Inter- and intraindividual variation in energy supply of dairy cows and the potentials of animal individual energy balancing

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Vorwort

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List of Abbreviations

ADF	Acid detergent fiber
ADFD	Acid detergent fiber digestibility
ADFom	Acid detergent fiber expressed exclusive of residual ash
ADL	Acid detergent lignin
AIA	Acid-insoluble-ash
AIC	Akaike’s information criterion
BHB	β-hydroxybutyrate
BW	Body weight
CP	Crude protein
CPD	Crude protein digestibility
CV	Coefficient of variation
CVAS	Cumberland Valley Analytical Services
d	Day
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
EB	Energy balance
ECM	Energy corrected milk
EE	Ether extract
ERM	Energy requirement for maintenance
ERP	Energy requirement for production
ESC	Ethanol-soluble carbohydrates
HF h (GER+AT)	Holstein Frisian breed on a high management level, in Germany (GER) and Austria (AT)
iNDF	Indigestible aNDFom
kg	Kilogram
LMM	Linear mixed model
MJ/kg	Mega joule per kilogram
MY	Milk yield
n	Number
NDF	Neutral detergent fiber
NDFD	Neutral detergent fiber digestibility
NDFom	Neutral detergent fiber not assayed with a heat stable amylase and expressed exclusive of residual ash

NEB	Negative energy balance
NEFA	Non-esterified fatty acids
NEL	Net energy lactation
NFC	Non-fiber carbohydrates
NIRS	Near infrared spectroscopy
OM	Organic matter
OMD	Organic matter digestibility
Pd	Production disease
SARA	Sub-acute ruminal acidosis
SD	Standard deviation
SE	Standard error
SP	Soluble protein
TC	Total collection
TDN	Total digestible nutrients
TER	Total energy requirement
TES	Total energy supply
TMR	Total mixed ration

List of Publications

This thesis is based on the work contained in the following publications referred to by Roman numerals in the text:

I. Rumphorst, T., Scheu, T., Koch, C., Sundrum, A., 2021. Intra- and interindividual variation in nutrient and energy availability in dairy cows during early lactation. (*submitted)*

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Abstract

Inter- and intraindividual variation in energy supply of dairy cows and the potentials of animal individual energy balancing

In recent decades, a massive increase in milk yield per cow per year has been observed, which could initially be considered positive due to economic advantages, but at the physiological level a corresponding increase in energy intake has not occurred. This situation of negative energy balance increasingly confronts cows with metabolic challenges, which, however, usually remain unnoticed due to a lack of animal-specific considerations, and thus increasing rates of disease and malting can also be observed. The objective of the present work was to determine intra- and interindividual variation in nutrient availability, energy availability, and energy balance within and between dairy cows during early lactation in order to identify animals that are particularly challenged in their ability to adapt. By looking at the energy balance, it is possible to quantify the energetic supply deficit.

In the present study, the individual areas of nutrient and energy supply were considered and analyzed and compared with the energy requirement to be able to depict the energetic situation at the individual animal level. In the first step, the nutrient availability was considered. Feed intake and nutrient digestibility were determined for 28 dairy cows from 2 to 15 weeks after calving to provide an indication of the level of nutrient availability of each animal during early lactation and the variation within and between individual animals (Paper I). Furthermore, feeding behavior during early lactation was analyzed and the influence of feeding behavior parameters on feed intake and nutrient digestibility was determined (Paper II). By comparing the parameters used to assess energy requirements and energy supply, it was possible to perform animal-specific energy balancing and to assess the energy balance between individual animals at the same stage of lactation and within individual animals during early lactation (Paper III).

Variation in feed intake as well as nutrient digestibility were substantial and resulted in equally large variation within and between animals in the amount of available nutrients and energy. In this context, the internal marker iNDF240 was identified as a suitable tool for determining animal-specific nutrient digestibility under farm conditions. There was also a wide variation in the parameters of feeding behavior within and between individual animals. Milk yield, meal frequency, feeding time, feeding rate and meal size were determined to be significant factors influencing the amount of feed intake. Nutrient digestibility was predominantly influenced by feed intake and week of lactation. Variables of intake behavior had no significant effect on nutrient digestibility. All animals, whether having high, medium or low feed intake on average during early lactation, differed significantly in the level and variation of nutrient digestibility.

The described variation in feed intake behavior shows that animals of the same breed, at the same stage of lactation and in the same environment follow different strategies for nutrient and energy intake. The individuality of the feed intake behavior conflicts with herd-based efforts of predicting feed intake. To reduce the metabolic challenges and the associated health risks at the beginning of lactation, it is necessary to assess animal-specific demand and supply levels, and thus energy balances, at least during the critical lactation phase. By calculating the energy balance, besides a very deficient situation on average, a very large variation between individual animals at the same stage of lactation as well as within individual animals during early lactation could be observed.

Individual determination of energy balances and comparison of the energy-producing and energy-consuming parameters offer the possibility of estimating the individual energetic situation during lactation as well as an objective comparison to other animals of the herd, and of identifing individual animals with a particularly pronounced negative energy balance. By looking at the individual parameters, possible causes for the extent of the deficit situation can be identified, and based on this, options for action can be developed. Despite the higher effort compared to the conventional approach of aggregation using average values, the individual animal energy balancing can be seen as a starting point for sustainable improvement of animal health.

Keywords: variation; dairy cows; digestibility; dry matter intake; intake behavior; energy supply; energy balance

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Zusammenfassung

Intra- und interindividuelle Variation in der Nährstoff- und Energieverfügbarkeit von Milchkühen und Potentiale in der tierindividuellen Energiesaldierung

In den letzten Jahrzehnten konnte ein massiver Anstieg der Milchleistung pro Kuh und Jahr beobachtet werden, der zunächst aufgrund wirtschaftlicher Vorteile als positiv bewertet werden konnten. Auf physiologischer Ebene blieb jedoch eine entsprechende Steigerung der Energieaufnahme aus. Diese Situation des negativen Energiesaldos stellt die Kühe vermehrt vor metabolische Herausforderungen, die aufgrund mangelnder tierindividueller Betrachtungen jedoch meist unbeachtet bleiben und so steigende Erkrankungs- und Merzungsraten zur Folge haben. Ziel dieser Arbeit war es, die intra- und interindividuelle Variation in der Nährstoff- und Energieverfügbarkeit sowie des Energiesaldos innerhalb und zwischen Milchkühen während der Frühlaktation zu ermitteln. Durch die Bestimmung des Energiesaldos ist es möglich, die energetische Versorgungslücke zu quantifizieren.

Im Rahmen der vorliegenden Untersuchung wurden die einzelnen Bereiche der Nährstoff- und Energieversorgung betrachtet und analysiert und in Abgleich mit dem Energiebedarf gebracht, um auf tierindividueller Ebene die energetische Situation abbilden zu können. Im ersten Schritt erfolgte die Betrachtung der Nährstoffverfügbarkeit. Für 28 Tiere wurden in der 2 bis 15 Woche nach der Kalbung die Futteraufnahme und die Nährstoffverdaulichkeit bestimmt. Damit sollte zunächst die Höhe der Nährstoffverfügbarkeit der einzelnen Tiere während der Frühlaktation sowie die Variation innerhalb und zwischen den Tieren ermittelt werden (Paper I). Weiterhin wurde das Fressverhalten im Laufe der Frühlaktation analysiert und der Einfluss der Parameter des Fressverhaltens auf die Futteraufnahme und die Nährstoffverdaulichkeit bestimmt (Paper II). Durch die Gegenüberstellung der Parameter, die zur Beurteilung des Energiebedarfs und der Energieversorgung dienen, konnte eine tierindividuelle Energiesaldierung vorgenommen werden und das Energiesaldo zwischen Einzeltieren im gleichen Laktationsstadium sowie innerhalb von Einzeltieren während der Frühlaktation beurteilt werden (Paper III).

Die inter- und intraindividuelle Variation der Futteraufnahme sowie der Nährstoffverdaulichkeit war beträchtlich und führte zu einer ebenfalls großen Variation in der Verfügbarkeit an Nährstoffen und Energie. In diesem Zusammenhang erwies sich der interne Marker iNDF240 als ein geeigneter Marker zur Bestimmung der tierindividuellen Nährstoffverdaulichkeit unter betrieblichen Bedingungen. Auch in den Parametern des

Fressverhaltens bestand innerhalb und zwischen Einzeltieren eine große Variation. Milchleistung, Häufigkeit der Mahlzeiten, Fresszeit, Fressgeschwindigkeit und Größe der Mahlzeiten konnten als signifikante Einflussfaktoren auf die Futteraufnahme identifiziert werden. Die Nährstoffverdaulichkeit wurde vor allem durch die Futteraufnahme und die Laktationswoche beeinflusst. Die Variablen des Aufnahmeverhaltens hatten keinen signifikanten Einfluss auf die Nährstoffverdaulichkeit. Tiere mit im Durchschnitt hoher, mittlerer oder geringer Futteraufnahme während der Frühlaktation unterschieden sich signifikant in der Höhe und der Variation der Nährstoffverdaulichkeit. Die Variation im Futteraufnahmeverhalten zeigt, dass sich Tiere der gleichen Rasse, im gleichen Laktationsstadium und in der gleichen Umgebung zur Nährstoff- und Energieaufnahme unterschiedlich verhalten. Die Individualität des Futteraufnahmeverhaltens steht im Widerspruch zu den bisherigen Bemühungen, die Futteraufnahme vorherzusagen.

Um die metabolischen Herausforderungen zu Beginn der Laktation und die damit einhergehenden gesundheitlichen Risiken zu reduzieren, ist es erforderlich, die tierindividuellen Bedarfs- und Versorgungsniveaus und damit die Energiesalden zumindest in der kritischen Laktationsphase einzuschätzen. Die Berechnung der Energiesalden förderte nicht nur eine im Durchschnitt sehr defizitäre Energieversorgung, sondern auch eine sehr große Variation zwischen den Einzeltieren zu Tage. Die tierindividuelle Bestimmung der Energiesalden und die Gegenüberstellung der energieliefernden und energiezehrenden Parameter bietet eine Möglichkeit eine Einschätzung der tierindividuellen energetischen Situation im Laktationsverlauf sowie in Relation zu anderen Tieren der Herde zu bekommen und Einzeltiere mit besonders ausgeprägtem negativen Energiesaldo zu identifizieren. Durch die Betrachtung der Einzelparameter können mögliche Ursachen für das Ausmaß der defizitären Lage identifiziert und darauf aufbauend Handlungsoptionen entwickelt werden. Trotz des, im Vergleich zur herkömmlichen Herangehensweise der Aggregierung und Verwendung von Durchschnittswerten, höheren Aufwandes kann die tierindividuelle Energiesaldierung als Ausgangspunkt für eine nachhaltige Verbesserung der Tiergesundheit angesehen werden.

Für meine Familie.

1. General Introduction

In recent years, economic pressure in dairy farming has increased massively. Low milk prices and high costs are forcing farmers to adapt their production processes in order to survive on the market in the long term (Bartova et al., 2009; Barkema et al., 2015; Donnellan et al., 2015). The most common adaptation processes include an increase in herd size and milk yield (Bewley et al., 2001; Wolf, 2003; Clay et al., 2020). Due to the increase in herd size, the keeping of animals in groups has become more and more prevalent (Raussi, 2003). This allows great savings in labor time and manpower in one fell swoop as it is much easier e.g. to clean stalls or do the milking or feeding processes via mechanized routes. It also very much accommodates the animals' natural herd instinct (Cabrera and Kalantari, 2014; Barkema et al., 2015). Despite technological facilitation, farms are increasingly reliant on non-family labor due to increasing herd sizes. However, the supply of skilled labor in agriculture remains limited (Bewley et al., 2001; vonKeyserlingk et al., 2013). In order to be able to cover the costs incurred despite low milk prices, a further increase in milk yield per cow has been pushed. Intensive breeding selection for milk yield along with improvements in husbandry, feeding and management have increased milk yield by 20% in the last 10 years (VandeHaar and St-Pierre, 2006; FAO, 2010, 2019). However, the initially apparent positive economic benefits of increased milk yield have been accompanied by growing metabolic challenges for the individual animal (Habel and Sundrum, 2020). The increase in milk production has lead to a massive increase in the energy requirements of the animals but not to an equivalent increase in energy intake (NRC, 2001; Sundrum, 2015; Souza et al., 2019). During early lactation the cow's metabolism changes from a non-lactating state to a state of intensive milk production within a very short time. For example, daily requirements for glucose, amino acids and fatty acids during this period are, for example, respectively more than 2.7, 2.0 and 4.5 times higher than the uterine requirements during the last third of gestation (Bell, 1995). The increase in entry yields that also occur as milk production increases exacerbates this metabolic challenge (Lean et al., 1989). To overcome this metabolic challenge, the animal requires a high level of energy which cannot be adequately provided due to physiological constraints e.g., limited rumen volume (Bauman and Currie, 1980; Bell, 1995; Sordillo and Raphael, 2013). This inevitably leads to a massive energy deficit or negative energy balance (NEB) and an increased need for metabolic adjustment and regulation by the cow (Sundrum, 2015). When energy which can be ingested is insufficient to meet the demand, the body begins to mobilize body fat reserves, resulting in increased lipolysis

(Sordillo and Raphael, 2013). Over time, the increasing hepatic uptake of non-esterified fatty acids (NEFA) from the blood can lead to the development of fatty liver, which in turn can have far-reaching negative consequences on overall body function (Knight et al., 1999; Collard et al., 2000; Sundrum, 2015). In addition to metabolic disorders, negative associations between energy deficit and fertility (Butler and Smith, 1989; Nebel and McGilliard, 1993; Domecq et al., 1997) and hoof health (Collard et al., 2000) have been described (Compton et al., 2017; Probo et al., 2018). The sharp increase in milk yield also results in insufficient glucose availability to maintain the immune system as the intermediate metabolism cannot provide enough glucose to simultaneously meet the needs of milk and immune cells, resulting in additional health risks to the animals (Habel and Sundrum, 2020). Thus, disorders and diseases can be understood as the result of an overload of bodily functions and as a failed adaptation process (Saborido and Moreno, 2015). Therefore, it is especially necessary at the beginning of lactation to make the most possible accurate assessment of the animal individual energy requirements supply situation to identify and support the animals which have a risk of failing (Habel and Sundrum, 2020).

Estimation of energy requirements for maintenance and production is fairly straightforward due to modern techniques for milk volume and milk content recording and determination of body weight by weighing (Thorup et al., 2012). However, estimating energy and nutrient supply presents a unique challenge to the dairy farmer. Although modern laboratory analyses make it possible to determine the ingredients of a feed and a ration quite accurately (Sniffen et al., 1992; Fox et al., 2003), measuring the level of feed intake or the degree of digestibility of the feed in the individual animal is hardly possible under practical conditions at present. Nevertheless, in order to have a rough tendency for calculating the amount of feed to be submitted, estimation formulas based on herd averages which assume an equal feed intake based on a fictive average animal for all animals in the group or herd are used to estimate feed intake (NRC, 2001; Gruber et al., 2004; Souza et al., 2019). This approach contributes on the one hand to considerable labor relief but on the other hand to a massive over- or underfeeding of most of the cows because of the variation, e.g. in feed intake, among animals (Azizi et al., 2009; Maltz et al., 2013; Huhtanen et al., 2015; Cabezas-Garcia et al., 2017). Moreover, estimation of digestibility is conventionally done using tabulated values based on digestibility trials with sheep, or with a few animals under standardized conditions (Schneider and Flatt, 1975; Yan et al., 2002; Morris et al., 2018). However, studies show that large discrepancies exist between the assumed and actual values (Colucci et al., 1989; Yan et al., 2002). The extent of variation between animals and the deviation from the assumed estimate is usually not known but is nevertheless accepted for reasons of effort reduction.

The consequences that an inappropriate nutrient and energy supply can have on an animal are shown by the results of recent studies which have identified a high level of health impairments and production diseases on dairy farms (Hoedemarker et al., 2020). In addition, high numbers of cullings during early lactation are also repeatedly disclosed, which are particularly worrying against an economic background. A large proportion of animals leave the farm before the costs incurred have been recovered through animal performance (Sundrum et al., 2021). The extent of overburdened animals is demonstrated by a 2013 study showing that 30-50% of dairy cows are affected by some form of metabolic or infectious disease around the time of calving (LeBlanc, 2013). Behind these numbers are individual animals that have failed to cope with metabolic stress. When many individual animals in a herd are unable to cope with the metabolic challenge, become ill or have to be culled in an early stage, it is imperative to reconsider the previous approach of aggregation, averaging and performance improvement. A high incidence of diseased or even prematurely slaughtered animals is especially unacceptable for animal welfare, food safety and social acceptance reasons, but also because of the negative consequences for the profitability of farms (Langford and Stott, 2012; van Soest et al., 2019; Habel et al., 2021a).

