairy cows grazed part-time (either during the day or during the night) in a rotational system. For these two farms, detailed information on herd structure, ingredient and nutritional composition of the animals' diet during the stall-feeding and grazing period, monthly and annual milk production and composition, and the milk yield, DMI, and N excretion of individual cows during the grazing period was available.

Table 4.4. Dairy herds used for assessing the modified LIVestock SIMulator for temperate, low-input dairy production systems (LIVSIMtemp) using herds representing a grassland-based (GRASS) and a mixed-farming system (MIXED), and a herd representing the average farm and herd structure across temperate, low-input grazing-based dairy cattle farms (STANDARD).

Herd	GRASS	MIXED	STANDARD
Breed	Simmental	Brown Swiss	Holstein-Friesian
Herd size (n)	48	79	80
Dairy cows, multiparous	23	43	38
Dairy cows, primiparous	5	6	12
Heifers	13	8	23
Calves	7	22	7
Dairy cow characteristics			
Days in milk (n)	148 ± 102.2	197 ± 160.2	206 ± 124.4
Parity (n)	4.0 ± 2.28	4.2 ± 2.57	3.3 ± 2.11
Calving interval (d)	390 ±39.5	407 ± 55.2	411 ± 72.9
Age at first calving (yr)	2.83 ± 0.227	2.66 ± 0.213	2.63 ± 0.262

Reference herd for sensitivity and scenario analysis

For both, scenario and sensitivity analysis, a third reference dairy herd (STANDARD) was used which was not one specific farm but composited to be representative of the average farm structures and grazing management of low-input dairy cattle farms in Southwest Germany (Table 4.4). The third herd comprised Holstein-Friesian cattle, which was the third most important cattle breed in the interviewed farms, following the Simmental and Brown Swiss breed. The STANDARD herd was composited according to the mean proportions of calves, heifers, and dairy cows observed across farms in the study region Velasco et al. (2021). Further, their mean and standard deviation in days in milk, parity, calving interval, and age at first calving per herd were considered to randomly assign similar dairy cow characteristics to the cows of the STANDARD herd using the *rnorm* function in R Version 4.2.0 (R Core Team, 2022).

4.2.2.2 Model evaluation based on observed herd data

The key outputs of the adapted modules (i.e., herd composition, DMI of pasture herbage, milk yield, and N excretion parameters) were evaluated to assess LIVSIM's accuracy. For each reference herd (GRASS and MIXED), 100 iterations à 15 years were simulated to account for the stochastic nature of the module for mortality in LIVSIMtemp. The observed herd composition, feeding and herd management of the farms GRASS and MIXED were used as respective input factors to initialise LIVSIMtemp simulations (Tables 4.4 and 4.5). Missing dietary information (i.e., dietary starch concentrations and its degradability in the rumen, dietary fatty acid concentrations, dietary concentrations of rumen-degradable and -undegradable CP, intestinal digestibility of N, and concentrations of fermentation products in silages, and nutritional value of feedstuffs offered during the stall-feeding period), was retrieved from feeding tables (Feedbase: Agroscope, 2016; INRA, 2019).

Model outputs were averaged across the 100 simulations. For evaluation of reproductive characteristics, outputs of the last five years of each simulation were averaged across the 100 simulations. To evaluate herd composition and animal performance parameters (i.e., DMI, milk yield, and N excretion), outputs of the last year of each simulation were considered. Because the reference values for evaluating the animal performance parameters were derived from trials conducted during the grazing seasons of 2019 and 2020, only animal performance outputs derived from the grazing season (April to October) were evaluated.

Table 4.5. Dietary composition and quality used as input factors for evaluating the modified LIVestock SIMulator for temperate, low-input dairy production systems (LIVSIMtemp) using herds representing a grassland-based (GRASS) and a mixed-farming system (MIXED).

	Offer, kg DM/cow and d		DM, g/kg FM		OM, g/kg DM		CP, g/kg DM		RUP, g/kg DM		ME, MJ/kg DM		NDF, g/kg DM	
	GRASS	MIXED	GRASS	MIXED	GRASS	MIXED	GRASS	MIXED	GRASS	MIXED	GRASS	MIXED	GRASS	MIXED
Stall-feeding period														
Concentrates	1.5	0.0	934	--	886	--	97	--	28	--	12.1	--	99	--
Grass hay	20.0	18.0	888	888	885	885	149	149	49	49	10.1	10.1	481	481
Alfalfa pellets	2.0	0.0	900	--	880	--	230	--	91	--	9.8	--	337	--
Grazing period														
Concentrates	0.0	0.0	--	--	--	--	--	--	--	--	--	--	--	--
Pasture herbage	15.0	10.0	236	255	899	886	145	189	44	58	9.5	9.0	457	482
Grass hay	3.0	2.0	850	865	920	930	84	119	28	39	9.2	9.7	537	511
Fresh forage	5.0	10.0	236	193	914	906	121	199	38	50	9.0	10.3	504	364

CP = crude protein; DM = dry matter; FM = fresh matter; ME = metabolisable energy; NDF = neutral detergent fibre; OM = organic matter; RUP = rumen-undegradable CP.

The reference data used for model evaluation did not have the same temporal resolution as model simulations (i.e., in animal-individual, monthly resolution). Animal-individual data for animal performance, DMI, and N excretion was solely available on the basis of 6-d trial periods (n = 105 animals). Further, annually aggregated data covering the milk performance and herd composition over three years (2017 – 2019) per dairy farm was available. It was, thus, not possible to statistically analyse the accuracy of LIVSIMtemp predictions by one-to-one comparisons with monthly observations (e.g., via the root mean squared error (**RMSE**) or coefficient of determination [$\boldsymbol{R^2}$]). Instead, the relative difference (= [observed – predicted output]/observed output * 100) was calculated to evaluate model predictions per herd, where a relative difference between -10 and 10 % was considered as indicator for accurate predictions.

4.2.2.3 Sensitivity analysis

The sensitivity of LIVSIMtemp to changes in model coefficients and inputs was quantified to understand whether LIVSIMtemp responds to factors that are decisive for animal performance and N excretion, and to identify those factors for which precise information is especially needed. For this, selected input variables and model coefficients (Table 4.6) were increased or decreased by ± 10 % (with the exception of average days open which were increased or decreased by ± 30 d) and the effects tested on the model outputs BW change, milk yield during the grazing period, DMI, of pasture herbage, and urinary N excretion. For each modified model input or coefficient, 100 iterations with 15 years each were simulated using a scenario where dairy cows are supplemented with meadow-grass and concentrates during grazing (see MeadowConc in 2.3.4) as the default scenario. The sensitivity of LIVSIMtemp was then evaluated using the Sensitivity Index (**SI**) and the percentage variation (**PV**) following Félix and Xanthoulis (2005). The SI and PV were calculated using the equations 11 and 12:

$$SI = \frac{\left(\frac{y_2 - y_1}{y_{mean}}\right)}{\left(\frac{x_2 - x_1}{x_{mean}}\right)} \qquad (11)$$

$$PV = \left(\frac{y_2 - y_1}{y_{mean}}\right) * 100 \qquad (12)$$

where x_1 and x_2 were the default and modified input variable or model coefficients, respectively; xmean was the mean of x_1 and x_2; y_1 and y_2 were the outputs of the default and modified simulations, respectively; and ymean was the mean of y_1 and y_2.

An SI = 1 signifies that the modification of the model input or coefficient caused the same variation in the output variable (Laguionie et al., 2014), whereas an SI > 1 or < -1 indicates an increased sensitivity of the model output as a response of changes in the model input or coefficient (Graux et al., 2011). The PV was used as measure of the variation in the output variables as a response of the modification of the model input or coefficient.

Table 4.6. Default and modified model coefficients and inputs for evaluating the sensitivity of the modified LIVestock SIMulator for temperate, low-input dairy production systems (LIVSIMtemp).

	Unit	Default [1]	+10%	-10%
Model parameters				
Potential peak milk yield, multiparous	kg/d	33.7	37.0	30.3
Potential peak milk yield, primiparous	kg/d	25.2	27.7	22.7
Milk fat concentration	g/100 g milk	4.4	4.9	4.6
ME requirements for maintenance	MJ/kg $BW^{0.75}$ and d	0.65	0.72	0.59
ME utilisation for lactation [2]	MJ/MJ	0.73	0.80	0.66
Input variables[3]				
Average days open	d	90	60	120
ME	MJ/kg DM	9.5	8.6	10.6
NDF	g/kg DM	431	474	388
CP	g/kg DM	148	162	133
RUP	kg/kg CP	0.28	0.31	0.25
Offered pasture biomass	kg DM/cow and d	6.91	7.60	6.22
Offered concentrates	kg DM/cow and d	1.91	2.10	1.72

BW = body weight. CP = crude protein. ME = metabolisable energy. NDF = neutral detergent fibre. RUP = rumen-undegradable CP.
[1] Default values for STANDARD herd offered diet of MeadowConc scenario.
[2] Utilisation of ME for milk energy synthesis (= lactation energy/ME).
[3] Dietary input variables: weighted averages based on offered pasture biomass, concentrates, and forage; averaged across all months and animal groups.

4.2.2.4 Scenario analysis

To test the ability of LIVSIMtemp to model the effect of changes in the feeding management on animal performance and N excretion of grazing dairy cows, four scenarios were created. Scenarios differed in supplementation composition during the grazing period, where animals either received no supplementation (**NoSup**), or were supplemented with meadow-grass from permanent grasslands and concentrates (**MeadowConc**), concentrates (**Conc**), or with a total mixed ration

(**TMR**) (Table 4.7). The diets for growing cattle and for all animal groups during the stall-feeding period (November to March) did not differ between scenarios. The four scenarios only differed in the nutritive value of diets for lactating dairy cows during the grazing season, particularly in the dietary energy and protein concentrations (i.e., rumen N balance). The nutritive value of dietary ingredients for the scenarios were either derived from the measurements across all trials (n = 9 farms à 1 to 4 trial periods) averaged per season for the grazing season (spring: April to July; summer: August to October) (Perdana-Decker et al., 2023), or from feeding tables (Feedbase: Agroscope, 2016; INRA, 2019). Herd-related inputs were derived from averaged interview data as outlined before.

Table 4.7. Ingredient composition, and nutrient and metabolisable energy (ME) concentrations of diets for lactating dairy cows during the grazing period (April to October) to simulate four different supplementation scenarios using the modified LIVestock SIMulator for temperate, low-input dairy production systems (LIVSIMtemp).

	Supplementation scenario			
	NoSup	MeadowConc	Conc	TMR
Dietary composition, kg DM/d				
Meadow grass	0.0	9.5	0.0	0.0
Maize silage	0.0	0.0	0.0	9.5
Concentrate	0.0	2.5	5.0	2.5
Pasture herbage	21.0	9.0	16.0	9.0
Nutrient (g/kg DM) and ME concentrations				
OM	890	906	908	929
CP	177	148	167	126
RUP	54.9	41.4	55.1	36.5
RNB	5.18	1.68	2.29	-2.40
NDF	459	431	398	402
ME, MJ/kg DM	9.0	9.5	10.0	10.4

CP = crude protein; Conc = supplementation solely with concentrates; DM = dry matter; NDF = neutral detergent fibre; OM = organic matter; RUP = rumen-undegradable CP; MeadowConc = supplementation with meadow-grass and concentrates; NoSup = no supplementation; TMR = supplementation with concentrates and maize silage).