Therefore, those animals suffering from an extreme energy deficit at the beginning of lactation require special observation, and targeted support if needed, from the beginning. However, due to the aggregation of animals into a group, the individual animal often only comes into focus when it deviates from the predicted performance, has obvious diseases or dies. In other words, the animals are not perceived as individuals until it is too late. This is shown by studies where increasing mortality rates were associated with increasing herd size (Shahid et al., 2015) and decreased animal health with increasing milk yield (Ingvartsen et al., 2003; Koeck et al., 2014; Barkema et al., 2015). Since every disease and every early slaughter, especially those in the first 100 DIM, directly cause economic losses (Habel et al., 2021a; Sundrum et al., 2021), the approach of aggregation, the work with average values, the focus on further performance increases and the disregard of the energy situation of the individual animal should be discussed critically. Especially the alleged benefits of further performance increase should therefore be questioned from an economic point of view.

To ensure sustainable and economic milk production in the long term, it is imperative to reintroduce the focus onto the individual animal and knowledge about the energy balance and situation of the individual animal (van Soest et al., 2019).

2. Aims and Hypotheses of the Thesis

The overall aims of this thesis were to determine the extent of intra- and interindividual variation in nutrient and energy availability and energy balance within and between dairy cows during early lactation to identify animals that are particularly challenged in their ability to adapt to and cope with the feeding conditions.

The specific aims were

i. to determine intra- and interindividual variation in nutrient digestibility and nutrient and energy availability between early lactating dairy cows;

ii. to determine variation in feed intake and feeding behavior during early lactation and to analyze the degree of interaction and influence of these factors on nutrient digestibility;

iii. to identify intra- and interindividual variation in sub-variables of energy balance and the extent of energy balance between early lactating dairy cows;

iv. to evaluate the approach of animal-specific energy balancing in terms of its suitability for determining the energetic situation of the individual animal over time and in comparison, to the rest of the herd, as well as its suitability for identifying animals with an especially high negative energy balance.

Hypotheses:

i. The iNDF240 fraction is suitable as an internal marker of feeds and can be used to reliably determine the apparent digestibility of feed rations and to reveal animal-specific differences in nutrient digestibility.

ii. The intraindividual variation in nutrient availability is influenced to a greater extent by variation in feed intake than by intraindividual variation in nutrient digestibility.

iii. The interindividual variation in energy balance exceeds the interindividual variation in feed intake.

iv. The intraindividual variation of feed intake has the largest effect on the intraindividual variation of energy balance.

3. I. Intra- and interindividual variation in nutrient and energy availability in dairy cows during early lactation

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Abstract

Knowledge of the nutrient and energy supply of dairy cows, especially during early lactation, is of crucial importance to identify those animals which are facing severe deficits and possible health risks. In practical animal nutrition, supply is usually based on the average requirements of all animals in a group. Individual differences between animals in nutrient uptake are often neglected, which can lead to an unbalanced supply in individual animals. Therefore, the aim of this study was to investigate the intra- and interindividual variation in energy and nutrient availability of dairy cows in early lactation. Animal-specific dry matter intake (DMI) was measured daily from twenty-eight multiparous German Holstein dairy cows, and nutrient digestibility of organic matter (OM), crude protein (CP), neutral detergent fiber (aNDFom) and acid detergent fiber (ADF) was assessed weekly using the intrinsic marker $iNDF_{240}$ (240-h-in vitro indigestible NDF) until the eleventh week postpartum (p.p.). The maximum coefficient of variation (CV) of feed intake between cows was 0.17. Nutrient digestibility of OM, CP, aNDFom and ADF varied only slightly, with mean CV values ranging from 0.02 (OM) to 0.08 (CP). An increase of one kg DMI was associated with a decrease of 1.35 g/kg in OM digestibility and 5.44 g/kg in CP digestibility. In addition, a positive effect of lactation week on CP digestibility (3.94 g/kg) but a negative effect on ADF digestibility (- 2.97) was observed. The data showed a large variation in nutrient availability per kg ECM between animals over the course of lactation, with CV values as high as 0.22 for available CP at wk 2, largely explained by variation in feed intake. Available energy from OM averaged 2.23 MJ

NEL/kg ECM, with CV values up to 0.18. The extent of variation and the generally low level of nutrient availability firmly show that these can no longer be ignored, and focus should shift to determining the supply situation of individual animals.

Keywords: variation; nutrient availability; dairy cows; early lactation

3.1. Introduction

Nutrient and energy availability in early lactation are of vital importance for a cow's ability to adapt to the environmental conditions and to defend against stressors and pathogens via a functional immune system (Habel and Sundrum, 2020). Especially high-yielding early-lactating dairy cows often incur varying degrees of nutrient and energy deficits due to the abrupt change from a non-lactating state to a high milk-producing state (Bauman and Currie, 1980; Sundrum, 2015). To deal with this metabolic challenge, animals require additional regulatory efforts. These lack of energy, decrease an animal's resistance towards stressors and increase the risk of metabolic and infectious diseases (Ingvartsen, 2006; Sordillo and Raphael, 2013; Habel and Sundrum, 2020). The occurrence of a disease indicates that a cow is overstressed by the need to adapt and cope with the living conditions (Sundrum, 2015). Frequent disease indicates conditions which do not appropriately cover animals´ needs. They can be interpreted as negative side effects of suboptimal production conditions, thus justifying including them as production diseases (Pds) (Allen and Piantoni, 2013; Sundrum, 2020). A high prevalence of Pds contradicts with good welfare and has severe economic impacts due to related failure and treatment costs (van Soest et al., 2019). Some animals suffer from Pds, sometimes resulting in early death, while other animals successfully overcome metabolic challenges of the same degree without clinical signs (Tremblay et al., 2018). This indicates a large variation between the animals in their ability to cope with nutritional deficits. The challenge in dairy cow management is to perceive the variation and to identify the animals that are particularly at risk due to exceeding nutrient and energy deficiencies. One possibility to identify such animals in the early stage of lactation is an animal-individual comparison of nutrient and energy requirements and availability. In contrast to nutrient and energy demand, which can be well mapped using milk recordings, milk checks and body weight data, there is still uncertainty in quantifying nutrient and energy availability. The respective amount of energy and nutrients available to the intermediate metabolism of the individual animal is directly related to the dry matter intake (DMI), the ingredients and digestibility of the diet and to their variation and interactions. Thanks to modern analytical methods, it is possible to comprehensively quantify the ingredients of the diet. However, it is still difficult to quantify the animal-specific level of feed intake and the animal-specific degree of digestibility under practical conditions. To obtain an estimate of DMI and digestibility for feeding management, estimation formulas, based on general assumptions derived from feeding trials (NRC, 2001; Jorritsma et al., 2003), herd averages and/or tabulated values are typically used. Because of interactions between diet- and animal-related factors, and due to the variation within each factor, an accurate estimate of the amount of energy and nutrients available to an individual animal using prediction equations or tabulated values often results in biased estimates (Colucci et al., 1989; Bruinenberg et al., 2002; Yan et al., 2002). What can be achieved is only approximations; identification of individual animals in a particularly deficient situation is not possible with this approach. Especially DMI of the individual animal is subject to a multitude of influencing factors. These include anatomical and physiological conditions, environmental conditions, management factors and social interactions (Grant and Albright, 2000), which all influence feeding behavior and thus the level of total feed intake and nutrient availability. In addition to feed intake, digestibility also plays a critical role in nutrient intake. Currently, digestibility is determined for feedstuffs or TMRs in laboratory analyses and reported as the total digestible nutrients (TDN) value (NRC, 2001) and equated for all animals. Up to now, less consideration has been given to animal influences and possible animal-specific variations in digestibility. But digestibility is a complex process, influenced by various animal-related factors like age, physiological stage or body weight as well as feed factors such as chemical composition or processing treatments (Cronjé and Boomker, 2000; Le Goff and Noblet, 2001). The standard procedure for estimating animal individual digestibility is the total collection (TC) of its feces (Schneider and Flatt, 1975). These types of in vivo studies are expensive and time-consuming as well as stressful for the animals as they must be isolated and caged (Schneider and Flatt, 1975; Cochran and Galyean, 1994; Morris et al., 2018). Instead, digestibility is calculated on a group base from tabulated coefficients of individual dietary ingredients, generally determined by feeding sheep or cows at maintenance level (INRA, 1989; Yan et al., 2002). However, numerous studies have shown that such group-based assumptions are not valid for all cows fed mixed diets intended for multiple levels of maintenance, resulting in biased estimates of nutrient availability (Colucci et al., 1989; Yan et al., 2002). An alternative approach to determine nutrient digestibility under practical conditions and on an individual basis is the use of indigestible internal markers (Satter et al., 1986). Commonly used intrinsic markers are lignin or acid-insoluble-ash (AIA) (van Keulen and Young, 1977; Lippke et al., 1986; Lee and Hristov, 2013). However, the measurability of lignin in feed analyses is not very precise and the partial digestibility of lignin reduces its suitability as a marker (Fahey and Jung, 1983; Schalla et al., 2012). An alternative is the indigestible NDF (iNDF) fraction. It is not chemically defined, but completely indigestible and thus provides more precise results. In different studies, iNDF was identified as a reliable marker and the results of digestibility estimations on an individual-animal base were similar to TC (Schalla et al., 2012; Lee and Hristov, 2013; Morris et al., 2018). Because reduced feed intake and digestibility result in less available nutrients and energy, knowledge of the extent and variation is crucial to identify affected animals (Habel and Sundrum, 2020). Knowledge of the level and variation in the sub-variables as well as nutrient and energy availability allows differentiation and identification of individual animals at risk of adaptive overload due to particularly suboptimal energy and nutrient supply (Sundrum, 2020). Despite its implications, variation in the supply situation of individual animals seems to have taken a back seat in animal nutrition in recent years. Only few studies address the variation between and within individual animals (Huhtanen et al., 2015; Cabezas-Garcia et al., 2017). Therefore, the objective of the present study was to quantify animal-specific nutrient and energy availability by measuring individual feed intake and nutrient digestibility using the internal marker iNDF, and to assess the extent of intra- and interindividual variation in DMI and OM, CP, NDF and ADF digestibility between and within individual dairy cows during early lactation.

3.2. Materials and methods

3.2.1. Animal ethics

The trial was conducted between April and October 2018 at the Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, Muenchweiler a.d. Alsenz, Germany. All animal experiments were performed according to the German Animal Welfare Act and approved by the local authority for animal welfare affairs (Landesuntersuchungsamt Rheinland-Pfalz; G 18-20-073) in Koblenz, Germany.

3.2.2. Animals, housing and treatments

A total of 28 German Holstein dairy cows ranging from the second to eighth parity (mean = 2.9; SD = 1.3) between wk 2 and 11 of lactation were used. The cows were housed together with nonexperimental cows in a free-stall barn with 60 cubicles and 30 feeding units. Cows had unlimited access to fresh water and were fed a total mixed ration (TMR) ad libitum (**Table 3.1**).

Table 3.1. Ingredient and chemical composition, and energy content of the total mixed ration.

Diet Composition (g/kg) [a]		**Chemical Composition** (g/kg) [b]		
			Mean	SD
Beet pressed pulp silage	188.3	Dry matter	402.0	13.8
Grass silage	97.0			
Grass hay	74.7	Organic matter	931.4	3.8
Maize silage	259.6	Crude protein	157.4	8.1
Concentrate	380.4	Soluble protein	63.2	7.3
		Ether extract	43.0	2.4
		aNDFom	355.1	8.8
		ADF	219.3	4.9
		Lignin	28.3	1.2
		$iNDF_{240}$	86.4	4.5
		Starch	182.3	14.8
		ESC	63.1	3.4
		TDN[c]	732.0	5.0
		Energy (MJ/kg DM)		
		NE_L[d]	7.0	0.0

[a,b] Diet and chemical composition reported on 105°C-dried matter. Averaged values based on weekly conducted feed analysis; diet was offered as TMR.

ADF, acid detergent fiber, expressed inclusive of residual ash; CV, coefficient of variation calculated as ratio of standard deviation to mean; ESC, ethanol-soluble carbohydrates; $iNDF_{240}$, indigestible aNDFom; standard deviation

[c] TDN (g/kg), Total digestibly nutrient values for TMR samples were calculated from the TDN value using Equations 2-5 by (NRC, 2001).

[d] NE_L for TMR samples were calculated from the TDN value using Equations 2-3 by (NRC, 2001).

The TMR was prepared in the morning and delivered twice daily (60 % of total daily amount at 6 am and 40 % of total daily amount at 11 am) after removement of diet orts. Individual feed intake was measured daily with the Insentec B.V. (Marknesse, the Netherlands) RIC (Roughage Intake Control) automatic weighing system. Cows were identified using individual collar-transponders which registered access to the feeding unit. Fresh matter consumed was automatically recorded for each visit. The daily DMI per cow was calculated by adding the recorded daily amount of fresh matter intake and multiplying the result by the diet DM content. Cows were milked twice daily between 5.00 and 7.30 am and between 3.30 and 6.00 pm. The daily milk yield was recorded electronically via the herd management system Dairy Plan C21 from GEA (Boenen). Milk aliquots from one evening and the next morning were taken once biweekly and pooled for further analysis of milk fat, protein and lactose by infrared spectrophotometry using a MilkoScan FT6000 (Foss Analytical A/S, Hillerød, Denmark). TMR samples were taken daily within one hour after feed delivery. These samples were combined on a weekly basis with 800 – 1,000 g TMR and taken as a representative sample for the determination of the weekly dry matter content. Starting at the day of calving, individual cow fecal samples were collected weekly two hours after morning milking via rectal palpation. The samples were labeled and stored frozen at - 20 °C until chemical analysis. Deviations in fecal content within a day, especially in aNDFom and $iNDF_{240}$ were reduced by defining a fixed time of feeding and sampling, which remained constant over the test period.

3.2.3. Chemical Analysis

Dry matter (DM) content was determined in a two-step process: thawed TMR and feces samples were first oven dried at 60 °C for 48 hours and ground to 1 mm particle size, followed by drying at 105 °C for 3 hours until constant weight was achieved. Organic matter (OM) was measured by ashing (550 °C) overnight. The dried and ground samples were submitted to Cumberland Valley Analytical Services Inc., Waynesboro, PA (CVAS) for chemical analysis. All TMR samples were analyzed for DM, OM (method 942.05; AOAC International, 2006), CP (method 990.03; AOAC International, 2006), soluble protein (SP, (Krishnamoorthy et al., 1982), ether extract (EE, method 2003.05; AOAC International, 2006), aNDFom using α-amylase, expressed exclusive of residual ash (Van Soest et al., 1991), ADF, expressed inclusive of residual ash (method 978.10; AOAC International, 2006), lignin (method 973.18; AOAC International, 2006), ethanol-soluble carbohydrates (ESC, Hall, 2000), starch (Hall, 2009) and $iNDF_{240}$ (Raffrenato, 2011). Dried and ground fecal samples were split into two subsamples. One subsample was analyzed with near infrared reflectance spectroscopy (NIRS) described by Althaus (2013). The analysis included DM, OM and CP. The other subsample was submitted to CVAS for determination of ADF, aNDFom and 240-h-in vitro indigestible NDF ($iNDF_{240}$).

3.2.4. Calculations

Nutrient digestibility was calculated from $iNDF_{240}$ as internal marker, and nutrient concentrations in TMR and feces was calculated using the following equation by Schalla et al. (2012):

$$\text{Apparent nutrient digestibility (g/kg)} = 100 - \{100 \times (\text{TMR iNDF}_{240} / \text{fecal iNDF}_{240}) \times [\text{fecal nutrient content (\% of DM)}/\text{TMR nutrient content (\% of DM)}]\}$$

Dry matter intake was averaged over one week, beginning with the calving day. The energy corrected milk yield (ECM) was calculated as follows Spiekers and Potthast (2004):

$$\text{ECM (kg/d)} = \text{milk yield (kg/d)} \cdot (([0.38 \cdot \text{fat (\%)} + 0.21 \cdot \text{protein (\%)}] + 1.05)/3.28)$$

ECM was calculated by multiplying the over one week averaged daily milk yield with the fat and protein content of the biweekly milk check. Nutrient availability was calculated by the following equation:

$$\text{Nutrient availability (g/d)} = \text{DMI (g/d)} \times \text{nutrient content in diet (g/kg DM)} \times \text{nutrient digestibility (g/kg)}$$

Energy availability was calculated by the following equation:

$$\text{Energy availability (MJ NEL/d)} = \text{DMI (kg/d)} \times \text{OM content in diet (g/kg DM)} \times \text{OM digestibility (g/kg)} \times \text{MJ NEL content in diet (MJ NEL/kg DM)}$$

3.2.5. Statistical Analysis

Data is presented as mean, standard deviation (SD) and coefficient of variation (CV). The differences in nutrient digestibility across the trial period were analyzed using the GLM Repeated Measures procedure of SPSS 25.0 software (IBM Company Inc., Chicago, IL, USA). Significant statistical effect was declared when probability was below 0.05. The individual comparisons were performed using post-hoc pairwise comparisons, with the Sidak correction applied, to determine which of the comparisons were significant. When the Mauchly's test indicated that the assumption of sphericity had been violated, the degrees of freedom were corrected using Greenhouse-Geisser estimates if the estimate was lower than 0.75, or the Huynh-Feld estimate if the estimate was greater than 0.75 (Girden, 2003). The effect sizes for main effects and interactions were determined by Partial eta squared (η^2) values. Partial eta squared (η^2) values were classified as small (0.010 to 0.059), moderate (0.060 to 0.137) and large (> 0.137).