For each scenario, again 100 iterations à 15 years were simulated. Model outputs were averaged across the 100 simulations across the last year per animal group (i.e., calves, heifer, cows) to demonstrate the differences in feed intake, milk yield, and N excretion between the scenarios. As indicator for N use, the N retention was calculated based on the modelled outputs as N intake minus urinary and faecal N excretion. The N use efficiency (in % of N intake), thus, represented the share of N which was retained as milk protein and in BW gain.

4.3 Results

4.3.1 Model evaluation

The dairy cow characteristics and herd composition were predicted accurately (i.e., with a relative difference ≤ 10 % between observed and predicted outputs) for the majority of outputs (Table 4.8). The days in milk and weeks in gestation of the reference GRASS herd was lower than of the reference MIXED dairy cows. At the end of the simulation, however, both variables did not differ anymore between the two herds, indicating that dairy cow characteristics converged during LIVSIMtemp simulations regardless of the initial herd demography. However, the variation in days in milk and weeks in gestation among cows within each herd remained the same after 15 years of simulation, as shown by a similar standard deviation for both variables for the predicted compared to the reference herd, suggesting that predicted calving dates were realistically spread across the year. In the simulated MIXED herd, more heifers compared to calves were present at the end of 15 simulation years than in the reference herd. This deviation in herd demography is likely owing to the farm's specific calf management, where calves are not sold immediately after calving but with approximately seven months. Overall, the relative differences for calving interval, age at first calving, replacement rate, culling-age, and life-time production ranged between -9.7 and 3.0 %, indicating that reproductive characteristics were accurately predicted. An exception were the predictions of lifetime milk production and replacement rate in the MIXED herd, which differed by 27.6 and 19.7 % from the observed values, respectively.

The predictions of annual milk yield and DMI of multiparous cows, and DMI of pasture herbage of all dairy cows during the grazing period were in close agreement with the observed values for both herds. Total milk yield of primiparous cows up until 305 d of lactation was accurately predicted for herd GRASS (2.7 %), but overpredicted for MIXED herd (-30.1 %). Faecal N excretion (g/d) was overpredicted in both herds (-15.3 and – 26.1 % for GRASS and MIXED, respectively), whereas the urinary N excretion was underpredicted by 49 and 36 % for the GRASS and MIXED herd, respectively. The simulated differences in N excretions between herds (i.e., farming systems), however, coincided with the observed differences between herds: the observed faecal, urinary, and total N excretion (in % of N intake) of the GRASS herd differed by 30, -23, and -7 %, respectively, from the observations of the MIXED herd, which is similar to the respective relative differences for the predicted faecal, urinary, and total N excretion of 32, -36, and -4 %.

Table 4.8. Evaluation of mean herd and performance characteristics as well as nitrogen (N) excretion of grazing lactating dairy cows from herds in a grassland-based (GRASS) and in a mixed-farming system (MIXED) simulated by the modified LIVestock SIMulator for temperate, low-input dairy production systems (LIVSIMtemp).

Herds	GRASS			MIXED		
	Reference	LIVSIMtemp	Diff, % [1]	Reference	LIVSIMtemp	Diff, % [1]
Dairy cow characteristics (± one standard deviation)						
Days in milk, n	148 ± 102.2	140 ± 105.2	5.41	197 ± 160.2	159 ± 120.4	19.3
Weeks in gestation, n	8.2 ± 12.67	13.4 ± 13.75	-63.4	10.8 ± 12.66	13.4 ± 14.03	-24.1
Parity, n	4.0 ± 2.28	4.1 ± 1.73	-2.50	4.2 ± 2.57	3.9 ± 1.55	7.14
Herd size, n [2]	47	50	-6.38	79	80	-1.27
Dairy cows	28	32	-14.3	49	52	-6.12
Heifers	13	14	-7.69	8	22	-175
Calves	7	3	57.1	22	6	72.7
Reproductive characteristics						
Calving interval, d	387	397	-1.79	407	419	-2.94
Age at first calving, yr	2.83	2.83	0.00	2.66	2.58	3.01
Lifetime milk production, kg	31,832	34,916	-9.69	20,171	25,735	-27.6
Culling age, yr	8.57	8.88	-3.62	6.91	7.30	-5.64
Replacement rate, % [3]	15.0	15.6	-4.00	14.7	17.6	-19.7
Annual productive characteristics						
Milk yield, kg/first lactation [4]	5,229	5,371	-2.72	4,136	5,383	-30.1
Milk yield, kg/yr, multiparous cows [5]	5,868	5,779	1.52	5,309	5,326	-0.32
Productive characteristics during the grazing period						
DMI, kg/d	21.2	20.2	4.72	18.9	19.6	-3.70
DMI of pasture herbage, kg/d	13.5	12.2	9.63	7.28	7.65	-5.08
N intake during, g/d	453	421	7.06	570	590	-3.51
Faecal N excretion, g/d	124	143	-15.3	115	145	-26.1
Urinary N excretion, g/d	220	111	49.5	350	224	36.0
Total N excretion, g/d	344	254	26.2	465	369	20.6
Faecal N excretion, % N intake	27.4	34.0	-24.1	20.2	24.6	-21.8
Urinary N excretion, % N intake	48.6	26.4	45.7	61.4	37.9	38.3
Total N excretion, % N intake	75.9	60.3	20.6	81.6	62.5	23.4

DM = dry matter; DMI = DM intake.
[1] Diff: relative difference = (observed output- predicted output) / observed output * 100.
[2] Animal classes: calves = animals < 6 mo; heifers = animals ≥ 3 mo until first parturition
[3] Replacement rate: number of replaced cows per year in % of number of dairy cows at end of the year
[4] Milk yield of first 305 d of first lactation. Average across all primiparous cows born during simulation. No annual milk yield of primiparous cows for year 15 of simulation reported because animals started calving at various time-points during year 15.
[5] Milk yield during year 15 of simulation: Only milk yield of animals remaining in farm until last month of simulation.

4.3.2 Sensitivity analysis

Body weight change was the most sensitive output of the selected LIVSIMtemp outputs and was strongly affected by changes in the potential peak milk yield of primiparous cows, the ME utilisation efficiency for lactation, the milk fat concentration, the average days open, dietary NDF concentration, and offered pasture biomass with SI values ranging between -3.95 to 3.45 (Table 4.9).

Daily milk yield and DMI of pasture herbage of dairy cows during the grazing season was mostly affected by changes in ME supply, ME requirements for maintenance, ME use efficiency for lactation, and offered pasture biomass (SI = -0.44 – 0.96). Additionally, increasing or decreasing the average days open by +/- 30 d moderately affected the daily milk yield (SI = -0.39 and 0.45). Urinary N excretion of grazing dairy cows was highly sensitive to dietary CP concentration (SI = 2.11 and 2.43, when modified by +10 or -10 %), and moderately sensitive to dietary rumen-undegradable CP concentration (SI = 0.52 and 0.48).

Table 4.9. Sensitivity index (SI) and percentage variation (PV) in body weight (BW) change across all animal groups, and milk yield, dry matter intake (DMI) of pasture herbage, and urinary nitrogen (N) excretion of lactating, multiparous (MP) cows during the grazing period, when modifying (+/- 10 %) selected model coefficients and inputs of the modified LIVestock SIMulator for temperate, low-input dairy production systems (LIVSIMtemp).

		BW change, kg/d				Milk yield, kg/d				DMI of pasture herbage, kg/d				Urinary N excretion, g/d			
		SI		PV, %		SI		PV, %		SI		PV, %		SI		PV, %	
	Unit	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Model coefficients																	
Potential peak milk yield, MP	kg/d	-0.12	0.79	-1.1	-8.3	0.13	0.27	1.2	-2.8	0.30	0.41	2.9	-4.4	0.16	0.22	1.5	-2.3
Potential peak milk yield, PP	kg/d	0.59	3.45	5.6	-36.3	0.02	0.26	0.2	-2.7	-0.01	0.14	-0.1	-1.5	-0.01	0.13	-0.1	-1.4
Milk fat concentration	g/kg	1.12	0.97	10.7	-10.2	-0.44	-0.38	-4.2	4.0	0.12	0.16	1.2	-1.7	0.06	0.07	0.6	-0.8
ME requirements for maintenance	MJ/kg $BW^{0.75}$ and d	-0.19	-0.06	-1.8	0.7	-1.02	-0.41	-9.7	4.3	-0.63	0.42	-6.0	-4.4	-0.29	0.36	-2.7	-3.8
ME utilisation for lactation [1]	MJ/MJ	-3.95	-1.30	-37.6	13.7	0.46	0.83	4.3	-8.7	-0.60	-0.35	-5.7	3.6	-0.54	-0.34	-5.1	3.5
Model inputs																	
Average days open [2]	d	-2.02	0.73	-19.2	-7.7	-0.39	0.45	-3.7	-4.7	-0.23	0.25	-2.2	-2.6	-0.11	0.14	-1.1	-1.5
ME of diet	MJ/kg DM	-0.43	-0.94	-4.1	9.9	0.96	1.74	9.2	-18.3	-1.19	0.28	-11.3	-2.9	-0.51	0.32	-4.8	-3.3
NDF of diet	g/kg DM	-0.61	0.70	-5.8	-7.3	-0.09	0.04	-0.8	-0.4	-0.10	0.03	-0.9	-0.3	-0.08	0.02	-0.8	-0.2
CP of diet	g/kg DM	-0.29	-0.09	-2.7	1.0	-0.03	0.03	-0.3	-0.4	0.00	0.02	0.0	-0.2	2.11	2.43	20.1	-25.5
RUP of diet	kg/kg CP	-0.32	0.05	-3.1	-0.5	0.02	0.02	0.2	-0.2	0.00	0.00	0.0	-0.0	0.52	0.48	4.9	-5.0
Offered pasture biomass	kg/d	-0.80	1.84	-7.6	-19.3	0.50	0.62	4.7	-6.5	0.68	0.73	6.4	-7.7	0.36	0.39	3.5	-4.1
Offered concentrates	kg/d	-0.49	-0.93	-4.6	9.8	0.24	0.50	2.2	-5.3	-0.13	0.61	-1.2	-6.4	0.04	0.47	0.3	-4.9

CP = crude protein. DM = dry matter. ME = metabolisable energy. NDF = neutral detergent fibre. PP = primiparous. RUP = rumen-undegradable CP.

[1] Utilisation of ME for milk energy synthesis (= lactation energy/ME).

[2] Modified by +/- 30 days.