A linear mixed model (LMM) was used to analyze the effects on nutrient digestibility. Akaike's information criterion and standard error (SE) were used for model evaluation and to find the best-fit model, respectively. The final model included the fixed effects of a week of lactation, DMI (kg/d), lactation number and a random intercept for cow. No covariates were used in the model. Further, linear regressions were performed to analyze the impact of variation in DMI, and of nutrient digestibility on the variation of nutrient availability.

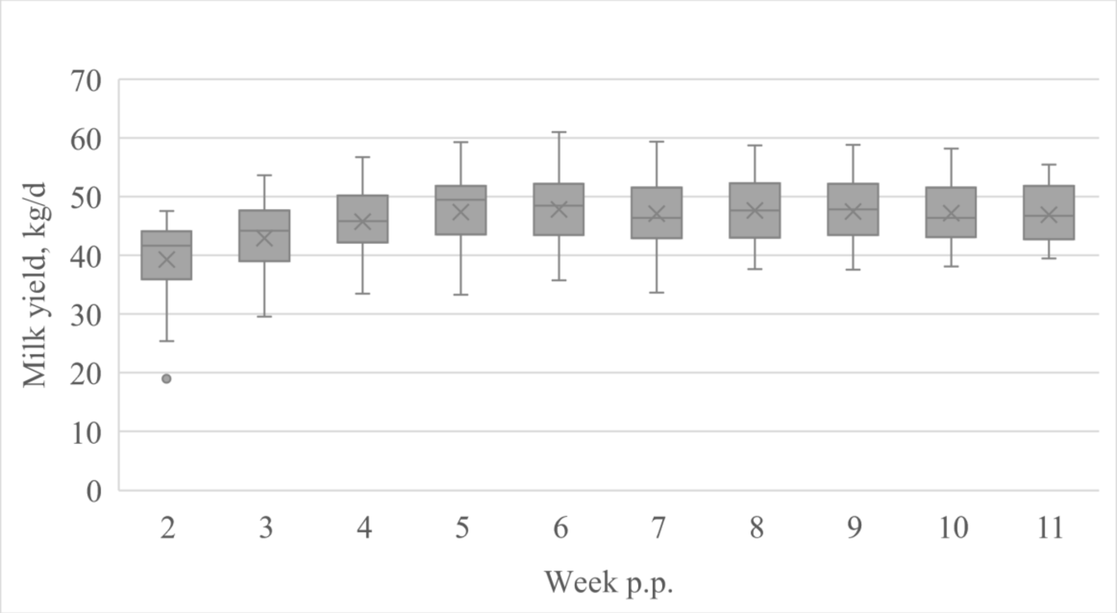
3.3. Results

3.3.1. Cow Performance Data

The mean daily milk yield averaged at 45.9 ± 6.2 kg/d, varying between 39.3 ± 6.8 kg/d in wk 2 and 47.8 ± 6.8 kg/d in wk 6, and 46.9 ± 4.9 kg/d in wk 11 (**Figure 3.1**A). CV was highest in wk 2 (0.17) and decreased to 0.10 in wk 11. For milk fat and protein content, the changes over time are shown in **Figure 3.1**B and **Figure 3.1**C. Milk fat and protein content showed the highest levels in wk 2 p.p. and then decreased, reaching relatively constant milk fat content by wk 6 and 7 p.p., or slightly re-increasing milk protein content thereafter. ECM reached mean peak value of 44.9 kg/d in wk 4 p.p. Thereafter, ECM declined to about 41.9 ± 3.9 kg/d until wk 11 (**Figure 3.1**D).

CV of ECM was highest in wk 2 (0.20), and decreased to 0.10 in wk 11, indicating quite a different level of energy and nutrient requirements between dairy cows fed the same diet.

A



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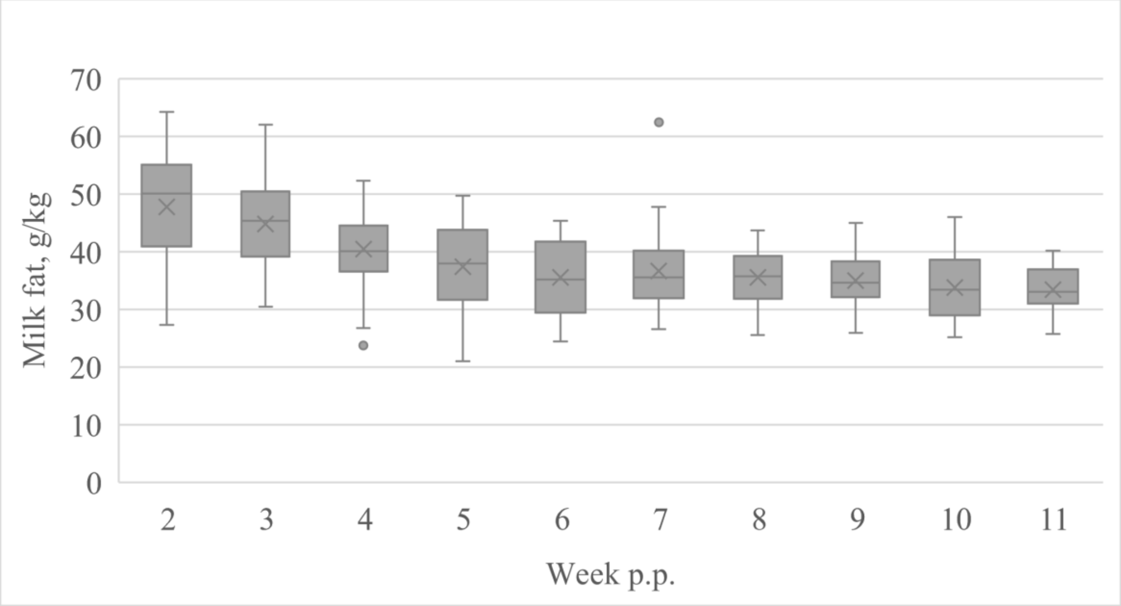

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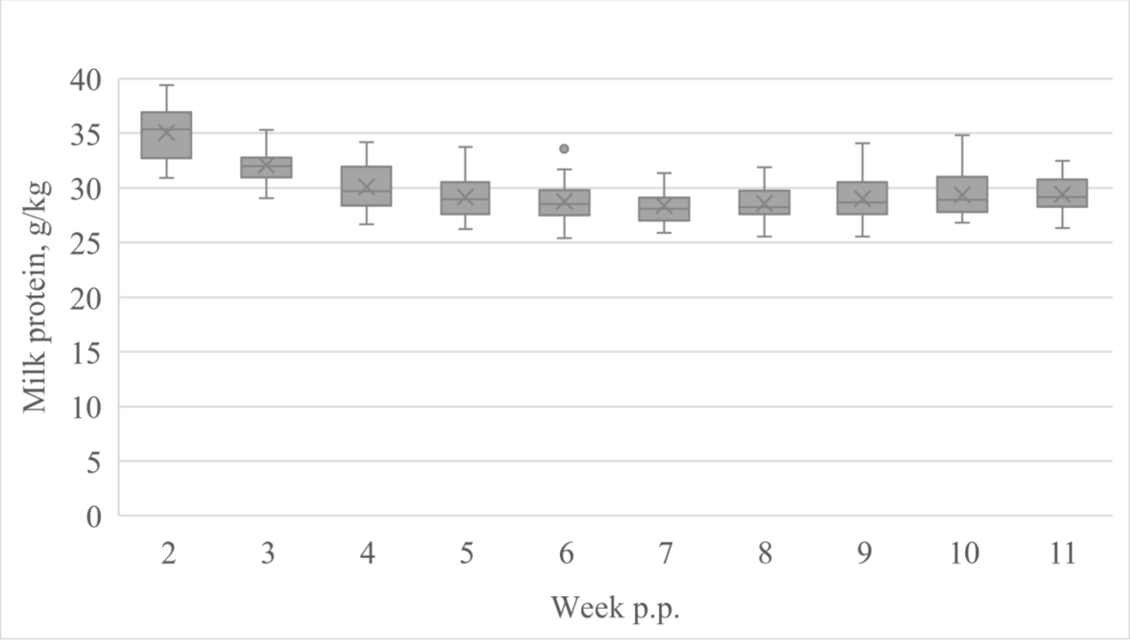

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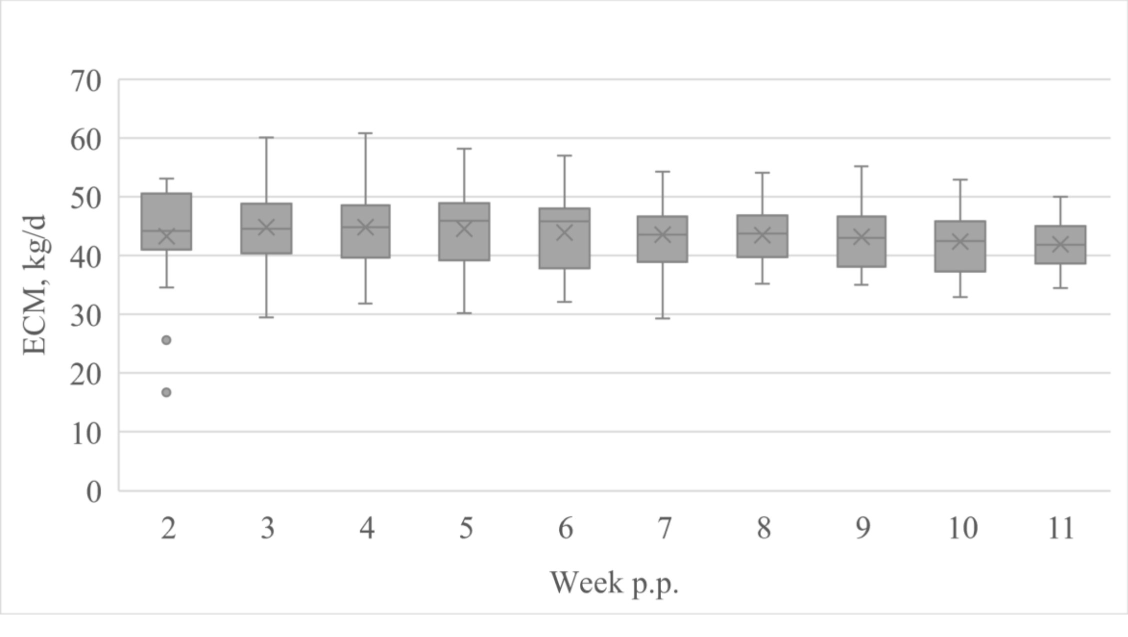


Figure 3.1. Mean milk yield, milk fat content, milk protein content and ECM yield measured for all 28 cows between wk 2 and 11 postpartum (p.p.). In each subfigure, the boxplots highlight, respectively, the median and upper and lower quartiles of each week. Small circles = outliers (1.5 × interquartile range (IQR)).

DMI significantly ($P < 0.01$; $\eta^2 = 0.697$) increased with a quadratic ($P < 0.01$; $\eta^2 = 0.642$) trend from a mean value of 14.3 ± 2.3 kg DMI per day in wk 2 up to 22.1 ± 2.3 kg in wk 11 (**Figure 3.2**). The highest increase compared to the previous wk was found in wk 3 (3.2 kg). CV of DMI was highest in wk 3 with a value of 0.17, and lowest in wk 9 with a value of 0.09. The widest range between minimum and maximum value was found in wk 7 with a minimum DMI of 13.9 and a maximum of 26.3 kg DMI.

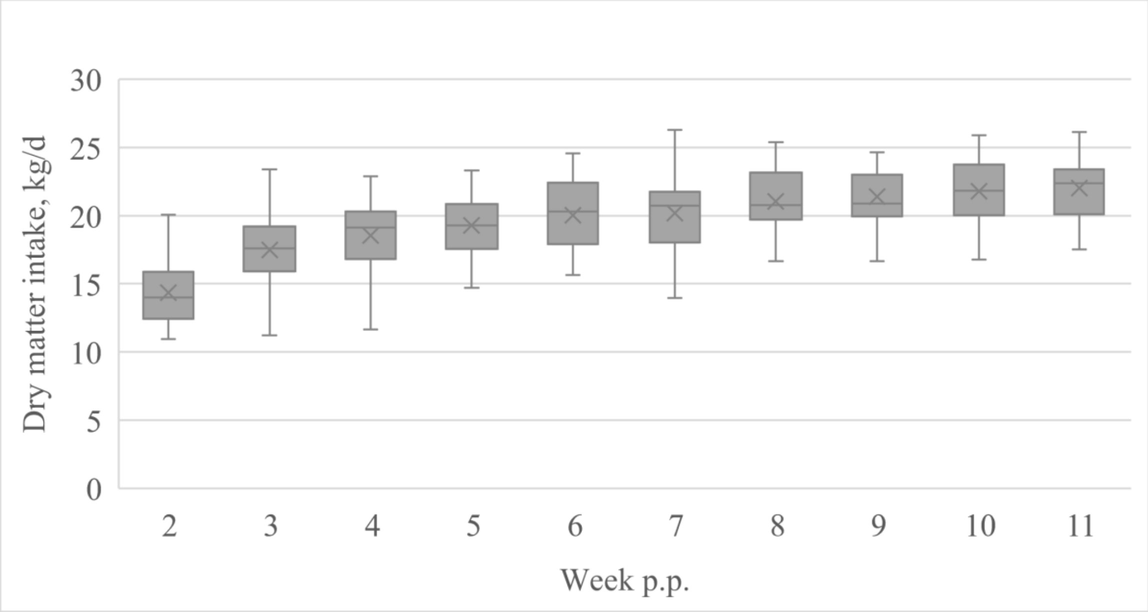


Figure 3.2. Mean values and the variation of DMI measured for all 28 cows between wk 2 and 11 postpartum (p.p.). In each subfigure, the boxplots highlight, respectively, the median and upper and lower quartiles of each week.

The variation of the DMI within individual cows is shown in **Figure 3.3**. The variation within one particular animal (no. 1563) during the test period was high, reaching up to 14.8 kg difference between minimum and maximum DMI between wk 2 and 11.

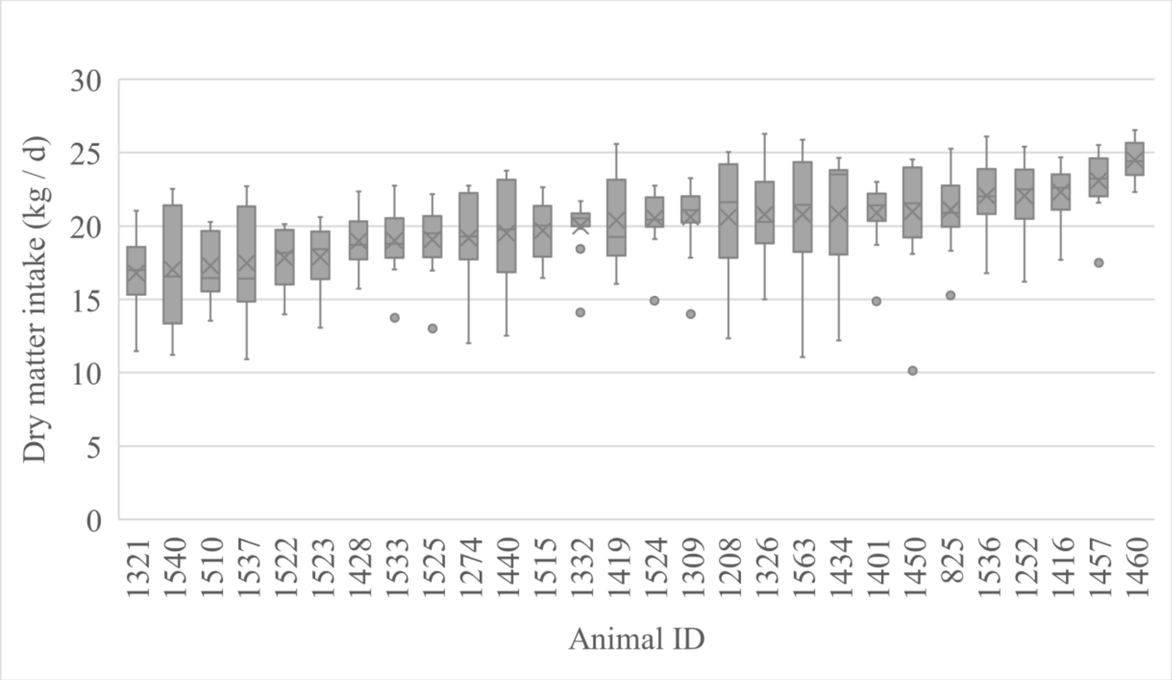


Figure 3.3. Intra- and interindividual variation in dry matter intake (DMI) measured between wk 2 and 11 postpartum (p.p.) for every cow. In each subfigure, the boxplots highlight, respectively, the median and upper and lower quartiles of each week. Small circles = outliers (1.5 × interquartile range (IQR)).

3.3.2. Apparent nutrient digestibility during early lactation

The highest mean value regarding the nutrient digestibility of the diet was found for OM (729.3 ± 19.6 g/kg), followed by CP, aNDFom and ADF with 625.07 ± 52.05, 585.1 ± 44.4 and 573.9 ± 34.7 g/kg, respectively. The mean, SD and CV of the nutrient digestibility per week are presented in **Table 3.2**. ADF digestibility ($P = 0.004$; $\eta^2 = 0.104$) decreased significantly with a linear ($P = 0.007$; $\eta^2 = 0.269$) trend over the time. No significant differences between the weeks of lactation for OM, CP and aNDFom digestibility were detected. Mean CV values for OM, CP, aNDFom and ADF digestibility were 0.03, 0.08, 0.07 and 0.06, respectively.

Table 3.2. Mean, SD and CV of nutrient digestibility (g/kg) between wk 2 and 11 postpartum (p.p.) (n = 28).

		Week p.p.									
		2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
OM	Mean	738.6	727.8	728.6	733.1	733.2	726.6	725.1	726.0	725.6	729.1
	SD	20.3	17.8	16.8	23.7	21.9	22.4	17.6	18.1	17.7	17.3
	CV	0.03	0.02	0.02	0.03	0.03	0.03	0.02	0.02	0.02	0.02
CP	Mean	653.4	617.1	617.8	622.8	619.9	619.7	618.9	618.6	624.0	640.6
	SD	46.9	59.5	46.1	58.1	57.1	58.1	41.7	60.9	41.9	38.7
	CV	0.07	0.10	0.07	0.09	0.09	0.09	0.07	0.10	0.07	0.06
aNDFom	Mean	607.4	587.9	585.7[a]	585.6	591.1	577.3	578.2	580.9	577.0	579.3
	SD	54.0	45.3	41.2	57.4	44.8	49.9	42.9	29.7	36.4	32.7
	CV	0.09	0.08	0.07	0.10	0.08	0.09	0.07	0.05	0.06	0.06
ADF	Mean	589.5	580.8	576.6	579.3	585.2	570.7	566.8	569.1	560.7	559.9
	SD	42.0	40.1	31.5	38.0	29.8	32.5	33.6	31.3	27.7	30.7
	CV	0.07	0.07	0.05	0.07	0.05	0.06	0.06	0.06	0.05	0.05

aNDFom, using α-amylase and expressed exclusive of residual ash (Van Soest et al., 1991); ADF, acid detergent fiber; CP, crude protein; CV, coefficient of variation calculated as ratio of standard deviation to mean; OM, organic matter; SD, standard deviation.

The influence of week of lactation and DMI on OM, CP, aNDFom and ADF digestibility is shown in **Table 3.3**. OM ($P = 0.01$) and CP ($P < 0.01$) digestibility were negatively related to DMI (kg/d). The rate of depression in OM and CP digestibility amounted to 1.35 and 5.44 units for each additional kg of DMI. Week of lactation was positively related to CP digestibility (3.94; $P < 0.01$). With progressing stage of lactation, ADF digestibility (- 2.97; $P < 0.01$) decreased.

Table 3.3. Influence of week of lactation and DMI on OM, CP, aNDFom and ADF digestibility, estimated by linear mixed model derived from 28 dairy cows between wk 2 and 11 p.p.

	OM digestibility			CP digestibility			aNDFom digestibility			ADF digestibility		
Variable	Estimate	SE[a]	P-value	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
Intercept	753.35	8.32	< 0.01	705.51	24.87	< 0.01	616.36	18.83	< 0.01	587.07	14.58	< 0.01
Week of lactation	0.33	0.53	0.53	3.94	1.37	0.004	- 1.04	1.13	0.36	- 2.97	0.88	< 0.01
DMI[b] (kg/d)	- 1.35	0.52	0.01	- 5.44	1.38	< 0.01	- 1.29	1.16	0.27	0.29	0.89	0.75
AIC[c]	2.371.45			2.889.40			2.778.77			2.648.49		

[a] Standard error

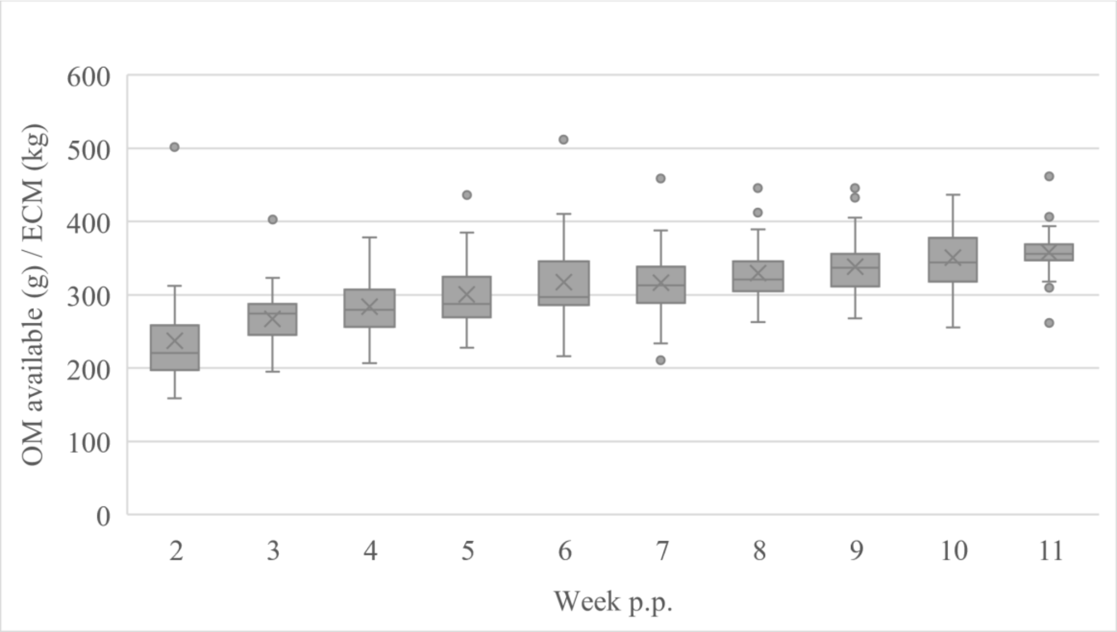
[b] Dry matter intake

[c] Akaike's information criterion

3.3.3. Individual nutrient and energy availability

The mean amount of available OM (avOM), CP (avCP), aNDFom (avNDF) and ADF (avADF) during the experimental period was 310.5 ± 59.3, 44.9 ± 8.8, 94.6 ± 16.2 and 57.3 ± 9.3 g/kg ECM, respectively. Weakly mean values of considered available nutrients were significantly different between weeks of lactation and increased continuously over time with the lowest mean amount in wk 2. AvOM increased from 236.9 ± 70.2 g/kg ECM with a quadratic ($P = 0.016$; $\eta^2 = 0.245$) trend up to 357.9 ± 35.9 g/kg ECM in wk 11 (**Figure 3.4**A). CV of avOM was highest in wk 2 (0.19) and decreased to 0.10 in wk 11. AvCP increased with a linear ($P = 0.001$; $\eta^2 = 0.796$) trend from 35.3 ± 11.7 (wk 2) to 54.3 ± 7.5 g/kg ECM (wk 11) and CV value decreased from 0.22 (wk 2) to 0.11 (wk 11). The widest weekly ranges were detected in wk 6 for avOM, with a value of 194.5 g/kg ECM, and for avCP in wk 9 p.p., with a value of 33.6 g/kg ECM (**Figure 3.4**B). The mean amounts of avNDF and avADF in relation to week number are shown in **Figure 3.4**C and **Figure 3.4**D. They both increased, with a linear ($P < 0.01$; $\eta^2 = 0.792$) trend for avNDF, and a quadratic ($P < 0.01$; $\eta^2 = 0.239$) trend for avADF. Mean avNDF and avADF showed the widest range with respectively 69.6 and 41.5 g/kg between lowest and highest amount of absorbed nutrients per kg ECM in wk 10. Mean CV for avNDF and avADF was 0.15, with a range from 0.23 (wk 2) to 0.11 (wk 11) for avNDF and 0.22 (wk 2) to 0.10 (wk 11) for avADF.

A



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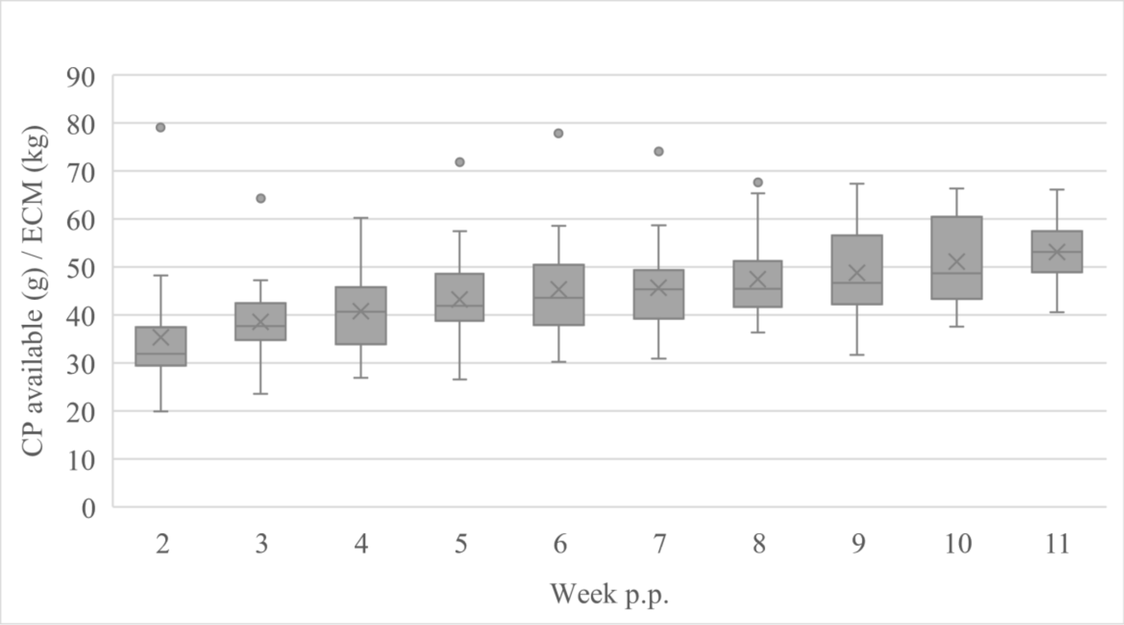
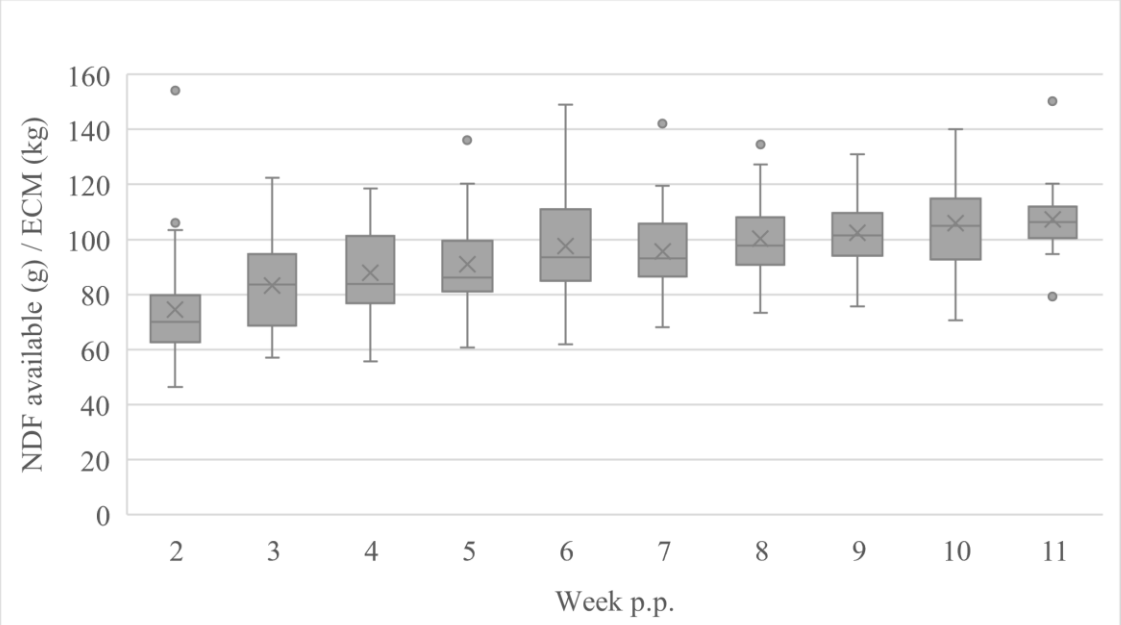

CP available (g) / ECM (kg)
0
10
20
30
40
50
60
70
80
90
2
3
4
5
6
7
8
9
10
11
Week p.p.

C


NDF available (g) / ECM (kg)
0
20
40
60
80
100
120
140
160
2
3
4
5
6
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9
10
11
Week p.p.

D

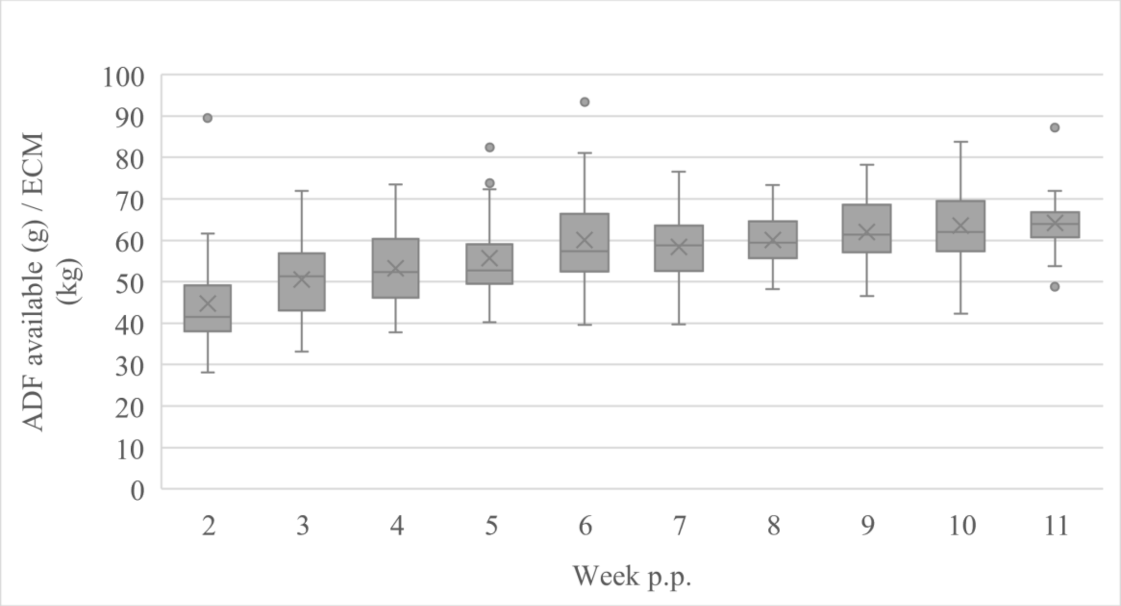

Figure 3.4. Box and whisker plots indicating the mean available amount of OM, CP, aNDFom and ADF per 1 kg of ECM measured for all 28 cows between wk 2 and 11 postpartum (p.p.). In each subfigure, the boxplots highlight, respectively, the median and upper and lower quartiles of each week. Small circles = outliers (1.5 × interquartile range (IQR)).

Linear regressions were performed to analyze the impact of variation in DMI and nutrient digestibility on the variation of nutrient availability per kg ECM. They showed a significant positive trend ($P < 0.05$) for CV of DMI and a non-significant ($P > 0.05$) trend for CV of nutrient digestibility (**Table 3.4**).