4.3.3 Scenarios

The scenarios differed in the type and inclusion level of different supplement feedstuffs, and, hence, in the dietary concentrations of CP, rumen-undegradable CP, ME, and rumen N balance. During the stall-feeding period, dairy cows received the same diet across all scenarios. Accordingly, milk yield, DMI, and N excretion did not differ between the scenarios during the stall-feeding period (Figure 4.1). With the onset of the grazing period, visible differences in the monthly milk yield, DMI and faecal N excretion of each cow between different scenarios became apparent. These differences increased with the change from spring to summer pasture conditions. Cows of the TMR scenario had the lowest pasture DMI, N intake, and urinary N excretion and the greatest N use efficiency, whereas the opposite was the case for NoSup dairy cows.

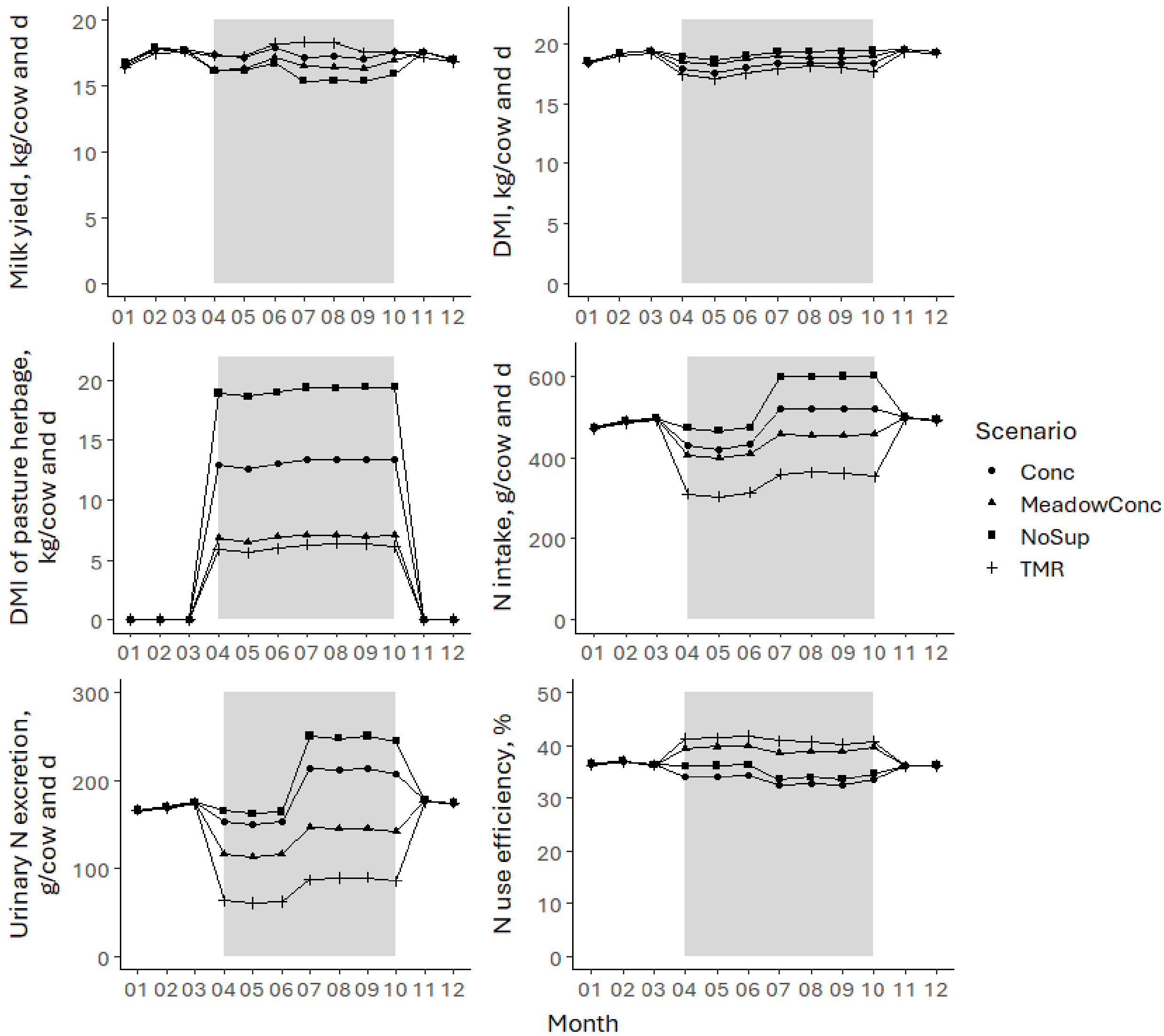

Figure 4.1. Milk yield, nutrient intake, and nitrogen (N) use of dairy cows across the year for four supplementation scenarios, simulated by the LIVestock SIMulator adapted for temperate grazing-based low-input dairy farms (LIVSIMtemp). The months highlighted in grey refer to the grazing period. Conc = supplementation solely with concentrates; DMI = dry matter intake; MeadowConc = supplementation with meadow-grass and concentrates; NoSup = no supplementation; TMR = supplementation with concentrates and maize silage.

The share of N retained as milk protein or BW gain did not differ considerably between the scenarios across the year (Figure 4.2A). Differences between scenarios in N retention and excretion were more pronounced during the grazing period, when animals in the MeadowConc and TMR scenario used more of the ingested N for milk protein synthesis or BW gain than the Conc and NoSup animals (Figure 4.2B). Urinary and faecal N excretion differed between MeadowConc and the TMR scenarios with a greater faecal to urinary N ratio for the TMR scenario.

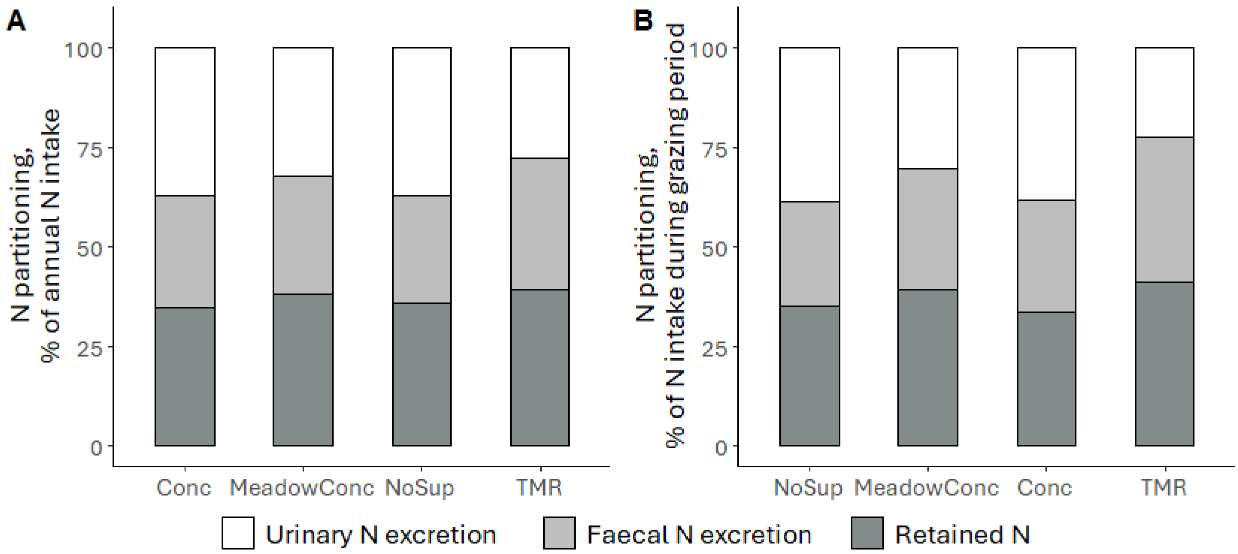

Figure 4.2. Nitrogen (N) partitioning as proportion of N intake across the year (A) and during the grazing period (B) for dairy cows during the last year of a simulation across 15 years for different supplementation scenarios. Conc = supplementation solely with concentrates; MeadowConc = supplementation with meadow-grass and concentrates; NoSup = no supplementation; TMR = supplementation with concentrates and maize silage.

4.4 Discussion

The present study adapted an existing dynamic ruminant herd model to predict the performance and N excretion of dairy cattle on temperate, low-input, grazing-based farms. The LIVSIM-mod was adapted by changing modules simulating lactation, DMI, ME requirements, N excretion, and herd composition, and by parameterising three common European dairy cow breeds (Holstein-Friesian, Simmental, and Brown Swiss).

4.4.1 Limitations of the reference dataset

Predictions of urinary N excretion were evaluated based on reference values calculated as balance between measured N intake, milk N secretion, and faecal N excretion. The uncertainty related to the measurements of N intake and faecal N excretions for the reference dataset might thus limit the evaluation of predicted urinary N excretion. Chapter 2, however, demonstrated that the reference urinary N excretion data used for the present study correlated highly with the urinary ratio of N to creatinine (r = 0.56) and the milk urea-N concentration (r = 0.57). Both, the ratio of N to creatinine in urine and the milk urea-N concentration, are independent and reliable indicators of daily urinary N excretions in lactating dairy cows Chizzotti et al., 2008; Huhtanen et al., 2015), demonstrating that the chosen method to calculate urinary N excretion in the reference dataset reflected sufficiently differences in urinary N excretions between different farm types and feeding strategies.

In the present study, the evaluation of LIVSIMtemp's prediction accuracy was limited to dairy cows. Furthermore, BW changes were not evaluated due to the lack of reference data with a monthly resolution and covering a longer timeframe. The LIVSIMtemp projected that multiparous cows mobilised on average 10 % of their BW during early lactation, which was within the range of values reported in previous studies. Depending on the production intensity, dual-purpose Simmental or Normande cows mobilise between 8 and 11 %, and highly productive milk-specialised Holstein-Friesian dairy cows between 11 and 15 % of their pre-partum BW (Gappmaier et al., 2021; Jorge-Smeding et al., 2022). Assuming that BW changes in multiparous cows are mainly a result of BW mobilisation, it can be concluded that LIVSIMtemp simulated BW changes of multiparous cows accurately. Nevertheless, predictions of BW changes by LIVSIMtemp should be evaluated in future studies, considering that BW, BW change, and energy balance are key determinants of feed intake and animal performance (Köck et al., 2018). For this, a dataset covering a longer timeframe (e.g., including the stall-feeding period) at a monthly resolution, on changes in BW of dairy cows and growing animals is therefore needed for drawing final conclusions on the modelling accuracy of LIVSIMtemp.

4.4.2 Model assessment

4.4.2.1 Model accuracy

Model evaluation has confirmed the hypothesis that LIVSIM was adaptable for low-input grazing-based dairy cattle systems in Central Europe. The evaluation based on two reference dairy herds representing two types of grazing-based organic dairy cattle farms in Southern Germany (i.e., GRASS and MIXED) showed close agreement between observed and simulated average herd composition and milk performance across the year. Similarly, reference and predicted feed intake and milk yield of dairy cows during the grazing period were very similar. The mechanistic nature of the model as well as the lower input requirements of LIVSIM compared to other ruminant livestock models (Bateki and Dickhoefer, 2020) implies that it can be easily parameterised for different production systems. Besides, the model structure allows for easy exchange or integration of modules relevant for specific production situations (e.g., N excretion).

Nevertheless, LIVSIMtemp did not predict each output of the reference herds accurately, such as the milk yield of primiparous cows in the MIXED herd, parameters related to lifetime production, or N excretions. Predictions of milk yield of primiparous cows exceeded observations for the MIXED herd by 30.1 %. The potential peak milk yield – a breed-specific input factor based on literature values – might not have been suitable for the respective primiparous cows in the MIXED reference herd. Primiparous cows in the MIXED herd had a particularly low milk yield during the first lactation (4,136 kg) compared to previous findings for Brown Swiss cattle in a low-input production system (5,094 kg; Jeretina et al., 2013). This may be related to farm-specific management decisions (e.g., targeted BW at first conception) or differences in genotype, which were not captured by the model. However, for the sake of developing a universally applicable model, no further parameter calibration was conducted to avoid overfitting the model to the specific characteristics of the farms chosen for the present study.

The predicted lifetime performance of cows was greater than the reference values, in particular for the MIXED herd. This overestimation may partly be related to the greater predicted milk yield of primiparous cows in that herd, and a slightly overestimated culling age of cows in both herds. Other reproductive performance parameters that may influence the lifetime performance such as age at first parturition and annual milk yield of multiparous cows were well predicted by LIVSIMtemp. The model reproduced the low replacement rate (15 %) and high average number of parities (4.1 lactations) of the reference herds, which are typical of low-input dairy herds with low milk production intensity (Knaus, 2009; Ivemeyer et al., 2018). Such great compliance in observed and predicted reproductive performance values might at least partly be due to the fact that these parameters are greatly determined by the initial herd demography as well as the input factors for mortality, maximum lactation length, maximum herd size, and conception probability, which were adjusted to those values commonly found on the studied type of farms. For a more holistic assessment of the sustainability of different ruminant production systems, however, there is certainly merit in considering the effects of differences in genotypes, feeding and herd management and their effects on milk yield, body composition, and energy balance to simulate the animals' lifetime performance (Phuong et al., 2016; Grandl et al., 2019). There are examples of ruminant livestock models which give a more precise representation of herd dynamics by simulating the reproductive process in response to breeding and feeding management (e.g., Phuong et al., 2016; Ruelle et al., 2016). However, such models disregard the aspect of resource losses such as N excretions which are a key aspect of grazing-based systems (Hoekstra et al., 2007). Yet, their elements and approaches may be integrated into LIVSIMtemp in the future.

LIVSIMtemp was capable of simulating the relative differences in N excretions between treatments as shown by the evaluation of the GRASS and MIXED simulations, and the scenario outputs. The absolute total N excretion, however, was underpredicted for both reference herds mainly due to the underprediction of urinary N excretion. Part of the prediction bias might originate from the uncertainty in the observed values of urinary N excretion as outlined above. Additionally, dietary inputs used for the simulations may not fully represent the nutritional value of ingested diets, for instance, because manually harvested samples of pasture herbage may not reflect the herbage truly ingested by the animals due to selective grazing. Yet, the latter source of error applies to both, the reference values as well as the prediction of urinary N excretion. For the GRASS herd, the lower predicted N intake might partially explain the underprediction of urinary N excretion. Additionally, predicted faecal N excretions was greater than observations for both reference values. Nevertheless, these differences do not fully explain the underestimation of urinary N, suggesting that there are also systematic errors in the adopted model. The adopted INRA model (2019) predicts urinary N based on the N recycled and excreted in urine, N losses from metabolisable protein utilisation, endogenous N excreted in urine, microbial nucleic acids flow, and the N retention in BW gain. The latter is derived from the energy balance (i.e., ME intake not used for maintenance and lactation) that is deposited as body tissue, assuming a fixed protein concentration in BW gain. Urinary N excretion in the reference dataset was calculated as the N intake of animals that is not excreted via faeces or retained in milk protein. Hence, underestimation of urinary N excretion by the LIVSIMtemp model was likely due to the fact that N retention in BW gain was not considered in the reference dataset and/or overestimated by the N excretion model.

Despite the underprediction by the N excretion module by INRA (2019), it was retained, because of its semi-mechanistic nature and the fact that it considers the variation in the efficiency of metabolisable protein use. Empirical models can perform well for grazing-based dairy production systems (e.g., Beck et al., 2023: relative prediction error = 11.3 %). Their disadvantage, however, is their restriction to the specific conditions of their development dataset. Additionally, empirical models for urinary N predictions commonly rely on milk urea-N as input factor (Beck et al., 2023), which is not a model output of LIVSIMtemp. Hence, to enable an easier and uniform application of LIVSIMtemp across different production systems (i.e., without the need of fitting different empirical models per study focus), a semi-mechanistic model to predict N excretion was chosen. Salazar-Cubillas et al. (2024) further found that predictions of urinary N excretion by the chosen INRA model are highly sensitive to changes in the use efficiency of metabolisable protein. Similarly, Lapierre et al. (2018) demonstrated that models using a variable efficiency respond more accurately to variations in the dietary CP and energy supply and that constant efficiency coefficients are unsuitable when predicting the metabolisable protein use in diets with an unbalanced protein and energy supply. Such unbalanced nutrient supplies can particularly be found where semi-natural grasslands constitute the main feed base of dairy cows, causing high seasonal and spatial variation in nutrient supply. Therefore, a preliminary analysis was conducted in the present study to test two approaches (fixed or variable) for estimating the use efficiency for metabolisable protein. Accordingly, a wide range of efficiencies (0.67 – 0.83) was estimated using eq. 17.7 of INRA (2019) for the preliminary evaluation dataset. Using a fixed coefficient of 0.67 as suggested by multiple national recommendation systems (e.g., NRC, 2001; Cornell Net Carbohydrate and Protein System: van Amburgh et al., 2015), on the other hand, produced a lower RMSE (i.e., greater accuracy) for daily urinary N excretion (RMSE = 22.9 % of observed mean) than the use of the variable efficiency factor (RMSE = 32.2 %). But the fixed efficiency coefficient also caused a greater slope bias for urinary N excretion (in % of N intake), mainly, because the variation in observed urinary N excretion (coefficient of variation [CV] = 18.4 %) was reproduced more accurately using the variable efficiency factor (CV = 19.4 %) than with the fixed factor (CV = 10.7 %). Hence, a lower prediction accuracy (i.e., greater RMSE) for urinary N excretion was accepted in favour of being able to more accurately reproduce the relative differences in N excretions across a broad range of feeding situations (i.e., varying in supply of protein and energy).

For future applications of LIVSIMtemp to assess the environmental burden of certain strategies, predictions with a greater accuracy for N excretions will be required. Therefore, further attempts should be made to improve the accuracy of N use predictions due to the varying degree of susceptibility of faecal or urinary N to volatilisation and leaching (Powell et al., 2010). A potential alternative represents the newly updated German feeding recommendations (GfE, 2023).

4.4.2.2 Model sensitivity

A sensitivity analysis was conducted to assess which model inputs (e.g., dietary factors) and coefficients (e.g., potential peak milk) most affected the core model outputs (i.e., BW change, milk yield, DMI of pasture herbage, and N excretion). In general, model outputs responded most sensitively to parameters related to ME use, ME requirements, and the ME and CP concentrations of the animals' diets. Body weight change was highly sensitive to model parameters that foremostly play a role in prediction of milk yield (e.g., peak milk yield) or feed intake (e.g., offered pasture biomass), likely because BW predictions are derived from a combination of the ME intake and ME requirements for lactation, determining the extent of energy mobilisation or deposition in BW gain. Accordingly, the sensitivity of DMI of pasture herbage and milk yield to changes in dietary ME concentration and model parameters characterising ME requirements (e.g., ME utilisation for lactation) was expected, because in LIVSIMtemp and LIVSIM-mod, milk yield and voluntary DMI are mainly governed by the animal's physiological energy requirements.

Besides the ME-related model parameters, DMI, milk production, and BW changes were also affected by differences in the amount of offered pasture herbage. This was likely due to the fact that herbage offer on pasture was not sufficient to cover the requirements of cows during peak lactation. In this line, herbage biomass may only limit predicted DMI and animal performance for those conditions in which the necessary herbage intake from pasture is high (e.g., at high animal performance or low supplementation levels) and/or the available herbage biomass is very low (i.e., dry spells).

Predictions of urinary N excretion responded sensitively to differences in concentration and ruminal degradability of dietary CP. For instance, an increase or decrease by 10 % in the dietary CP concentration increased or decreased the daily urinary N excretion by -20 or 26 %, respectively. Correspondingly, previous studies demonstrated that dietary CP concentrations or N intake are factors explaining the greatest share of variation in urinary N excretion for stall-fed diets (Spek et al., 2013), as well as for grazing-based diets (Beck et al., 2023). Faecal N excretion solely reacted sensitively to increases in the dietary ME concentration (SI = -0.50). None of the remaining model parameters greatly affected faecal N excretion, likely because the main factors regulating faecal N excretion (i.e., DMI and diet digestibility) were not tested within the present sensitivity analysis. Additionally, at such high dietary N concentrations than in the investigated farm systems and feeding practices, faecal N excretion varies only minorly (Schuba et al., 2017). In contrast, it cannot be precluded that for other feeding situations, e.g. with lower N intakes, faecal excretion may vary and respond more sensitively to dietary and animal factors. In this line, findings of the present sensitivity analysis are restricted to the specific production and feeding system underlying the analysis, in which dairy cows were mainly fed on forages (pasture herbage and supplementation) from semi-natural grassland.

Under on-farm conditions, ME and CP concentrations of dairy cattle diets commonly vary by 10% or more, particularly in grassland-based feeding systems, due to a great spatial and seasonal variability in the nutritional value of pasture herbage. For instance, forage CP concentrations on semi-natural pastures of organic dairy farms in the present study region varied by 13 % across the grazing seasons of 2019 and 2020 (data not shown). The sensitivity analysis demonstrated that the model is sufficiently sensitive to such changes in the feeding management which is essential to capture the effects of seasonal differences in pasture herbage and of supplementation strategies on intake, milk yield, and N excretion of grazing dairy cows. Finally, the sensitivity of LIVSIMtemp outputs to changes in offered pasture biomass and/or dietary ME, CP, and rumen-undegradable CP concentrations demonstrates that precise measurements or estimates of the available forage quantity and quality are a prerequisite for reliable predictions. While information on the amount and nutritional value of supplement feeds offered in the barn are more readily available, reliable estimates of the available herbage on pasture are difficult to obtain. Hence, the application of LIVSIMtemp might be complemented with sensor- or modelling-based approaches for more precise estimates of pasture forage quality.