Table 3.4. The effect of variation in DMI and nutrient digestibility on variation in nutrient availability per kg ECM (n = 28).

	organic matter*			crude protein*			aNDFom*			ADF*		
Variable	Estimate	SE[a]	P-value	Estimate	SE[a]	P-value	Estimate	SE[a]	P-value	Estimate	SE[a]	P-value
Intercept	0.061	0.036	0.105	0.058	0.070	0.420	0.067	0.050	0.196	0.095	0.030	0.004
CV DMI[b]	0.574	0.168	0.002	0.623	0.263	0.026	0.679	0.199	0.002	0.662	0.167	0.001
CV nutrient* digestibilty[c]	0.217	1.133	0.849	0.332	0.616	0.594	- 0.236	0.605	0.700	- 0.875	0.527	0.109
R²	0.318			0.196			0.333			0.388		

[a] Standard error

[b] Variation coefficient of dry matter intake

[c] Variation coefficient of nutrient digestibility

The mean amount of available energy from OM (avEnergyOM) per kg ECM, was 2.23 MJ NEL/kg ECM, and it was significantly different between the weeks of lactation ($P < 0.01$; $\eta^2 = 0.688$). AvEnergyOM increased with a quadratic ($P = 0.017$; $\eta^2 = 0.241$) trend from 1.77 ± 0.53 MJ NEL/kg ECM in wk 2 up to 2.67 ± 0.24 g/kg ECM at wk 11 (**Figure 3.5**), on average 2.31 ± 0.36 MJ NEL. CV value of avEnergyOM was highest in wk 6 (0.18) and lowest in wk 11 (0.10). The widest range between minimum and maximum with 1.33 MJ NEL/kg ECM was detected in wk 10, indicating quite a different level of available energy from OM per kg ECM between dairy cows fed the same diet in the same stage of lactation.

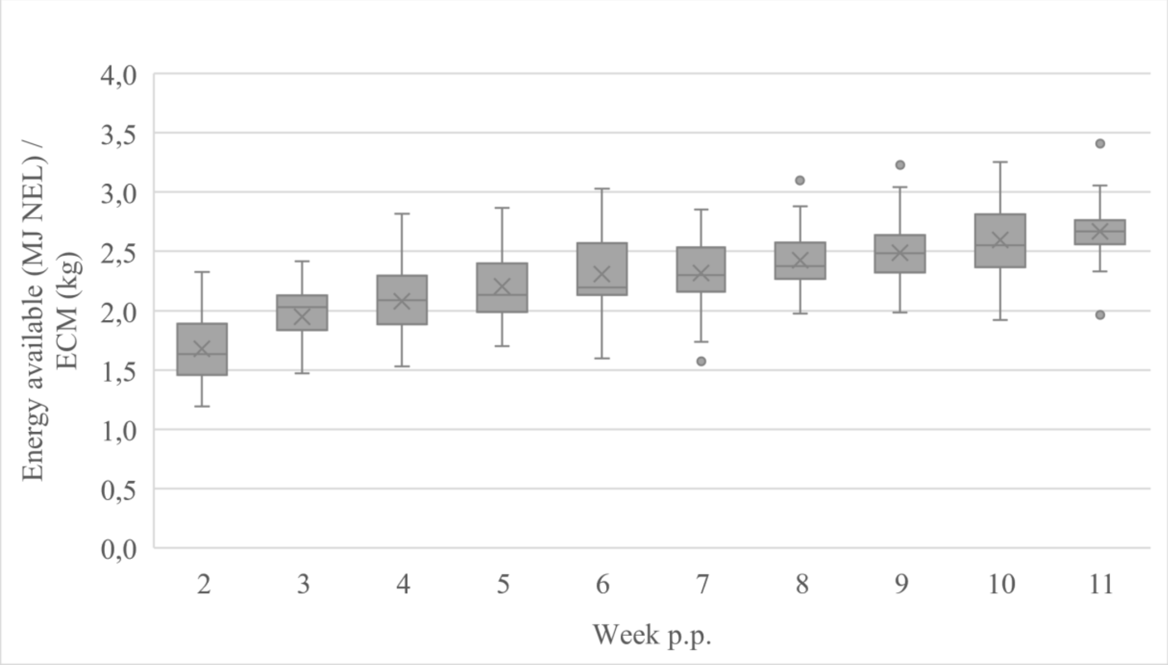

Figure 3.5. Variation over time in the available amount of energy (MJ NEL) derived from feed intake and feed digestibility per kg of ECM measured for all 28 cows between wk 2 and 11 postpartum (p.p.). In each subfigure, the boxplots highlight, respectively, the median and upper and lower quartiles of each week. Small circles = outliers (1.5 × interquartile range (IQR)).

The variation of the amount of available energy per kg ECM between wk 2 and 11 between individual cows is shown in **Figure 3.6**. The variation within one particular animal (no. 1536) during the test period was high, reaching up to 1.74 kg difference between minimum and maximum available energy value per kg ECM between wk 2 and 11.

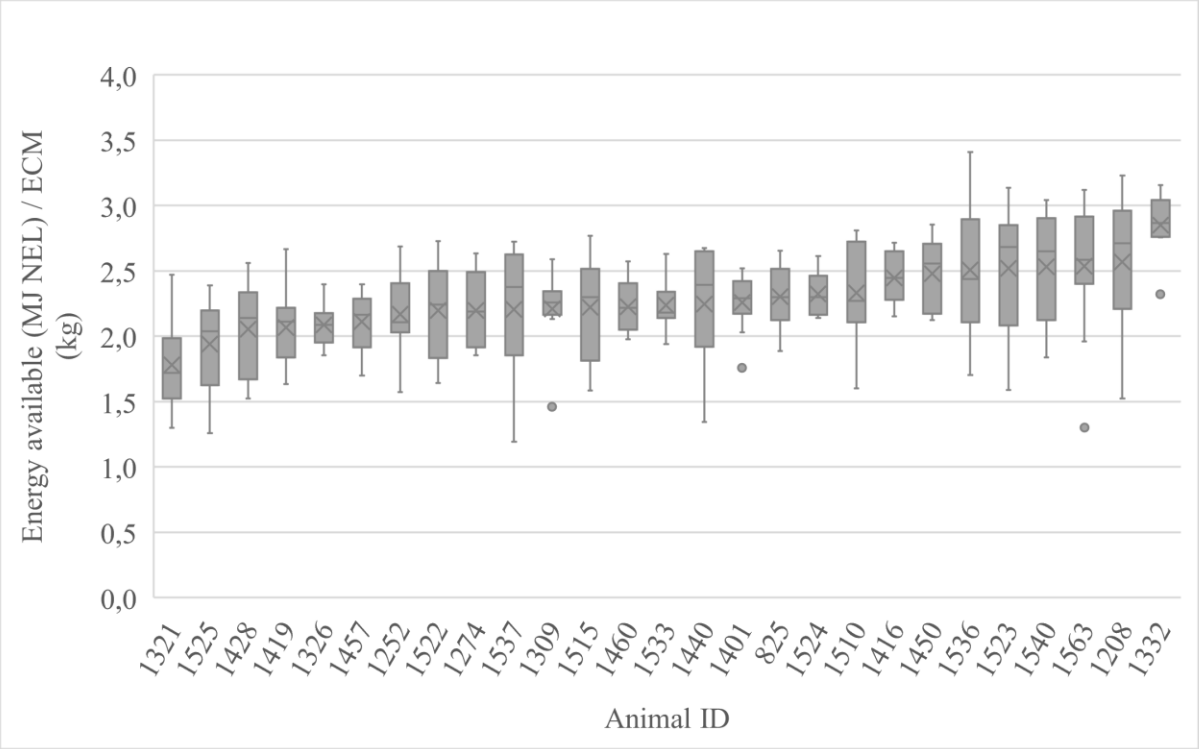

Figure 3.6. Intra- and interindividual variation in the availability of energy (MJ NEL) per 1 kg of ECM measured between wk 2 and 11 postpartum (p.p.) for every cow (n = 28). In each subfigure, the boxplots highlight, respectively, the median and upper and lower quartiles of each week. Small circles = outliers (1.5 × interquartile range (IQR)).

3.4. Discussion

Contrary to the common trend of aggregating data from individual animals, the present study focuses on the intra- and interindividual nutrient and energy availability as well as the variation within and between individual dairy cows in early lactation. Making use of longitudinal individual animal data measured during the first eleven weeks of lactation, considerable individual animal variation in feed intake, digestibility, and nutrient and energy availability was determined. Feed intake varies considerably between individual animals over the course of lactation, leading to large variations in nutrient and energy availability. The magnitude of variation is particularly important in the context of the metabolic challenge at the beginning of lactation and critically challenges the conventional approach of assessing supply situation using mean values, estimation equations and herd-based tabulated values.

3.4.1. Variation in DMI and nutrient digestibility during early lactation

To be able to trace variation in the measured parameters back to the differences between individual animals, possible external influencing factors caused by breed, diet or environment were reduced as far as possible. Slight deviations in the chemical composition of the diet during the trial period are caused by the ensiling process from the field through the feeding phase. However, by doing weekly feed analyses, deviations were detected promptly and compensated for by adjusting the rations as needed. Milk production was high and was similar to values of other experiments where cows were fed a diet with 160 g/kg CP and 7.0 MJ NEL/kg DM during early lactation (Komaragiri and Erdman, 1997). Azizi et al. (2009) also reported similarly high ECM yields (on average 44.49 kg/d) during early lactation in multiparous dairy cows by a similar energy concentration of the ration (6.99 MJ NEL/kg DM). Feed intake, defined as DMI per day, is one of the most important components for available nutrient and energy in dairy cows. Mean DMI increased up to 22.1 ± 2.3 kg in wk 11 and was slightly lower than the DMI detected by Azizi et al. (2009) with a mean value of 23.4 kg/d. The mean between-cow CV in DMI during the trial period was similar to the between-cow CV value of 0.14 that we estimated from the meta data set of Huhtanen et al. (2015) and slightly lower than the CV value of 0.18 that we estimated from the meta data set of Cabezas-Garcia et al. (2017). The high CVs indicate a high difference in the amount of DMI between and within cows in the same stage of lactation and in the same environment. Reasons for variation in DMI are manifold. Besides the feed composition, DMI is affected by the number, length and eating rate of meals per day as well as by different mechanisms which may control total daily DMI between cows of different parities, with different rumen capacity, body weight or growth requirements (Dado and Allen, 1994; Grant and Albright, 2000). Therefore, working with averages on a herd basis is not exactly reliable in terms of estimating the supply situation. To improve the assessment of nutrient and energy intake, future research should place strong focus on developing technical hard- and software on-farm solutions to record the level of animal-specific daily DMI. This would enable identification of dairy cows that vary or deviate greatly in their nutrient and energy intake, and thus identify animals at increased risk due to inadequate nutrient and energy intake (Sundrum, 2015; Maltz, 2020). Conceivable approaches would be sensor-based systems on or in the animal or weighing systems that record the amount eaten by the cow (Bikker et al., 2014; Ruuska et al., 2016; Carpinelli et al., 2019). In addition to feed intake, nutrient digestibility is of particular importance for calculating the available nutrient and energy amount in dairy cattle.

To the best of our knowledge, there is no literature which reports on nutrient digestibility over a longer period during early lactation. The mean values of OM, CP and aNDFom digestibility in the current study were comparable to the mean values of a meta-analysis by Cabezas-Garcia et al. (2017), who reported mean values of different diets for OM, CP and NDF of 740, 684 and 648 g/kg, respectively. Mean values of OM digestibility were close to the TDN value of the TMR (732 g/kg). Similar results for OM (728 g/kg) and NDF (594 g/kg) digestibility were presented in a meta-analysis by Huhtanen et al. (2010). Lee and Hristov (2013) compared acid-insoluble ash (AIA¸ digestion with 2 N HCl) and iNDF (12-d ruminal incubation in 25-µm-pore-size bags) as intrinsic digestibility markers with total fecal collection (TC) in comparable dairy cows fed corn silage and alfalfa haylage-based diets. The apparent digestibility of CP measured with iNDF (626 g/kg) was quite similar to the present study. However, digestibility of OM (654 g/kg), NDF (509 g/kg) and ADF (459 g/kg) were lower and, in fact, closer to the TC results of Lee and Hristov (2013). The mean between-cow CV of OMD during the trial period was 0.03. In recent meta-analysis, lower CV values for OMD of 0.014 (Guinguina et al., 2020) and 0.013 (Cabezas-Garcia et al., 2017) were reported. The results demonstrate that there is little variability among early-lactating dairy cows in their ability to digest the organic matter of a given diet. Despite the low variation and the closeness to the TDN value, digestibility calculated by iNDF from a small random sample of fecal samples, could be used to control the digestibility of the diet, for example after a change of the diet. By using iNDF$_{240}$ instead of tabulated digestibility coefficients, it is possible to identify nutrient digestibility at animal level, and thus the level of nutrients available to individual animals, rather than only the feed level of total digestible nutrients. Therefore, it could be a useful on-farm tool for continuous diet control. Despite the small variation in nutrient digestibility, it is important to know which animal-related factors affect digestibility and to what extent. The presented results verify the relationship between the nutrient digestibility and week of lactation and DMI. Colucci et al. (1989) also reported a negative relationship between nutrient digestibility and DMI. They attributed this to the increasing passage rate due to increasing feed intake and the resulting shortening of the residence time of the feed in the rumen. Huhtanen et al. (2009) observed a relative decrease in OMD when the feeding level was increased to a 2.0% multiple of the maintenance requirement (15 g/kg). In the case of grass silage-based diets, Yan et al. (2002) revealed a decrease in digestibility of 2.5% following an increase in DMI. Tyrrell and Moe (1975) reported significant depression in digestibility on average of about 4% of each increase in intake and they also found a higher correlation between ADF digestibility and dry matter intake in comparison to the other nutrients. Discrepancies in the relationship between digestibility and DMI among studies could partially be accounted for by differences in the physiological state of the animals as well as the type of diets fed. The considerably weaker relationship between DMI and nutrient digestibility in this study shows that generalizations based on studies under standardized conditions are not reliable for assessing the actual situation of the individual animal, especially in the first weeks p.p. However, based on an estimated feed intake for an average cow and tabulated values for digestibility, it is almost impossible to evaluate the existing variation between individual animals in a herd and to make a statement about the individual amount of energy and nutrients available to each animal in the group. Predictive equations have limited robustness due to extreme intra- and interindividual variation at the animal level and provide only an initial benchmark that should be verified by single animal controls. Animal-specific recording of daily DMI remains a major challenge. One approach to meet this challenge are developments in various technologies of camera and sensor technology for recording DMI of animals at the feedbank under practical conditions. These approaches should be further advanced to enable better decisions about the supply situation of dairy cows in the future.

3.4.2. Variation in nutrient and energy availability

Decreasing variation in the availability of energy per kg ECM between animals over time was found for CP, NDF and ADF as well. The ranges between lowest and highest amounts of available OM, CP, NDF and ADF indicate a considerable degree of variation in nutrient availability in early lactating dairy cows. In general, variation in DMI has a significant impact on variation in nutrient availability. The large variation in available OM is accompanied by a high variation in available energy from OM (avEnergyOM). Mean values of 2.37 ± 0.41 MJ NEL per g/kg ECM of available energy from OM were not sufficient to cover the energy demand of 3.28 MJ NEL per kg ECM. This corresponds with results obtained by Beerda et al. (2007) and Kessel et al. (2008), who also reported a large energy deficit and a large variation between animals during this period. The maximum range of 1.33 MJ NEL/kg ECM between lowest and highest amount of available energy between cows at the same stage of lactation demonstrates the extent of variation in energy availability between individual dairy cows even when fed the same diet.

Only by focusing on the individual animal and considering the animal-specific variation, is it possible to obtain a more accurate assessment of the energy supply of the animals. Knowledge about the nutrient and energy supply of individual animals, especially during early lactation, is of crucial importance since a massive energy deficit has far-reaching consequences for the health and performance of the animals, and is therefore also of economic interest (Mostert et al., 2018).

In order to control the variation and reduce the number of animals with insufficient nutrient and energy supply, the establishment of feeding groups based on the nutrient and energy demand is one approach among others (St-Pierre and Thraen, 1999). Nutritional grouping and multiple TMR undoubtedly increase management efforts as well as managerial and labor costs. However, feeding cows according to their individual requirements is essential to enhance animal health and welfare as well as the productivity of dairy cows, which in the long run affect the profitability and sustainability of a farm (VandeHaar and St-Pierre, 2006; Cabrera and Kalantari, 2014). Another approach is to feed supplements individually by using a computerized feeding system that recognizes cows and dispenses specific mixes at timed intervals throughout the day (VandeHaar et al., 2016). The high variation in available energy per kg ECM within cows during early lactation indicates the necessity to consider the available nutrient and energy amount on an individual-animal base as well as the necessity of continually adjusting demand and supply (Sundrum, 2020). The identification of animals with a high risk of Pds is only possible if the individual animal itself is the reference system rather than the average of the herd. Only by knowing the level of each parameter can an assessment of the extent of intra- and interindividual variation be made. Animals with relatively large variation should then be watched and controlled more closely. As long as feed intake cannot be assessed regularly on commercial-farms, animal-specific energy requirements can be estimated by checking, for example, body condition score and live weight on a daily, animal-by-animal basis. Continuous monitoring allows comparisons of animals as well as recognition of changes and trends, which can identify those that respond particularly strongly to metabolic challenges. In the end, all supply and demand data should be pooled, for example, in a software tool, which would allow for daily identification of those animals having a large discrepancy between nutrient and energy requirement and demand. To achieve a long-term reduction in production diseases by the early detection of animals at particularly high risk of production diseases, knowledge of individual energy and nutrient availability is essential, especially at the time of energy deficiency in early lactation.