4.4.2.3 Model plausibility

Model plausibility was tested by scenario analysis, which further demonstrated that the effect of different feeding strategies on animal performance and N excretion can be portrayed by LIVSIMtemp. The four scenarios tested the effect of supplementation ingredients (i.e., concentrates, meadow-grass, or maize silage) mainly differing in concentrations of ME and CP, and the origin of forages (i.e., arable land or grassland) on milk production, feed intake, and N excretions. It was expected that a wide range in N use would be observed between scenarios due to their difference in rumen N balance and ME concentration, causing differences in the urinary N excretion (Ferreira et al., 2023), and microbial protein and milk protein synthesis (Almeida et al., 2020). Diets in the TMR scenario had a rumen N balance of -46.7 g/d, whereas rumen N balances were positive in the MeadowConc (35 g/d), Conc (44 g/d), and NoSup (102 g/d) scenarios. Accordingly, N use efficiency during the grazing season was greatest for the TMR scenario (42.2 % of N intake), exceeding that of NoSup animals (36.8 % of N intake) by 14 %. Almeida et al. (2020) determined similar differences in the N use efficiency (17 %) while testing the effect of supplementing Holstein x Jersey cows grazing an oat-ryegrass pasture with maize silage (3.1 kg DM/cow and d), where difference in dietary CP concentration between animals without or with supplementation (219 vs. 169 g/kg DM, respectively) was comparable to the differences between the NoSup and TMR scenarios. Together with the results of the sensitivity analysis, the scenario evaluation, thus, showed that LIVSIMtemp is sufficiently sensitive to changes in the type, amount, and nutritional value of both, supplement feeds and pasture herbage, and able to produce plausible predictions of animal performance and N excretion for low-input grazing systems.

4.5 Conclusion

The adapted version LIVSIMtemp of the dynamic herd model LIVSIM-mod accurately predicts feed intake, animal performance, and herd composition of cattle on different types of grazing-based dairy farms in central Europe. Additionally, effects of different supplemental feeding strategies are simulated plausibly. Relative differences in N use and excretion are predicted reliably, however, absolute total and urinary N excretion are underpredicted. Hence, further model improvements should focus on increasing the accuracy of N excretion and its partitioning due to the varying degree of susceptibility of faecal or urinary N to volatilisation and leaching. Core input and model coefficients are the dietary ME, CP, and rumen-undegradable CP concentrations, and the offered pasture biomass, for which precise measurements are a prerequisite. The mechanistic nature of LIVSIM-mod enabled a comparatively easy adaptation of LIVSIM to temperate, low-input grazing-based systems. There is further potential for adopting LIVSIMtemp for production systems beyond the ones investigated in the present study.

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Chapter 5 | General Discussion

During the past decades, the growing demand for animal-based foods has led to livestock and grazing-based research which focused on increasing the productivity i.e., product output per input or agricultural area (Capper and Bauman, 2013; Roche et al., 2017). This dissertation, in contrast, aimed at shifting the attention to low-input grazing-based systems, due to the environmental and socio-economic benefits of such grassland-based ruminant production systems (Dentler et al., 2020; Schils et al., 2022). The present thesis addressed this gap by adapting an existing livestock model for investigating the nitrogen (**N**) use and excretion of dairy cattle on the herd-level focusing on production systems using semi-natural grasslands for grazing. Such low-input grazing-based systems do not only employ different types of grassland (i.e., semi-natural instead of improved grassland) but are also characterised by different farm and feeding conditions than high-input grazing systems with, for instance, more roughage-based feeds and a lower reliance on external inputs (Poetsch, 2007; Scollan et al., 2017). It was, thus, hypothesised (1) that strategies to mitigate N losses to the environment established for high-input grazing conditions (e.g., Moorby and Fraser, 2021; Nguyen et al., 2022) would not be transferable to low-input grazing systems, and (2) that a model specifically adapted to low-input grazing-based systems will be required. The first hypothesis was tested by investigating the N use and excretion of lactating dairy cows across a diverse set of commercial dairy farms in the case study region of Baden-Württemberg (Chapter 2). Furthermore, an existing dynamic livestock herd model, the LIVestock SIMulator (**LIVSIM**) was adapted and evaluated for low-input grazing systems (Chapters 3 and 4). In the following, the findings on the N use and excretion of grazing cows in organic dairy farms in Southern Germany will be discussed. Then, the resulting implications for modelling will be addressed: firstly, by referring to the general challenges of modelling grazing dairy cattle on semi-natural grassland; secondly, by comparing different existing grassland-based livestock models, and thirdly, by recommending paths for further model advances.

5.1 Nitrogen use in low-input grazing-based systems

5.1.1 Differences between low-input and high-input grazing-based systems

Grazing-based diets are prone to greater losses of N via urinary N excretions than balanced stall-fed diets that are adjusted to the animals' nutrient requirements (Hoekstra et al., 2007). Chapter 2 demonstrated that this issue does not necessarily pertain to low-input grazing systems, which further emphasises the ecological benefits of using semi-natural as opposed to improved grasslands for grazing. The mean milk N use efficiency (**MNE**) of cows grazing semi-natural grasslands (24.7 g/100g N intake ± 5.91) was within the upper range of observations gathered in high-input grazing systems in Northern Germany (Löw et al., 2020: 22 – 25 g/100 g N intake) or Ireland (Doran et al., 2022: 12 to 25 g/100 g N intake). The observed milk urea N concentration

(**MUN**) (10.1 mg/dL ± 4.23) served as another indicator for the more efficient N use in low-input grazing-based systems. Ayers et al. (2022) reported similar values for dairy cows grazing multi-species pastures (68% grass, 18% legume, 8% forbs, and 6% dead material) on organic commercial dairy farms in the Northeast United States (grazing season before trial: 11.0 mg/dL; n = 6 farms). Their average MUN observations and the ones of Chapter 2, are within the recommended range of 8 – 12 mg/dL suggesting sufficient N supply to rumen microbes without adverse effects on animal health or the environment via excessive ruminal ammonia levels (Nousiainen et al., 2004; Ayers et al., 2022). In contrast, observations of a dataset covering ten pasture- or fresh forage-based experiments exceeded the recommended MUN range (Beck et al., 2023: n = 33 treatments, MUN = 16.7 mg/dL ± 4.07), confirming that feeding systems foremostly reliant on ryegrass pastures are at higher risk for excessive environmental N losses. The review articles by Bougouin et al. (2022) and Beck et al. (2023) further affirmed that MUN represents a robust indicator for urinary N excretion of cattle irrespective of the feeding systems i.e. for both, diets based on total mixed rations and fresh forages. The lower MUN of the investigated farms, thus, demonstrated the lower risk for N losses via urinary N excretions in low-input than high-input grazing conditions.

The use of the semi-natural grassland, as opposed to improved or temporary grasslands, mainly explains the greater N use in the observed farming systems. On average, 83.8 % of the ingested DM originated from the farms' own semi-natural grassland in form of grazed herbage, fresh meadow grass, grass silage, or grass hay. Thereby, the observed animals received pasture herbage and supplements (forages and concentrates) with a moderate to low crude protein (**CP**) concentration (16.7 ± 2.93, and 14.2 g/100 g DM ± 4.37, respectively). Additionally, according to the feeding tables by INRA (2019), the herbage of the observed semi-natural pastures had a lower rumen CP degradability (between 64 - 68 g/100 g CP) than of perennial ryegrass pastures (between 71 – 79 g/100 g CP). Hence, the reliance on semi-natural grasslands as main source for grazed or supplemented forages allows diets with a lower surplus in ruminal N, explaining the more efficient N use and lower urinary N excretion than in animals grazing improved or temporary grasslands.

The hypothesis that N mitigation strategies established for high-input grazing conditions were not applicable for low-input grazing systems was, thus, partly confirmed, precisely because several suggested strategies are already implemented by using semi-natural grasslands. For instance, the most straight forward strategy suggested for reducing N losses via leaching (Vogeler et al., 2013) or volatilisation during grazing (Li et al., 2013) is the reduction of the herbage CP concentration by decreasing the N fertilisation rate, which is the key characteristic distinguishing semi-natural and improve/temporary grasslands. Another strategy receiving increased attention is the integration of forbs into the pasture sward, such as plantain (*Plantago lanceolata* L.), for lowering the herbage CP, and increasing mineral and secondary plant metabolite concentrations. A meta-analysis of Nguyen et al. (2022) demonstrated that grazing pastures containing on average 434 g plantain per kg DM increased milk yield by 1.02 kg/cow and d, and reduced total daily urinary N excretions by 22 %. Yet, temperate, semi-natural grasslands naturally contain greater shares of herbs. The grazed paddocks in Chapter 2, for instance, contained mean proportions of grasses, clover, and other herbs of 50.8, 20.4, and 28.8 % of the entire aboveground herbage mass, respectively. The present study, thus, demonstrated the lower risk for N losses in grazing-based farming systems using semi-natural grasslands. Nevertheless, high variation in the observed N-related indicators highlighted that still a high risk for harmful N emissions pertained to at least a part of the observed farms or trial periods.

5.1.2 Observed variation in nitrogen use and excretion

A high variation in the N-related indicators were observed which can be ascribed to both, the variability in farm types and management across the observed farms, and the seasonal (inter- and intra-annually) differences in N use. For instance, the MUN averaged per farm, period, and animal group ranged between 5 and 23 mg/dL. This variation demonstrates that there were periods within farms with a lack of dietary CP (< 8 mg/dL MUN), and several periods within farms in which the high N supply from pasture herbage and/or the additionally supplemented fresh forages could not be compensated. Much of this variation in the N-related indicators in Chapter 2 can be attributed to the large variability in agro-ecological conditions and farm types across low-input grazing-based dairy farms (Velasco et al., 2021), which adjust their farm-individual farm and grazing management to their own farm layout and the locally available resources (van den Pol-van Dasselaar et al., 2020). Similarly, Ayers et al. (2022) observed a high variation in average herd MUN between organic commercial dairy farms during the grazing period in the Northeastern United States (5 to 15 mg/dL). Such large variation was also observed by Dentler et al. (2020) for the protein conversion efficiency (1 – 10.2 kg human-edible protein input/kg human-edible protein output) of low-input grassland-based dairy farms in Baden-Württemberg, suggesting that the observed variation in N use on the animal-level is also translatable to the farm-level.

A bootstrapping analysis in Chapter 2 suggested that the supplementation strategy explained most of the variation in N use and excretion in the present thesis, proving that dietary interventions are universally applicable strategies for improving the N use of grazing animals (Hoekstra et al., 2007). For the investigated grazing systems, greater MNE and lower urinary N excretion can be attained by moderate supplementation with fresh forages or grass hay.

Concentrate supplementation further decreased urinary N excretion. Similarly, Akert et al. (2020) observed a greater MNE and lower MUN for part-time grazing cows, mainly because the substitution of grazed herbage by more mature fresh meadow-grass decreased the overall N intake. However, contradictory to findings by Akert et al. (2020), full-time grazing was likewise related to a greater N use and lower urinary N excretion in the present thesis. This discrepancy could not be explained by factors such as pasture CP concentrations, concentrate supplementation, or other management factors which might have differed between the observed farms employing full-time or part-time grazing. Full-time grazing was suggested as key management factor for reducing the environmental impact of grazing-based dairy farms via allowing optimum use of the locally available pasture resources (Chobtang et al., 2017). Therefore, further investigations are needed to unravel the local and management conditions which allow full-time grazing for maintaining maximum pasture intake without excessive urinary N excretion or hampering milk protein yield.

Chapter 2 contributed to enhancing the understanding on the N use in low-input grazing-based systems by investigating the animal-based N flows of lactating dairy cows on commercial farms in South Germany. At the same time, more aspects requiring further investigation (e.g., the feasibility of full-time grazing) were revealed. Considering that the applied experimental approach was time-consuming, a modelling approach may simplify the investigation of more feeding scenarios, their interactions, different local conditions, and considering the spatial and temporal variation of pasture herbage quality and yield. Thus, the second purpose of the dataset gathered for the present thesis was its application for adapting a mathematical model to simulate dairy cattle herds on semi-natural grassland. The general advantage of modelling is that multiple treatments and their interactions can be tested for different local conditions (Christie et al., 2014). Further, longer timeframes and periods varying in resource availability can be tested to prove the robustness of management strategies on different aspects of resource use. This is particularly important for grassland systems which are characterised by high spatial and temporal variation, where context specific strategies need to be identified to improve the system, e.g. regarding its environmental conformity (Scollan et al., 2017).

5.2 Challenges for modelling low-input grazing-based dairy farming systems

The general challenge of developing or adapting a mathematical model for simulating livestock production systems is that modellers need to strike a balance between capturing the complex interacting processes underlying the respective system at hand while ensuring that sufficient reliable data is available for model calibration, evaluation, and application (Snow et al., 2014). In the following, this trade-off between model complexity and data availability is discussed for the context of ruminant livestock models for low-input grazing-based systems.

5.2.1 Modelling complexity

Simulating ruminants under year-round housing conditions is already complex as such, but even more so under grazing conditions. When focusing on a livestock model, the key differences and challenges lie within modelling the nutritional aspects of grazing ruminants. More factors play a role in simulating the nutritional processes of grazing ruminants than under stall-feeding conditions, namely the greater variation in feed intake, effects of environmental stressors (e.g., heat or parasites), and additional energy expenditures for the physical activities during grazing (Tedeschi et al., 2019). While stall-fed animals receive diets which are adjusted to the animal's requirements allowing less room for selection, grazing-based diets are less uniform enabling greater selective grazing (Gregorini et al., 2015). On the one hand, grazing-based diets are less uniform, because they are subject to a greater variation in nutritional quality and biomass availability from pasture herbage due to the interannual and seasonal weather variability and resulting changes in plant growth (Bell et al., 2018). On the other hand, in low-input grazing-based situations where semi-natural grasslands constitute the main feed base, a greater herbage abundance and spatial variation in feed resources on pasture can be found (Tonn et al., 2019). Low stocking rates and limited nutrient inputs typically lead to grassland swards with a mosaic structure containing short and tall patches, which is maintained by the animal's preference to graze short patches more frequently than tall patches (Tonn et al., 2019). The differences in botanical composition between short and tall patches, in turn, cause a high spatial heterogeneity in nutrient availability across semi-natural grasslands, affecting the selectivity, nutrient intake as well as movement energy expenditures during grazing (Hamidi et al., 2021). The grassland botanical composition and yield, in turn, is impacted by the animal's selection behaviour and nutrient excretions. Finally, the influence of the grassland-animal interaction on feed intake is also influenced by the grazing management, such as the grazing intensity (McCarthy et al., 2011; Hamidi et al., 2021) or paddock layout (Auerswald et al., 2010). Hence, modelling the dynamic interacting effects of weather variability, multi-species grasslands, the animal's grazing behaviour, and grazing management on grassland and animal performance, is a complex task particularly pertaining to low-input grazing-based farming systems.

To capture all interactions between the underlying processes within a modelled system, model complexity is commonly increased. For instance, the dry matter intake (**DMI**) of grazing animals can be simulated mechanistically using a bite-based approach, which would consider the underlying biophysical predictors (Snow et al., 2014). However, this requires accurate predictions for bite mass, which is a result of the animal's body size (Erlinger et al., 1990), and sward height and density (Boval and Sauvant, 2019). The sward density, in turn, differs throughout different sward strata and is dependent on the botanical composition, which is particularly difficult to estimate for diverse swards (Snow et al., 2014). Predicting the nutrient intake using a bite-based approach further requires simulating the animal's selective behaviour. For this, predictions of the animal's preferences for certain nutrients (e.g., protein) or plant parts in dependence of the temporally and spatially variable availability of nutrients across the pasture area and sward strata is needed (Gregorini et al., 2015).

This serves as an example of how efforts leading to more complex models also require the availability and accessibility of the respective inputs (e.g., sward density and nutrient distribution across different sward strata). Additionally, for the long-term perspective of integrating multiple models to enlarge the modelling scope (e.g., by inclusion of a grassland model) it further needs to be considered that the required input factors need be delivered by the coupled model. The data availability poses a contrasting challenge particularly pertaining to models for low-input grazing-based farming systems.

5.2.2 Modelling data requirements

The development, adaptation, evaluation, and application of mathematical models require large volumes of data, because the data needs to represent the variability of the targeted inputs and outputs for a robust representation of the targeted farming system. This pre-requisite is particularly challenging to fulfil for low-input grazing-based systems where (1) the high variability in farm, weather, and grazing conditions requires extensive datasets which capture the breadth of existing farming systems, (2) generally, a greater uncertainty in data is found, and (3) data availability is restricted owing to the lower research interest low-input farming received in the past decades.

Regarding issue (1), the diversity in farm structure and management observed through the observed dataset demonstrates that research revolving around (low-input) grazing systems cannot solely rest upon findings from few research farms (Kapp-Bitter et al., 2021). Scollan et al. (2017) emphasised that solutions to improve the competitiveness of low-input and organic dairy production systems need to be tailor-made for the region and context. Data gathered on commercial farms, thus, helps to represent the true variation in grazing conditions and common grazing management practices and the effect of changes in management, thereupon. Hence, to capture the breadth of farm types and grazing management systems across low-input grazing-based farming systems, data gathered on commercial dairy farms, as opposed to research farms, was used in the present thesis. The farms involved in the studies further benefited directly from the trial periods by gaining a deeper understanding of their own system (Doole et al., 2023). Another purpose of on-farm research is that it strengthens the credibility of research findings among practitioners. Nevertheless, it certainly needs mentioning that this on-farm research approach entails limitations such as the challenge of tightly controlling all influencing factors of the respective research question or the lack of measurements which are routinely gathered on research farms (e.g., animal-individual body weight (**BW**) and intake measurements) (Doole et al., 2023). A trade-off of on-farm approaches is therefore the lower accessibility of data which can be used as potential model inputs or outputs.

With respect to issue (2), there is partly a sheer lack of methodologies and partly practical reasons which restrict the applicability of standard methodologies in a grazing context. Hence, for modelling grazing-based farming systems, the inevitable uncertainty in data needs to be considered. This issue is illustrated by three examples from the present thesis, i.e., the feed intake, rumen-undegradable CP in pasture herbage, and urinary N excretions. Regarding the feed intake, it can be argued that there is no golden standard for determining intake on pasture because there is no way to quantify the actual DMI and nutrients of grazing ruminants (Hellwing et al., 2015; Smith et al., 2021). Instead, there are several estimation methods based on markers, sensors, or measurements on the paddock level, and each of these methods ensue estimation errors (Smith et al., 2021). For instance, in Chapter 2, estimations of DM intake of pasture herbage were estimated using a double-marker technique, where uncertainty can be expected from the limitations related to total marker recovery and use of faecal spot samples (de Souza et al., 2015; Guinguina et al., 2019).

The rumen-undegradable CP concentration (**RUP**) of forages serves as exemplary source of uncertainty owing to the practical and methodological restrictions to determine forage RUP. The *in-situ* methodology is considered as a standard method to determine feed RUP, but it is labour intensive and requires rumen-fistulated animals, which restricts its applicability as routine measurement technique (Edmunds et al., 2012). The RUP was, therefore, determined *in-vitro* for a subset of pasture herbage samples (n = 21) using protein fractionation and an equation developed using data from temperate, multi-species grassland (Kichhof, 2007; assuming a passage rate of 5%/h). The values determined *in-vitro* were greater (343 g/kg CP) than literature values by INRA (2019; 306 g/kg CP) which would have further increased the underprediction of urinary N excretions in Chapter 4. Considering the low coefficient of determination (**R^2**) for the equations by Kirchhof (2007: R^2 = 0.51 – 0.55) compared to her *in-situ* values and the lack of more robust alternative equations to estimate the RUP of fresh forage samples (Edmunds et al., 2012), the literature values by INRA were, thus, preferred. The findings of Chapter 4, however, highlighted the sensitivity of the predictions of urinary N excretion by the adapted LIVSIM model (**LIVSIMtemp**) to the RUP inputs. The chosen literature values, which may not reflect the true values found in the investigated semi-natural grasslands, consequently, add uncertainty to model predictions of urinary N excretions.

In Chapters 2 and 4, the uncertainty in the reference observations for urinary N excretion were discussed, because they were estimated as the balance between measured N intake, milk N secretion, and faecal N excretion. Under stall-based research conditions, urinary N excretions can be determined via total collection, which is hardly feasible under grazing conditions. The fast developments and increased accessibility of sensors can facilitate the retrieval of animal-individual, site-specific data in a grazing context (e.g., Aquilani et al., 2022). For instance, the use of non-invasive sensors depict an emerging alternative for monitoring urine volume and urinary N excretions on pasture (Betteridge et al., 2010; Mangwe et al., 2019), which has already found its way into grazing trials (e.g., Misselbrook et al., 2016; Marshall et al., 2020). Their large-scale applicability for an on-farm context, however, needs to be contemplated e.g., because commercially kept animals are less accustomed to wearing bulky sensors, and it requires the farmers' acceptance to use sensors which are glued to the animal. Given the increasing interest for low-input dairy farming systems (e.g., Capper and Bauman, 2013), issue (3) may be resolved over time by closing the knowledge gap on low-input grazing-based farming systems in combination with the ongoing advances in sensor-based data collection.

The preceding examples demonstrate the challenges of accumulating suitable datasets within low-input grazing-based production systems, because key variables such as the feed intake or forage RUP concentrations can add uncertainty to model predictions, while parameters such as urinary N excretions are difficult to obtain across a variety of grazing conditions. Since increasing model complexity for the sake of capturing more of the underlying processes requires more inputs, the bias from uncertain data might accumulate, potentially rendering more detailed but inaccurate models. Therefore, there is a trade-off between model complexity and simplicity. Both can restrict the applicability of the model either due to access to all variables, or the risk of missing the main interacting factors (Snow et al., 2014).