The extent of variation indicated the importance of implementing a practical and feasible method of estimating the energy and nutrient supply of individual animals.

3.5. Conclusion

The common approaches of assuming uniform feed intake and digestibility or basing assumptions on similar milk yields among animals at the same stage of lactation neither allow for the determination of the supply situation of an individual animal nor the timely identification of animals at increased health risk due to inadequate nutrient and energy availability. The results of the present study show that there is considerable intra- and interindividual variation in nutrient and energy availability per kg ECM in early lactation which should not be ignored when trying to optimize nutrient and energy supply. The variation is primarily caused by the large variation in DMI, which is often disregarded in animal nutrition at the farm level. Due to the interaction and variation of feed intake and digestibility, the variation in availability becomes even greater. While methods of practical quantification of individual feed intake are still under development, digestibility can already be determined for groups of animals using internal markers to provide a more accurate indication of the level of digestibility of the currently fed diet.

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4. II. Inter- and intra-individual variation in the behavior of feed intake on nutrient availability in early lactating dairy cows

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Simple Summary

The problem of nutrient and energy deficiency and the associated risk of disease in fresh dairy cows has been known for many years. Previous approaches to reducing the risk have been almost exclusively on a herd basis approach but have so far not been sufficiently effective. The present study revealed large variation between individual animals during the first weeks after calving, particularly with respect to dry matter intake (DMI). In addition to the large variation in intake behavior, feed intake and nutrient digestibility, interactions between parameters refute the traditional feeding regimes, based on the mean requirement values at herd level. Inter-individual variation indicates that each animal follows an individual strategy in optimizing DMI. Only if constant access to the feed bunk and balanced diets are made possible, all animals can follow their individual strategy, maximize individual DMI and come closer to the goal of an adequate supply. As there is only a low risk for excessive DMI during early lactation, feeding regimes should not be oriented towards the assumed average level of feed intake but towards the animals with a low level of dry matter intake. Otherwise, it will not be possible to improve the nutrient supply for all animals.

Abstract

Since energetic deficits in dairy cows can only be reduced at an animal level, the objective of the present study was to determine the extent of variation in intake behavior within and be-tween animals during early lactation, to explore the magnitude of interactions between feed in-take, intake behavior and nutrient digestibility, and to identify levers for maximizing feed intake at the individual animal level. Feeding behavior, intake and nutrient digestibility of 28 German Holstein dairy cows, fed TMR with 7.0 MJ NEL, were studied between the 2nd and 15th week after calving. Dry matter intake was assessed daily and nutrient digestibility weekly, with iNDF240 as an intrinsic marker. Results showed high intra- and inter-individual variation in intake behavior parameters with coefficients of variation (CV) up to 0.58 in meal frequency. Nutrient digestibility varied only slightly with CV values up to 0.10 in crude protein. Milk yield, meal frequency, feeding time, feeding rate and meal size had significant positive effects on DMI ($p < 0.01$). To achieve long-term improvements in feed intake, it is important to optimize feed intake and feeding be-havior of individual animals by improving feeding conditions and develop technical tools to identify animals with insufficient feed intake.

Keywords: dairy cows; variation; digestibility; dry matter intake; intake behavior; nutrient availability

4.1. Introduction

Many high-yielding dairy cows suffer severe nutrient and energy deficits in early lactation [1,2]. These deficient states stress body functions and reduce an animal's resistance to stressors while increasing the risk of metabolic and infectious diseases [3–5]. Especially in early lactation, many factors affect the performance and health status of dairy cows, but the level of influence can vary between individual animals (**Figure 4.7**).

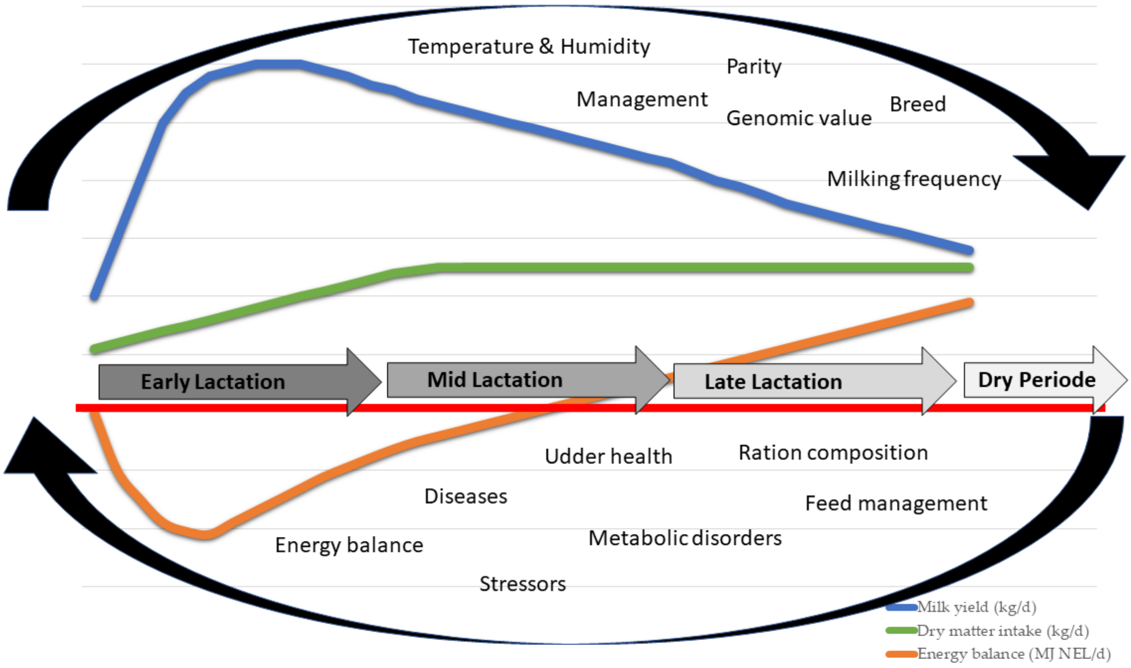


Figure 4.7. Schematic representation of factors affecting milk yield, dry matter intake and energy balance of dairy cows during different states of lactation [6].

The magnitude of nutrient and energy deficiencies also varies between dairy cows and depends on nutrient and energy intake and requirements. In addition, there is a large variation between individual cows in their ability to cope with metabolic imbalance [2]. The individual availability of nutrients and energy results from the combination of feed components in the diet, the level of feed intake and the degree of digestibility of the feed in an animal, as well as the variation and interaction within and between these factors. Of these factors, feed intake has proportionately the greatest impact on the level of nutrient and energy availability. Accordingly, research in recent years has focused on optimizing diet composition in relation to the average requirements of the dairy cows in the feeding group to achieve a high level of dry matter intake (DMI) [7–9]. While feeding behavior showed a considerable effect on the level of DMI [10,11], it is often not adequately considered in ration calculation, feeding management or nutrient supply. In addition, it is difficult to assess feeding behavior in practice. Feeding behavior can be described by the number, duration and size of individual meals per day [12]. DMI, as the sum of single meals, is influenced both positively and negatively by the individual behavior patterns as well as their interactions [10]. Furthermore, an influence on the digestibility of the ingested diet has also been described. Baumont et al. [13] and Golden et al. [14] revealed that cattle and small ruminants which ate smaller and more frequent meals showed higher nutrient digestibility than animals that ate less frequent, but larger, meals.

Tyrrell and Moe [15] and Potts et al. [16] found that digestibility diminished with increasing feed intake. Higher feed intake results in a higher passage rate, which leads to a reduced digestion time. However, the extent of variation among individual animals and the degree of antagonistic relationships between feeding behavior, feed intake and digestibility have so far received little attention. Since individual deficiencies can only be reduced at the animal level, the objective of the present study was to determine the extent to which intake behavior and nutrient digestibility varies within and between animals during early lactation, to explore the extent of interactions between feed intake, intake behavior and nutrient digestibility, and to identify levers for maximizing feed intake and hence nutrient intake at an individual animal level.

4.2. Materials and Methods

All procedures described in this study were performed according to the German Animal Welfare Act and approved by the local authority for animal welfare affairs (Landesuntersuchungsamt Rheinland-Pfalz; G 18-20-073) in Koblenz, Germany.

4.2.1. Animal Housing and Diet

The study was carried out between April and October 2018 at the Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, Muenchweiler a.d. Alsenz, Germany. A total of 28 German Holstein dairy cows ranging from the 2nd to 8th parity (mean = 2.9; SD = 1.3) and between the 2nd and 15th week of lactation were used. German Holsteins are the most common dairy breed in Germany and play a crucial role in the German dairy industry. Parity and mean daily milk yield (kg/d) of every single cow during the course of study were shown in **Table 4.5**.

Table 4.5. Parity and mean daily milk yield (kg/d) of the trial cows between the 2nd and 15th week of lactation.

Animal	Parity	Mean Daily Milk Yield (kg/d)
825	8	50.3
1208	5	40.5
1252	4	49.0
1274	4	47.0
1309	4	48.2
1321	4	44.9
1326	4	51.4
1332	4	42.2
1401	3	49.4
1416	3	46.6
1419	3	46.5
1428	3	45.4
1434	3	34.4
1440	3	51.6
1450	2	43.5
1457	2	55.5
1460	2	51.4
1510	2	41.3
1515	2	47.4
1522	2	43.5
1523	2	38.5
1524	2	44.0
1525	2	52.1
1533	2	41.7
1536	2	44.6
1537	2	40.4
1540	2	35.7
1563	2	50.0

The cows were housed together with non-experimental cows in a free-stall barn with 60 cubicles and 30 feeding units. Cows had unlimited access to fresh water and were fed a total mixed ration (TMR) ad libitum (**Table 4.6**). The TMR was prepared in the morning and delivered twice daily (60% of total daily amount at 6 a.m. and 40% of total daily amount at 11 a.m.).

Table 4.6. Ingredient, chemical composition and energy content of the total mixed ration.

Diet Composition (g/kg DM) [1]	**Mean**	**SD**
Beet pressed pulp silage	188.3	
Grass silage	97.0	
Grass hay	74.7	
Maize silage	259.6	
Concentrate	380.4	
Chemical Composition (g/kg DM) [1]		
Dry matter	402.0	13.8
OM	931.4	3.8
CP	157.4	8.1
SP	63.2	7.3
EE	43.0	2.4
aNDFom	355.1	8.8
ADF	219.3	4.9
Lignin	28.3	1.2
iNDF$_{240}$	86.4	4.5
Starch	182.3	14.8
ESC	63.1	3.4
TDN [2]	732.0	5.0
Energy (MJ/kg DM)		
NE$_L$ [3]	7.0	0.0

[1] Diet and chemical composition reported on 105 °C dry matter. Averaged values based on weekly conducted feed analysis; diet was offered as TMR. ADF—acid detergent fiber, expressed inclusive of residual ash; aNDFom—neutral detergent fiber assayed with heat-stable amylase and expressed exclusive of residual ash; CP—crude protein; CV—coefficient of variation calculated as ratio of standard deviation to mean; EE—ether extract; ESC—ethanol-soluble carbohydrates; iNDF240—indigestible aNDFom; OM—organic matter; SD—standard deviation; SP—soluble protein. [2] TDN (g/kg)— total digestibly nutrient values for TMR samples were calculated from the TDN value using

Equations 2–5 by [17]. [3] NE_L—net energy for lactation, for TMR samples were calculated from the TDN value using Equations (2)–(3) by [17].

4.2.2. Data and Sample Collection

Individual feed intake was measured daily with the Insentec B.V. (Marknesse, the Netherlands) RIC (roughage intake control) automatic weighing system. Cows were identified using individual collar transponders, which registered access to the feeding unit. The connected computer recorded cow number, feeder number, trough weight and the time of the beginning and the end of each visit. TMR intake per visit was calculated from the differences in trough weight between start and end of the visit. Feeding time was calculated from the difference between start and end timepoint. Each visit resulting in a trough weight difference of more than 0.1 kg was considered a meal. All parameters were calculated on a dry matter base by multiplying the amount of fresh matter by the dry matter (DM) content of the TMR. Intake behavior was characterized for an individual animal over a day by the following parameters and definitions, according to Nielsen [12]: meal frequency (meals/d), defined as number of individual feeding bouts per day; meal duration (min/meal), defined as average time per meal, average meal size (kg DM/meal); daily feeding time (min/d), defined as the sum of the meal durations in a day; daily DMI (kg DM/d), defined as the sum of the meal sizes in a day and speed of food ingested; or feeding rate (g DM/min), defined as the ratio between meals size (kg DM/meal) and meal du-ration (min/meal). To account for the individuality of feed intake behavior, the general term “feeding behavior” is replaced by the term “intake behavior” in all that follows. The individual parameters of daily intake behavior were averaged for every cow per week of lactation. Cows were milked twice daily between 5.00 and 7.30 a.m. and between 3.30 and 6.00 p.m. The daily milk yield was recorded electronically via the herd management system Dairy Plan C21 (GEA Farm Technologies, Boenen, Germany). Milk aliquots from one evening and the next morning were taken biweekly and pooled for further analysis of milk fat, protein and lactose by infrared spectrophotometry using a MilkoScan FT6000 (Foss Analytical A/S, Hillerød, Denmark). TMR samples were taken daily within one hour of feed delivery. Daily samples were combined on a weekly basis with a representative TMR sample of 800–1000 g for determining the weekly dry matter content. Starting at the day of calving, individual cow fecal samples were collected weekly two hours after morning milking via rectal palpation. The samples were labeled and stored frozen at −20 °C until chemical analysis. Deviations in fecal content within a day, especially in aNDFom and iNDF240, were reduced by defining a fixed time of feeding and sampling, which remained constant over the test period.

4.2.3. Chemical Analysis

Dry matter content was determined in a two-step process: thawed TMR and feces samples were first oven dried at 60 °C for 48 h and ground to 1 mm particles, followed by drying at 105 °C for 3 h until constant weight was achieved. Organic matter (OM) was measured by ashing (550 °C) overnight. The dried and ground samples were submitted to Cumberland Valley Analytical Services Inc., Waynesboro, PA (CVAS), for chemical analysis. All TMR samples were analyzed for dry matter (DM), organic matter (OM) (method 942.05; [18]), crude protein (CP) (method 990.03; [18]), soluble protein (SP) [19], ether ex-tract (EE) (method 2003.05; [18]), neutral detergent fiber assayed with heat-stable amylase and expressed exclusive of residual ash (aNDFom) [20], acid detergent fiber (ADF), ex-pressed inclusive of residual ash (method 978.10; [18]), lignin (method 973.18; [18]), ethanol-soluble carbohydrates (ESC) [21], starch [22] and 240 h in vitro indigestible neutral detergent fiber (iNDF240) [23]. Dried and ground fecal samples were split into two sub-samples. One subsample was analyzed with near infrared reflectance spectroscopy (NIRS) described by [24]. The analysis included DM, OM and CP. The other subsample was submitted to CVAS for determination of ADF, aNDFom and iNDF240.

4.2.4. Calculations and Statistical Analysis

Sample size was determined by the capacity of the research facility. Our sample size of $n = 28$ was assumed as adequate to reliably detect differences between cows and weeks of lactation (>0.95 statistical power and $p \leq 0.05$ significance level; G*power 3 software; [25]). Organic matter digestibility (OMD), crude protein digestibility (CPD), neutral deter-gent fiber digestibility (NDFD) and acid detergent digestibility (ADFD) were calculated from iNDF240 as internal marker and nutrient concentrations in TMR and feces using the following equation, by [26]:

$$\text{Apparent nutrient digestibility (g/kg)} = 1000 - 100 \times \{[\text{iNDF240}]\ \text{Diet}/[\text{iNDF240}]\ \text{Feces}\} \times \{[\text{nutrient}]\ \text{Feces}/[\text{nutrient}]\ \text{Diet}\} \quad (1)$$

Data was analyzed using the SPSS 25.0 software (IBM Company Inc., Chicago, IL, USA). Each variable was checked for normal distribution by a histogram and a Q–Q plot, and the mean, range, standard deviation (SD) and coefficient of variation (CV) were calculated.

Differences were considered significant at a level of $p \leq 0.05$, and a tendency was considered at $0.05 < p \leq 0.10$. Changes of each parameter for all cows over the course of the study are shown by box plots, which represent the median, interquartile range and extreme cases of individual variables.

The differences in intake behavior and nutrient digestibility across the trial period were analyzed using the GLM repeated measures procedure. When Mauchly's test indicated that the assumption of sphericity had been violated, the degrees of freedom were corrected using Greenhouse–Geisser estimates if the estimate was lower than 0.75, or the Huynh–Feld estimate if the estimate was greater than 0.75 [27]. The effect sizes for main effects and interactions were determined by partial eta squared (η2) values. Partial eta squared (η2) values were classified as small (0.01 to 0.059), moderate (0.06 to 0.137) and large (>0.137).

Differences in intake behavior between second and greater than or equal to third lactating dairy cows were analyzed using an independent samples t-test. The effects of intake behavior on DMI and on the four measured variables (nutrient digestibility of OM, CP, NDF and ADF) were statistically tested using the linear mixed model (LMM) procedure. Analyses were carried out on the individual animal as the observational unit. Akaike's information criterion and relative standard error (RSE) were, respectively, used for model evaluation and to find the best-fit model. Correlations, calculated with Pearson correlation coefficient, between dependent variables were low ($R < 0.80$; [28]), indicating that multicollinearity was not a confounding factor in the analysis. The final model to test the effect of intake behavior on DMI included the fixed effects of milk yield, meal frequency, feeding time, meal size, feeding rate and a random intercept for the cow. Meal duration (min/meal) was used to calculate feeding time (min/d) and feeding rate (g/min) and was therefore excluded. There were no significant two-way interaction terms between the fixed effects. The final model to test the effect of intake behavior on nutrient digestibility of OM, CP, NDF and ADF included the fixed effects of week of lactation, DMI (kg/day), meal frequency (meals/d), feeding time (min/d), meal size (kg/meal), feeding rate (g/min) and a random intercept for the cow.

There were no significant two-way inter-action terms between the fixed effects. Meal duration (min/meal) was used to calculate feeding time (min/d) and feeding rate (g/min) and was therefore excluded from both calculations.

Cows were retrospectively grouped in quartiles based on average DMI between week 2 and 15 of lactation. The use of quartiles in the formation of groups has the advantage of providing greater differentiation between cows having high differences in DMI. The three groups were created based on the mean DMI of the cows during the trial period.

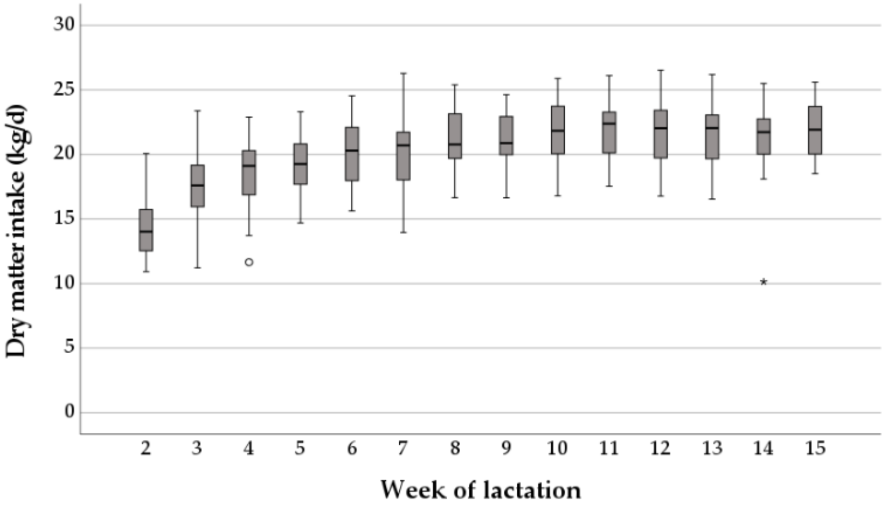
The first group was the lower quartile, consisting of the cows with the lowest mean DMI (<18.99 kg DMI/d, n = 7) between week 2 and 15 of lactation. The second and third groups consisted of the intermediate (between 19.00 and 20.93 kg DMI/d, n = 14) and upper (>20.94 kg DMI/d, n = 7) quartiles, respectively. Differences in intake behavior and nutrient digestibility were analyzed using ANOVA, with quartiles as fixed factor. The individual quartile comparisons were performed using post hoc pair-wise comparisons, with the Sidak correction applied.

4.3. Results

4.3.1. Interactions between Intake Behaviour and DMI during Early Lactation

The trends of intake behavior parameters and feed intake over the course of the study are shown in **Figure 4.8**. DMI significantly increased with a linear ($p < 0.001$; $\eta^2 = 0.75$) and quadratic ($p < 0.001$; $\eta^2 = 0.677$) trend from a mean value of 14.3 ± 2.3 kg DMI per day in week 2 up to 22.1 ± 2.3 kg in week 11 and 21.57 ± 2.3 kg in week 15 (**Figure 4.8**a). Mean CV of DMI ranged between 0.09 (week 9) and 0.17 (week 3). Daily meal frequency remained at the same level of 23.6 ± 11.5 meals over the course of study ($p > 0.005$; $\eta^2 = 0.122$; **Figure 4.8**b). Mean CV of daily meal frequency was lowest in week 3 (0.39) and highest in week 14 (0.58). Meal duration had a mean of 9.9 ± 4.0 min/meal and constantly increased with a linear ($p < 0.001$; $\eta^2 = 0.572$) and quadratic ($p < 0.001$; $\eta^2 = 0.223$) trend from 7.4 min/meal in week 2 p.p. to 11.9 min/meal in week 11 p.p. (**Figure 4.8**c). CV values ranged from 0.35 (week 14) to 0.45 (week 10). Feeding time per day increased significantly ($p < 0.001$; $\eta^2 = 0.298$) with a linear ($p < 0.05$; $\eta^2 = 0.385$) and quadratic ($p < 0.001$; $\eta^2 = 0.606$) trend from a mean value of 151.2 ± 31.6 min per day in week 2 up to 228.9 ± 48.8 min per day in week 10, and constantly decreased to a value of 209.1 ± 49.8 min in week 15 p.p. (**Figure 4.8**d).

CV values of total feeding time per day ranged between 0.19 (week 11) and 0.24 (week 7). The meal size had a mean of 0.96 ± 0.4 kg/meal and constantly increased with a linear ($p < 0.001$; $\eta^2 = 0.602$) trend between week 2 and 15 of lactation from 0.7 ± 0.3 kg/meal to 1.3 ± 0.6 kg/meal, respectively ($p < 0.001$; $\eta^2 = 0.602$; **Figure 4.8**e), with CV values between 0.35 (week 13) and 0.46 (weeks 8 and 15). The feeding rate remained at the same level of 93.41 ± 22.7 g per min up to week 15 p.p. ($p > 0.005$; $\eta^2 = 0.122$; **Figure 4.8**f) and CV ranged between 0.21 (week 13) and 0.30 (week 3).



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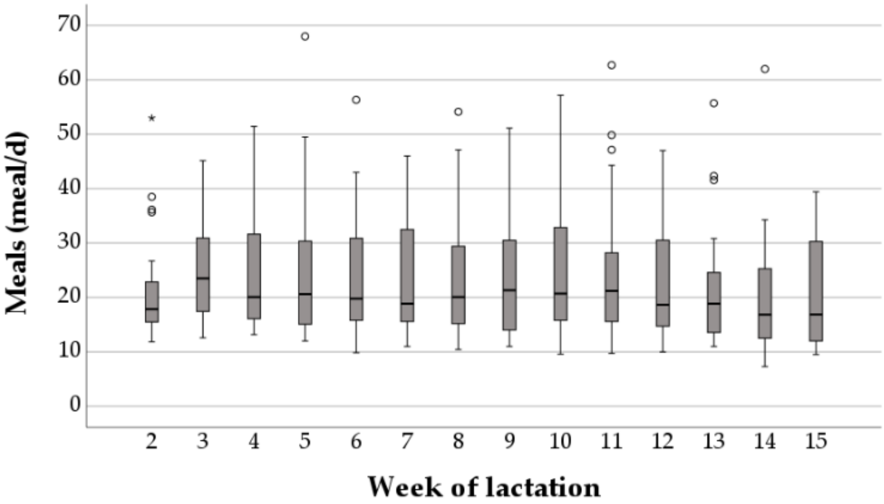

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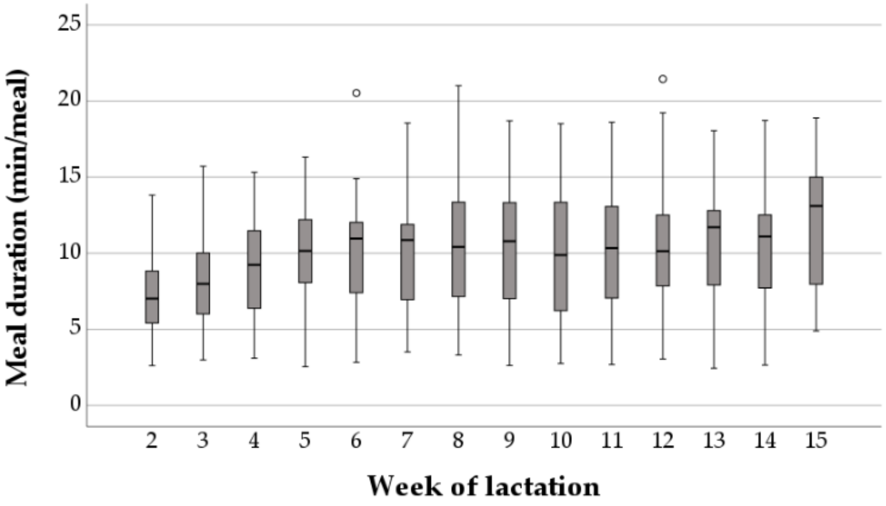
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25
20
15
10
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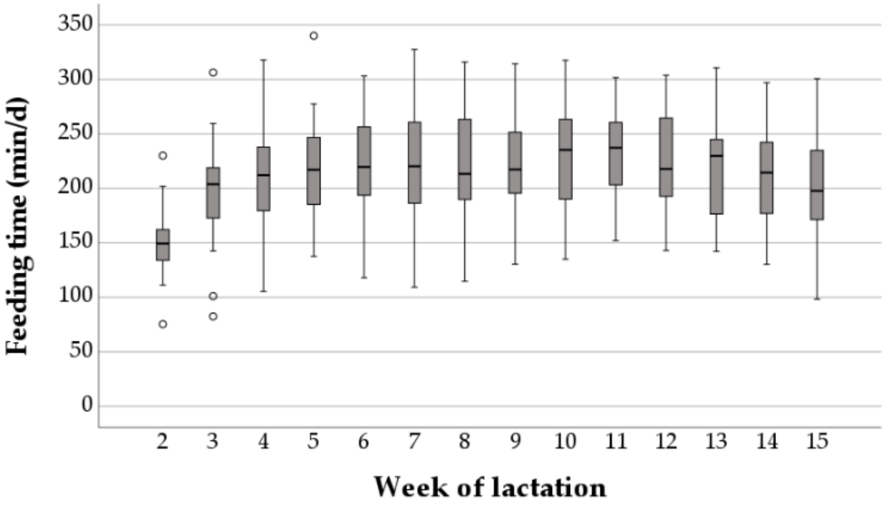
13
14
15
Week of lactation

(**c**)


Feeding time (min/d)
350
300
250
200
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100
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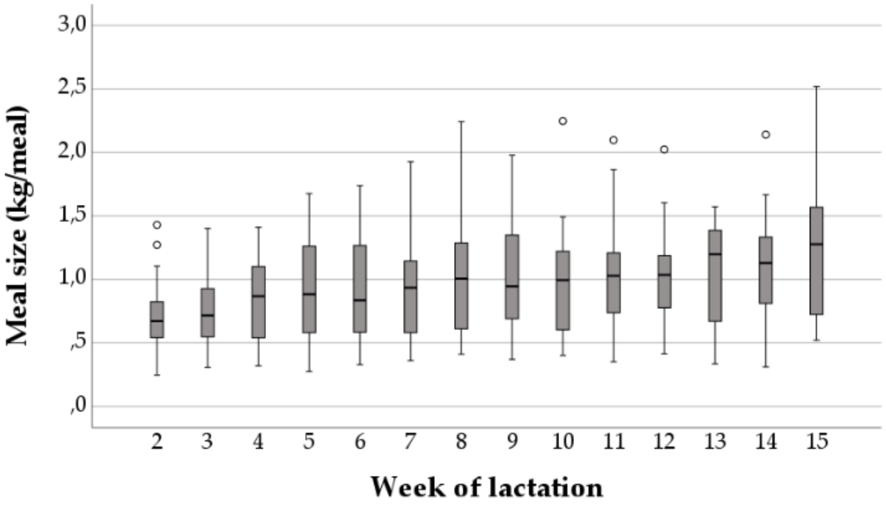
13
14
15
Week of lactation

(**d**)


Meal size (kg/meal)
3,0
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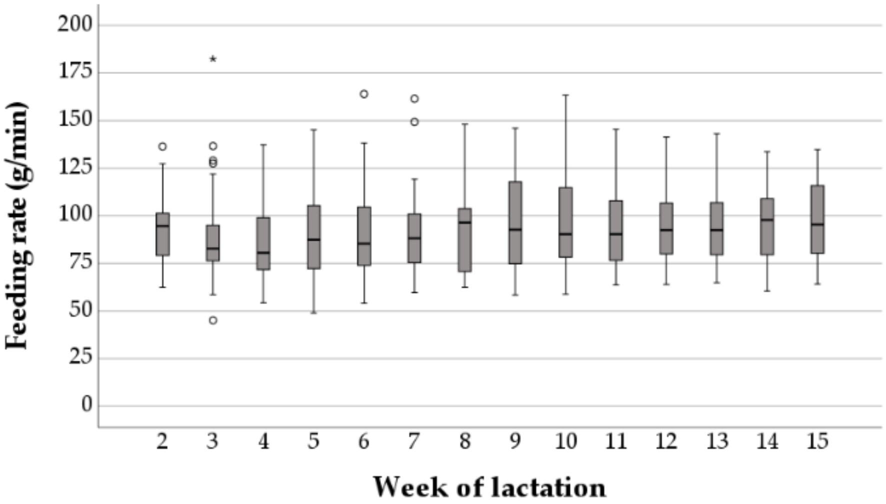
13
14
15
Week of lactation

(**e**)



(f)

Figure 4.8. Dry matter intake (kg/day) (a), meal frequency (meals/d) (b), meal duration (min/meal) (c), feeding time (min/d) (d), meal size (kg/meal) (e) and feeding rate (g/min) (f), measured for all 28 cows between week 2 and 15 postpartum (p.p.). In each subfigure, the boxplots highlight, respectively, the median and upper and lower quartiles of each week. Small circles = outliers (1,5 x interquartile range (IQR)); asterisks = extreme values (3 x IQR).

The LMM showed significant positive effects of milk yield, meal frequency, feeding time, feeding rate and a comparable high effect of meal size on daily DMI of dairy cows (**Table 4.7**).

Table 4.7. Influence of milk yield and intake behavior on dry matter intake during early lactation in dairy cows, estimated by the linear mixed model (LMM) procedure (n = 28).

	Daily DMI [1] (kg/Day)		
Parameter	**b [2]**	**SE**	***p*-Value**
Intercept	−7.60	1.02	<0.01
Milk yield (kg/d)	0.12	0.02	<0.01
Meal frequency (meals/d)	0.11	0.02	<0.01
Meal size (kg DM/meal)	4.89	0.41	<0.01
Feeding time (min/d)	0.05	0.00	<0.01
Feeding rate (g/min)	0.05	0.00	<0.01
AIC [3]	1322.59		

[1] Dry matter intake. [2] Parameter estimate. [3] Akaike’s information criterion.

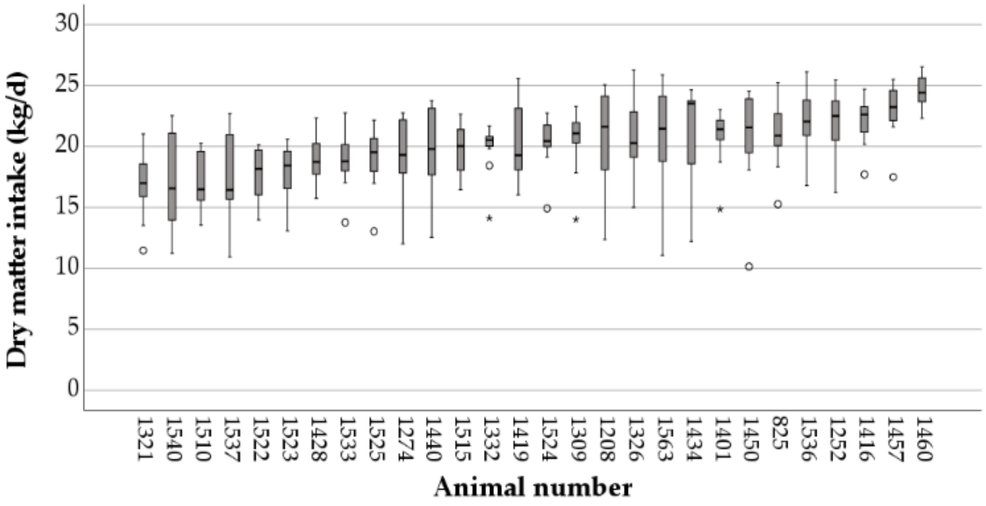
Table 4.8 illustrates the mean, SD and CV of DMI and variables of intake behavior for second and greater than or equal to third lactating dairy cows. Mean DMI and SD of meal frequency were significant lower in second lactating cow (*p* = 0.043; *p* = 0.021). No other significant differences were detected between parity groups.