5.3 Evaluation of models for low-input grazing-based farming systems

Low-input farming systems are commonly criticised for their lower productivity and the ensuing greater environmental impact per product output or hectare farm land (Capper and Bauman, 2013). Solely favouring a system due to its greater productivity, however, disregards the multifaceted aspects, in which low-input grazing-based systems can compete with high-input farming systems, such as land use efficiency (Dentler et al., 2020), lifetime production (Grandl et al., 2019), or provision of resilient, biodiverse feed resources (Schaub et al., 2020). Therefore, a mathematical grassland-based livestock model was adapted in the present thesis which does not only simulate animal performance but also considers aspects of resource use, such as N.

5.3.1 Alternative models

Temperate, grazing-based herd models with a similar scope as LIVSIMtemp are Dynamilk (Jacquot et al., 2015) and the one by Ruelle et al. (2016). Both of these process-based herd models simulate the variation in milk production and intake of dairy cows in grazing-based systems dynamically. They are also capable of simulating herd demography and animal-individual performance (intake, milk yield, and BW growth) from birth to death. Dynamilk was exclusively developed for rotational grazing systems, which is manifested in their intake, grassland, and grazing management modules, making it less suitable for low-input grazing-based systems where continuous grazing is also common. Ruelle et al. (2016), in contrast, predict herbage intake using the semi-mechanistic model GrazeIn (Delagarde et al., 2011; Faverdin et al., 2011) which considers both, continuous and rotational grazing systems. GrazeIn firstly estimates voluntary pasture herbage intake based on the animal's intake capacity and physical fill of forages and concentrates ingested in barn, using inputs on diet composition (proportions of forages and concentrates), and feed (digestibility, and energy and protein value) and animal characteristics (BW, lactation stage, production). Then, actual intake of pasture herbage is calculated accounting for the limiting effects of daily grazing time, and herbage availability. For the latter, empirically determined curvilinear relationships between herbage intake and pasture sward height (for continuous grazing) or herbage allowance (kg DM/animal and d; for rotational grazing systems) are applied. Chapter 3 exemplified that a model (e-cow; Baudracco et al., 2012) using empirically determined relationships between herbage allowance (kg DM/animal and d) and herbage intake developed for high-input grazing systems is not necessarily transferable to low-input grazing systems (relative prediction error = 45.3 %), likely owing to the differences in sward characteristics (e.g., sward height or diversity) and related behavioural responses by the animal (Boval and Sauvant, 2019, 2021). Similarly, GrazeIn's intake equation for rotationally grazing animals is based on a dataset with a herbage mass ranging between 800 – 5750 kg DM/ha, which is well above the herbage mass observed in the reference dataset (412 kg DM/ha ± 339). In contrast, their equation for continuously grazing animals also covers pastures with low herbage mass (sward height = 4.0 – 14.9 cm, measured with a HFRO sward stick; Barthram, 1985), which may suit the observed semi-natural pastures (compressed sward height = 4.1 cm ± 1.51). There is certainly merit in investigating whether the same or a similar approach could be implemented because the limiting impact of daily grazing time or herbage availability on herbage intake are not yet captured by LIVSIMtemp. Alternative existing dynamic process-based models such as the one by Ruelle et al. (2016), might be similarly suitable for predicting the feed intake and milk production within low-input grazing systems. However, one practical constraint of this model is its reliance on the French feeding values, which are not routinely determined in regions outside France. It further disregards the aspects of resource use such as N excretions.

5.3.2 The adapted LIVestock SIMulator (LIVSIMtemp)

The LIVSIM was chosen as suitable candidate model for multiple reasons. The LIVSIM enables the simulation of resource distribution across animal groups, space and time by considering the seasonally variable resource availability, differences in nutrient supply from different sources (on-farm and off-farm resources, and different pastures per farm), and allocation of feed resources to different animal groups. Moreover, considering the data requirements for displaying the breadth of grazing practices found in a low-input farming context, and the difficulties of collecting this data, a model with relatively low input requirements, such as LIVSIM, was needed for the present thesis. The LIVSIM was also chosen as candidate model because it can be coupled with a grassland module as demonstrated by Marohn et al. (2022), which will enable the simulation of the resource exchange between the animal and grassland scales. Ultimately, LIVSIM was also chosen as candidate model for practical reasons i.e., because code and expertise were available and accessible within the working group.

Comparisons with observed herd data, and scenario and sensitivity analyses confirmed that LIVSIMtemp is capable of plausibly simulating differences in animal performance (e.g., milk yield) based on differences in herd demography, supplement feeds and pasture herbage (Chapter 4) for the observed low-input grazing-based dairy farming systems. The LIVSIMtemp can further simulate the relative differences in N excretions between different treatments. The absolute total N excretion (g/d), however, was underpredicted by 23 % (= relative difference between observed and predicted values) mainly due to the underprediction of urinary N excretion by 43 %. Therefore, further attempts to improve the accuracy of N use predictions were suggested to reliably capture the varying degree of susceptibility of faecal or urinary N to volatilisation and leaching (Powell et al., 2010).

The LIVSIM was further chosen because it can be used for simulating further aspects of resource use such as the land use efficiency. As an example of this additional use case, an indicator for land use efficiency was calculated for the scenarios in Chapter 4. The proportion of milk derived from different agricultural land (arable land or grassland) was calculated according to its contribution to the animals' energy intake (Haas et al., 2007). This approach allocates the animals' energy demand for maintenance uniformly across all feed sources. The share of the daily milk yield derived from feedstuffs cultivated on arable land was greatest for the TMR scenario, both, across the entire year and the grazing season, due to the supplementation with maize silage in the TMR scenario (Figure 5.1). These findings on land use and on N use (Chapter 4), display the trade-off between different aspects of resource use. The TMR scenario clearly increased the use efficiency of ingested N decreasing the risk for losses of harmful N compounds (e.g., nitrate or nitrous oxide) to the environment. The reduction in potential N emissions, however, came at the expense that 2,941 kg or 44 % of the annual milk production was based on feedstuff cultivated on arable land. In the MeadowConc scenario, solely 1,091 kg or 18 % of the annual milk production was based on arable crops, while annual N use efficiency (in % of N intake) only differed slightly from the TMR scenario (0.8 percentage points). These scenarios, thus, demonstrate that LIVSIMtemp can be applied for gauging strategies to improve resource use of temperate, low-input dairy systems considering the interaction of multiple resources (e.g., grassland, arable land, and N).

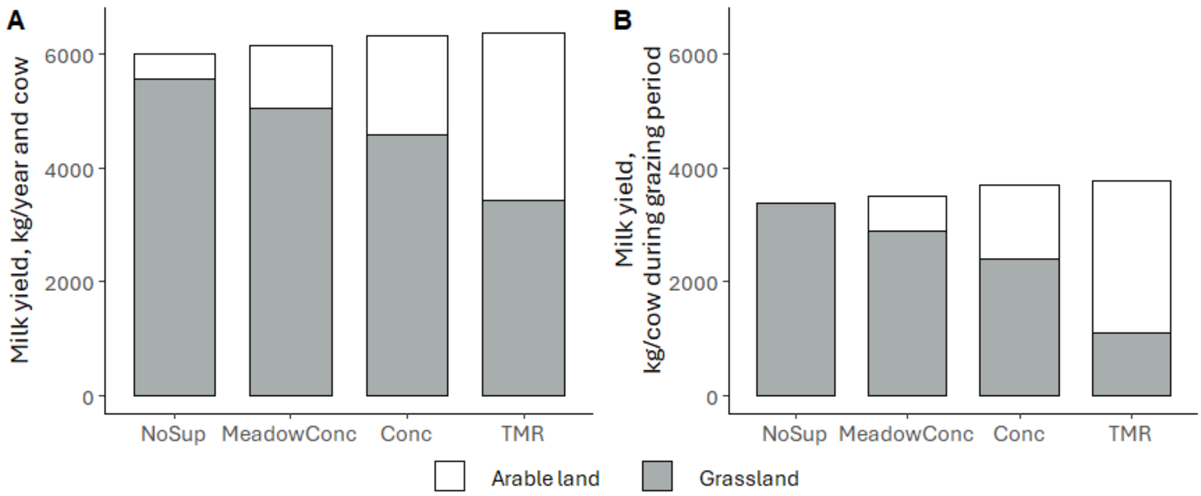

Figure 5.1. Partitioning of milk by feed origin across the year (A) or during the grazing period (B) for four supplementation scenarios, simulated by the LIVestock SIMulator adapted for temperate grazing-based low-input dairy farms (LIVSIMtemp). NoSup = no supplementation; MeadowConc = supplementation with meadow-grass and concentrates; Conc = supplementation with concentrates; TMR = supplementation with concentrates and maize silage).

5.4 Recommendations for modelling grazing-based dairy farming systems

Doubtless, further model modifications can improve LIVSIMtemp's ability to better capture the complexity of grazing-based cattle farming systems discussed earlier. Moreover, the present dissertation focused on the aspect of N use but it was stated earlier that there are multifaceted aspects, in which low-input grazing-based systems can compete with high-input farming systems. Hence, a non-exhaustive list of potential outputs (e.g., enteric methane), scales (e.g., grassland), and production systems (e.g., stall-based systems) for expanding LIVSIMtemp will be addressed, which will enable LIVSIMtemp to better evaluate the merits and drawbacks across different kinds of grassland-based cattle production systems.

5.4.1 Further outputs

The present thesis focused on the aspect of N use and excretion, whereas methane emissions are another key factor determining the environmental performance of ruminant livestock systems (e.g., Hristov et al., 2013). A methane sub-module is already integrated in LIVSIM which estimates enteric and manure methane emissions based on Tier 2 of the IPCC livestock inventory (IPCC, 2006). This module’s revision was beyond the scope of the present study, but is important for future applications, none the less. Multiple empirical models for estimating enteric methane emissions were evaluated by the GfE (2023) using a European dataset. Among the models for beef cattle, Animal_C by van Lingen et al. (2019) achieved the lowest RMSE (15.5 %). Animal_C mostly requires input factors, which are already integrated in LIVSIMtemp, except for dietary crude lipid concentration, which might be easily integrated. For dairy cattle, the model by Niu et al. (2018) was recommend by the GfE (2023) because it unites low input requirements (DMI, dietary NDF concentration) with a similarly high prediction accuracy (RMSE = 14.7 %) as more complex models. Although the suggested models seem promising, they have been predominantly developed and evaluated for animals fed conserved forages and concentrates. Hence, additional evaluation for grazing-based diets with high inclusions of fresh forage are required (Stergiadis et al., 2016).

Moreover, low-input farming systems can outperform high-input farms with regards to their lifetime production. The advances in greater animal-individual productivity have come at the price of hampering animal’s health and fertility via excessive BW mobilisation during peak lactation, impairing animal welfare (Knaus, 2009). The related shorter productive lifespan can further increase the environmental impact (e.g., methane emissions) and costs per kg milk, because the used resources and emissions during the rearing phase are allocated to a lower lifetime production (Grandl et al., 2019). Lifetime production and replacement rates also determine the number of beef calves, a co-product from dairy farming. Greater replacement rates in herds with greater productivity lead to less surplus calves for beef production. The lack of surplus calves is predominantly compensated by more meat production from suckler beef cow systems, which tend to have a greater environmental impact (e.g., via greater demand for land and greater methane emissions; Flysjö et al., 2012). Accurate predictions of lifetime production require capturing the combined effect of farm management, animal production, body condition, and health on the animal’s reproductivity (Phuong et al., 2016). Regarding LIVSIMtemp, this requires (1) an in-depth evaluation of the BW module, and (2) modifications in the determination of replacement/culling age. As outlined in Chapter 4, BW mobilisation was predicted accurately for the two reference (low-yielding) herds of the present study. However, further testing regarding differences between breeds, and whether BW and body condition during first lactation are represented reliably are required (Gruber et al., 2014). The model structure harbours good prerequisites for modelling differences in BW, body condition, and mobilisation between breeds because breed coefficients largely differ by their BW composition, age-dependent growth potential, and peak milk yield which are key traits determining energy partitioning between body weight and milk production (Ledinek et al., 2019). To better capture differences in BW mobilisation between genotypes, additionally, genetic scaling parameters might be implemented (Ruelle et al., 2016), which define a variable priority between BW gain and production. The reproductive performance and replacement management is presently an outcome of herd management factors (maximum age and herd size), and probabilities of conception and mortality in LIVSIMtemp. The conception probability is determined based on time after parturition, the farm-specific average days open, animal age, and a body condition index (Konandreas and Anderson, 1982). Here, it requires testing whether the body condition index reflects the effect of feeding level on conception probability. Further, the mortality rate was determined farm-individually by using the observed age-dependent death rates, which included culling due to various reasons (e.g., fertility issues, claw problems, etc.). The mortality rate as input factor, thus, fails to simulate the effect of feeding (i.e., energy and nutrient density) and production level on fertility and health.

5.4.2 Further scales

The present thesis focused on the N use and fluxes on the animal level. Predictions of urinary N excretion (daily or as share of N intake) are an important indicator for potential N losses. At the same time, faecal and urinary N excretions represent a valuable resource especially in the context of low-input grazing-based systems which are even more reliant on closing nutrient cycles. Here, part-time grazing is commonly practiced owing to the lack of sufficient grazable land (Gazzarin et al., 2021), which represents a positive side-effect of using semi-natural grasslands. Part-time grazing allows excretions to partly take place in barn, enabling manure collection and more even cattle excreta distribution to match plant requirements. Part-time grazing was, thus, shown to reduce nitrous oxide emissions by 7 to 11 % (Klein et al., 2006; Luo et al., 2008; Li et al., 2013) and N leaching by 30 to 60 % (Environment Waikato, 2008; Vogeler et al., 2013) in high-input grazing-based systems. This reduction potential is likely lower in low-input grazing-based systems, for which Chapter 2 demonstrated a generally lower risk for N losses via urinary N excretion. Nevertheless, on the observed farms, animals grazed part-time (on average 8 hr/d ±

2.2) during 26 of the 33 trial periods. Hence, less than half of the excretions were deposited directly on pasture which potentially reduces N losses further, when evaluated on the grassland level.

Predictions on the location of deposition would, therefore, be valuable for estimating N use and losses across the farm. To keep it simple, excretions between barn and pasture might be distinguished according to the ratio of time per day spent on pasture or in barn (Löw et al., 2020). However, this will not account for the differences in diurnal excretion patterns, nor for the spots within the farm area with greater accumulation potential such as trails and waiting areas to milking parlour, preferred resting areas, or watering places on pasture (Auerswald et al., 2010; Misselbrook et al., 2016; Aarons et al., 2017). Also, the distribution of urine patches across the paddock and day could be considered (Auerswald et al., 2010; Misselbrook et al., 2016). Auerswald et al. (2010) demonstrated that cattle excreta distribution patterns across the pasture were similar across animals and grazing periods i.e., characterised by little random variation (< 5 %). Nevertheless, this will require knowledge of paddock structure (e.g., location of fences, slope, resting area), and does not imply that the same patterns pertain for different types of pastures (e.g., differing in slope, yield or botanical composition). This raises the question whether such increase in model complexity will substantially improve the model's value or decrease the model's flexibility to be adopted across different kinds of farming situations.

Finally, another important scale is the grassland. Its integration would allow simulating the interaction between the herd and grassland, such as the effect of excreted nutrients on biomass production, in turn, determining the nutrient supply from pasture herbage at different moments in time. This foremostly requires identifying a suitable model for modelling plant growth and resource use of diverse and changing plant communities, and modelling the interaction between animals and grassland, including the effect of selective grazing (Snow et al., 2014). It further requires changes in the livestock module to provide the inputs needed by the grassland module to simulate changes in plant N uptake or N losses in response to diet changes. For this, accurate predictions of the partitioning into faecal or urinary N excretion are required. For instance, Wachendorf et al. (2008) measured the N losses via leaching and volatilisation from pastures in autumn for sandy soils which received faeces or urine applications with 1052 and 1030 kg N/ha, respectively. While nitrous oxide emissions were negligible for both, faeces and urine application (0.5 and 0.35 g N/ha, respectively), they measured leaching losses of 38 and 654 g N/ha for soils applied with faeces and urine, respectively. This greater susceptibility of urine for environmental losses is mostly related to its high concentration of urea, varying between 52 to 94 %. Much of this variation is determined by the diet, and in turn dictates efficiency of plant N uptake or susceptibility to losses (Dijkstra et al., 2013; Bougouin et al., 2022). Predictions of urinary N composition based on dietary factors would provide a nuanced depiction of a broad range of feeding situations (i.e., varying in supply of protein and energy).

5.4.3 Further production systems

Lastly, there are no distinct differentiations between low- and high-input farming systems but rather a range of facets in between as illustrated in Figure 5.2 (adapted from Moorby and Fraser, 2021, p. 2). Also, the awareness regarding the environmental issues related to high-input, productivity-oriented grazing-based systems is increasing, giving rise to researchers advocating the use of multispecies grasslands (e.g., Delaby et al., 2020; Leiber, 2022). This may represent a sustainable intensification strategy for grazing-based systems using improved or temporary grasslands, while making use of the benefits of diverse i.e., resilient, pastures (Schaub et al., 2020). Similarly, Grassauer et al. (2022) investigated the eco-efficiency of dairy farms in Austria and concluded that there is not one singular strategy to attain high eco-efficiency. Instead, multiple management options exist which need to be amended according the farm's individual conditions. Greater eco-efficiency can be attained by both, increasing outputs (e.g., by greater concentrate supplementation to attain greater milk yield and farm income), or by decreasing inputs (e.g., by lowering concentrate supplementation to reduce feeding costs). For evaluating the benefits and barriers of certain grazing and farming practices based on the locally available resources, a model is needed which is applicable beyond the conditions of low-input grazing-based systems.

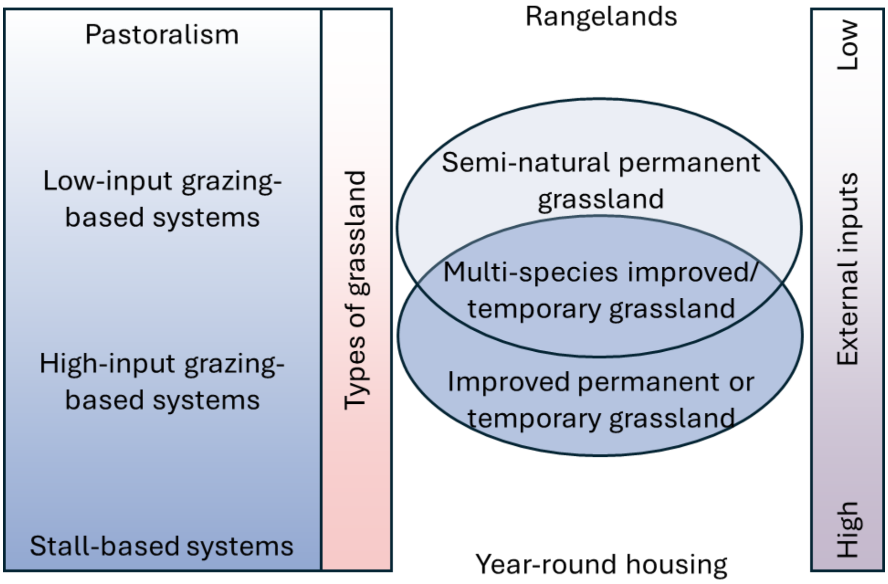

Figure 5.2. Range of ruminant production systems classified by housing situation or types of grassland (classification according Peeters et al., 2014), and reliance on external inputs (e.g., bought-in fertilisers and concentrates) (adapted from Moorby and Fraser, 2021, p. 2).

Model evaluation has proven that LIVSIM, a model foremostly developed for tropical ruminant production systems, was adaptable for temperate, low-input grazing-based systems. The mechanistic nature of the model as well as the lower input requirements of LIVSIM compared to other ruminant livestock models (Bateki and Dickhoefer, 2020) implies that it can be easily parameterised for further farming systems such as more intensive grazing-based or stall-based dairy farms. LIVSIM and LIVSIMtemp further pose the advantage of offering a modelling basis which can be used by research teams working across different agroecological systems, giving it a global perspective. Lastly, this conclusion opposes the second hypothesis of the present dissertation, stipulating that a model specifically adapted for low-input grazing-based systems was required. Instead, any endeavour for future evolutions of LIVSIMtemp and LIVSIM should ensure that its transferability to other farming systems is maintained or even improved.

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Chapter 6 | General Conclusions

Investigating the nitrogen (**N**) use and excretion of lactating dairy cows across a diverse set of commercial dairy farms in the case study region of Baden-Württemberg demonstrated that using semi-natural as opposed to improved grasslands favours a more efficient N use and lower urinary N excretions on an animal basis. Nevertheless, there is a high variation in N use and excretions between farms and seasons, demonstrating the need for a model than can assess more strategies to further reduce the N losses from grazing animals. Therefore, the dynamic process-based ruminant production model, the LIVstock SIMulator (**LIVSIM**) was adapted. The dry matter intake of grazing dairy cows is among the most decisive factors for determining animal performance and aspects of resource use, such as N excretions, and can be best predicted by a semi-mechanistic intake model, although it had been developed for stall-based feeding situations. Instead, models specifially developed for high-input grazing systems are not necessarily transferable to low-input grazing systems. Consequently, by integrating the identified intake mode, and modifying the modules for predicting energy requirements, N excretion, and herd management, LIVSIM was adapted to low-input grazing-based cattle farms. The adapted LIVSIM is capable of plausibly simulating differences in animal performance (e.g., milk yield) and nutrient excretions (e.g., urinary N excretion) based on differences in herd demography, supplement feeds and pasture herbage. Future model improvements should focus on increasing the prediction accuracy of N excretion and its partitioning due to the varying susceptibility of faecal or urinary N to volatilisation and leaching. There is further merit in adapting LIVSIM for production systems beyond the ones investigated in the present study, and in adding more outputs (e.g., enteric methane) and scales (e.g., grassland) to better capture the multifaceted aspects determining the sustainability of ruminant-based farming systems.