Table 4.8. Mean, SD and CV values of dry matter intake (DMI) and variable of intake behavior of early lactating dairy cows grouped in second and greater than or equal to third lactating dairy cows.

		Parity		
		2	**≥ 3**	***p*-Value**
DMI [1] (kg/d)	mean	19.9	20.3	0.043
	SD	3.4	3.0	0.375
	CV	0.2	0.1	0.301
Meal frequency (meals/d)	mean	21.6	25.7	0.112
	SD	9.0	13.3	0.021
	CV	0.4	0.5	0.427
Meal duration (min/meal)	mean	10.6	9.4	0.581
	SD	3.7	4.3	0.493
	CV	0.3	0.5	0.934
Feeding time (min/d)	mean	222.5	204.3	0.776
	SD	50.4	46.4	0.056
	CV	0.2	0.2	0.251
Meal size (g/meal)	mean	1.0	0.9	0.753
	SD	0.5	0.4	0.984
	CV	0.5	0.4	0.412
Feeding rate (g/min)	mean	86.9	99.9	0.668
	SD	21.5	22.1	0.476
	CV	0.2	0.2	0.324

[1] Dry matter intake.

In addition to the high variation in intake behavior between dairy cows at the same stage of lactation, the extent of variation in intake behavior during early lactation within individual animals was also great (**Figure 4.9**). Intra-individual CVs of DMI ranged between 0.05 and 0.25, CVs of meal frequency ranged between 0.11 and 0.36. For meal duration and feeding time, CVs ranged between 0.10 and 0.36 and between 0.08 and 0.26, respectively. The widest range in CVs was detected for meal size (0.1–0.42). Intra-individual CVs for feeding rate ranged between 0.07 and 0.30.



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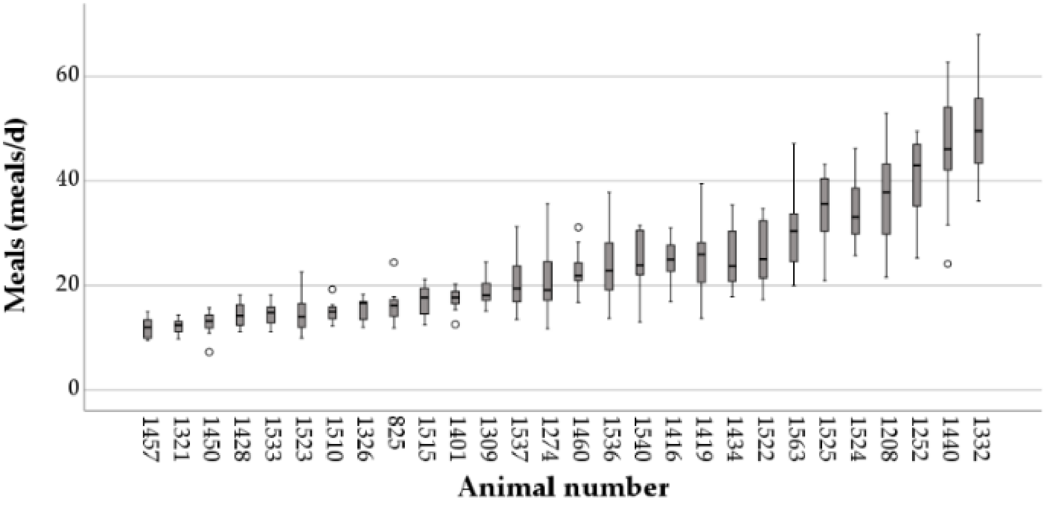

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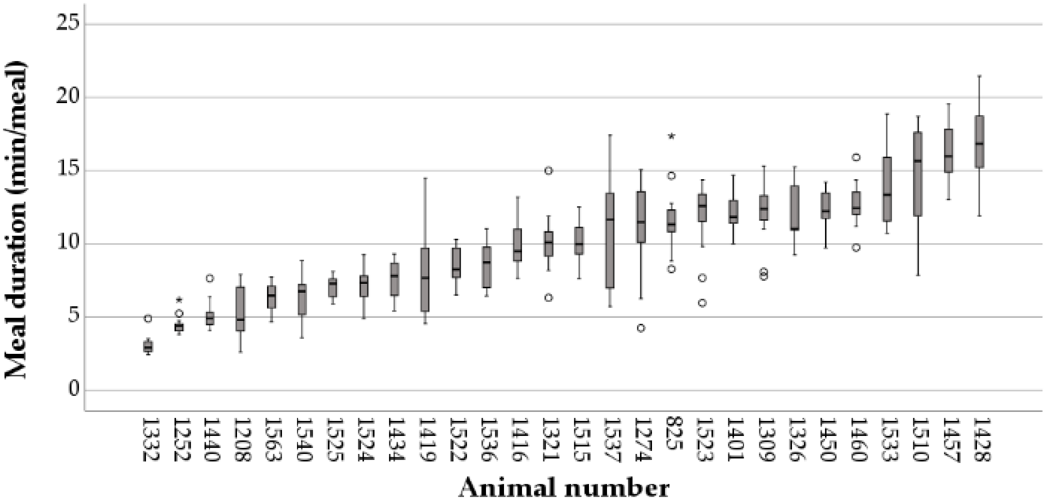
Meal duration (min/meal)
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1419
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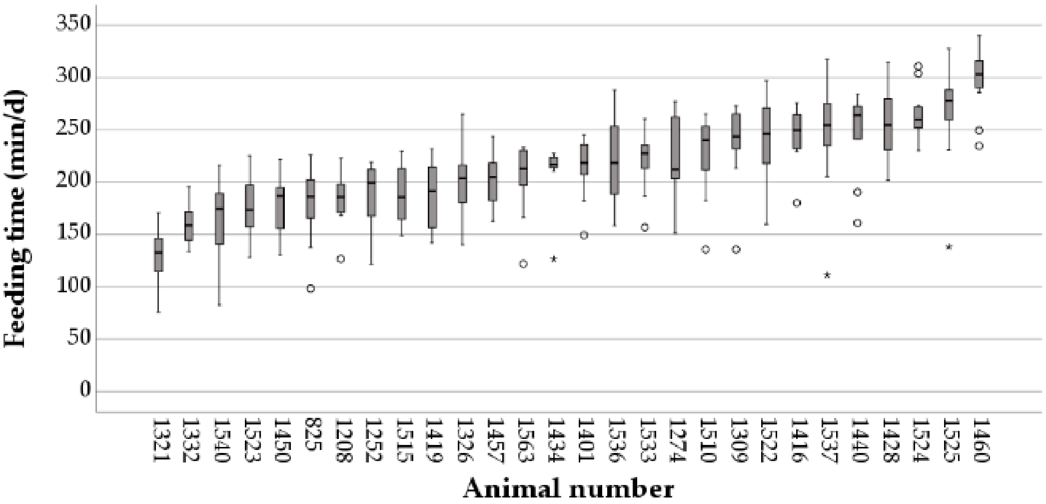
Animal number

(**c**)


Feeding time (min/d)
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1515
1419
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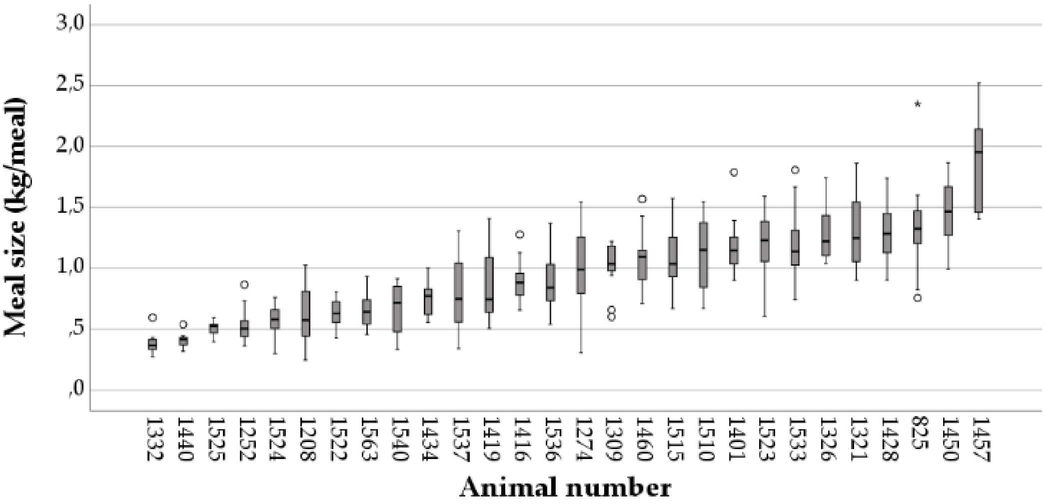
Animal number

(**d**)


Meal size (kg/meal)
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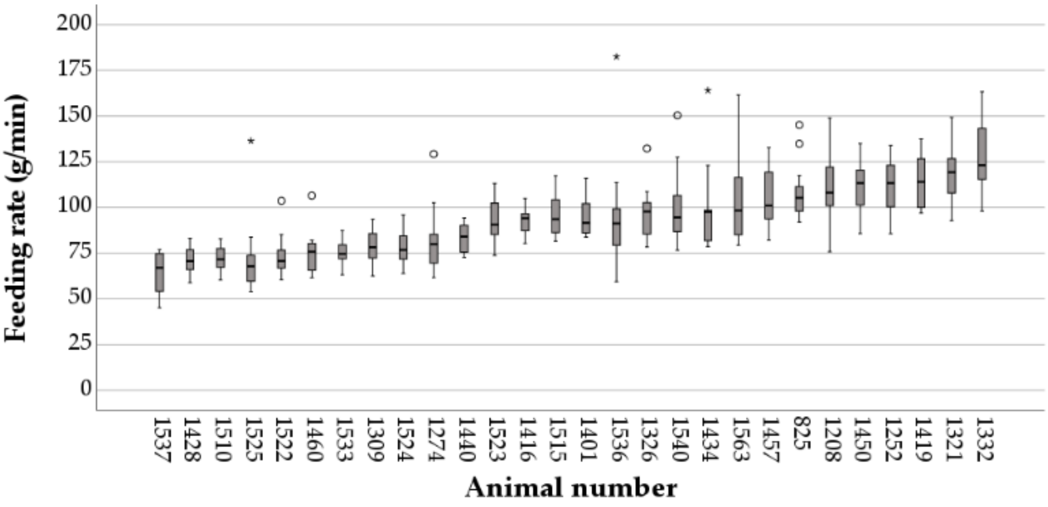

(f)

Figure 4.9. Dry matter intake (kg/day) (a), meal frequency (meals/d) (b), meal duration (min/meal) (c), feeding time (min/d) (d), meal size (kg/meal) (e) and feeding rate (g/min) (f), measured for individual animals (n = 28) between week 2 and 15 postpartum (p.p.). In each subfigure, the boxplots highlight, respectively, the median and upper and lower quartiles of each week. Small circles = outliers (1,5 x interquartile range (IQR)); asterisks = extreme values (3 x IQR).

When comparing mean and CV values of intake behavior parameters of cows with mean low, medium and high DMI, no significant differences could be detected between the quartiles (**Table 4.9**).

Table 4.9. Mean and CV values of DMI and intake behavior of early lactating dairy cows grouped in quartiles according to mean DMI observed between week 2 and 15 of lactation.

	Lower Quartile (<25%)	**Intermediate Quartile**	**Upper Quartile (>75%)**	***p*-Value**
Mean DMI [1]	17.61	20.14	22.30	>0.05
CV DMI [1]	0.15	0.14	0.10	>0.05
Mean meal frequency	18.24	27.62	21.83	>0.05
CV meal frequency	0.19	0.21	0.19	>0.05
Mean meal duration	11.35	8.67	10.85	>0.05
CV meal duration	0.22	0.20	0.15	>0.05
Mean feeding time	206.61	215.51	216.24	>0.05

CV feeding time	0.18	0.14	0.14	>0.05
Mean meal size	1.00	0.82	1.16	>0.05
CV meal size	0.26	0.23	0.22	>0.05
Mean feeding rate	0.08	0.10	0.10	>0.05
CV feeding rate	0.14	0.16	0.16	>0.05

[1] Dry matter intake.

4.3.2. Interactions between Intake Behavior and Nutrient Digestibility during Early Lactation

The highest mean value regarding the nutrient digestibility of the diet was found for OM (728.8 ± 19.8 g/kg), followed by CP, NDF and ADF with 625.4 ± 50.7, 585.1 ± 43.5 and 571.9 ± 34.3 g/kg, respectively. Over the course of the study, mean CV was highest for CP with a value of 0.08 (0.06–0.10) followed by NDF, ADF and OM with 0.07 (0.05–0.10), 0.06 (0.05–0.07) and 0.03 (0.02–0.03), respectively. The GLM repeated measures procedure reported significant differences between week of lactation for CP ($P = 0.032$; $\eta^2 = 0.164$) and NDF digestibility ($P = 0.00$; $\eta^2 = 0.427$) with a quadratic trend over the time for CP ($P = 0.041$; $\eta^2 = 0.212$) and NDF ($P = 0.039$; $\eta^2 = 0.215$). CP digestibility decreased from 653.4 ± 46.9 g/kg in week 2 to 618.6 ± 60.9 g/kg in week 9, and increased again up to 634.1 ± 48.8 g/kg in week 15. NDF digestibility decreased from 607.4 ± 54.0 g/kg in week 2 to 577.0 ± 36.4 g/kg in week 10 and increased again up to 58.5.4 ± 49.3 g/kg in week 15. No significant differences between the weeks of lactation for OM and ADF digestibility were detected. Variation in OMD within individual animals during early lactation was comparably low with CVs between 0.02 and 0.04. CVs of CPD ranged between 0.04 and 0.11 between animals. CVs of NDFD and ADFD ranged between 0.03 and 0.11, and between 0.03 and 0.08, respectively.

The effects of week of lactation, dry matter intake and intake behavior on nutrient digestibility are shown in **Table 4.10**. Significant fixed effects ($p < 0.05$) of DMI were found for nutrient digestibility of OM and CP. Week of lactation had a significant effect on CP and ADFD ($p < 0.05$). Variables of intake behavior had no significant effect on nutrient digestibility. Examination of the components of variance showed that the intercepts significantly varied across cows to the amounts of 50.4, 328.1, 281.1 and 271.4 for OMD, CPD, NDFD and ADFD, respectively. Allowing for all other effects in the model, parameter estimates from this model predicted that nutrient digestibility of OM and CP decreased, respectively, by 1.9 (SE 0.68) and 5.2 (SE 1.7) g/kg DM per kilogram of increase in DMI.

Table 4.10. Influence of week of lactation, dry matter intake and variables of intake behavior on digestibility of OM, CP, NDF and ADF during early lactation in dairy cows, estimated by linear mixed model (LMM) procedure (n = 28).

	OMD			**CPD**			**NDFD**			**ADFD**		
Variable	**Estimate**	**SE**	***p*-Value**	**Estimate**	**SE**	***p*-Value**	**Estimate**	**SE**	***p*-Value**	**Estimate**	**SE**	***p*-Value**
Intercept	754.57	11.56	<0.01	714.56	28.79	<0.01	637.31	25.25	<0.01	600.28	19.10	<0.01
Week of lactation	0.29	0.31	0.365	3.00	0.78	<0.01	0.39	0.68	0.561	−1.75	0.51	<0.01
Daily DMI [1] (kg/day)	−1.83	0.68	0.008	−5.15	1.70	0.003	−1.95	1.49	0.194	−1.34	1.15	0.244
Meal frequency (meals/day)	−0.06	0.22	0.787	0.11	0.53	0.831	0.09	0.47	0.836	−0.17	0.36	0.650
Meal size (kg/meal)	2.92	6.16	0.636	−1.29	15.37	0.933	0.63	13.56	0.963	2.24	10.44	0.830
Feeding time (min/d)	0.04	0.05	0.368	0.05	0.11	0.659	0.01	0.10	0.898	0.10	0.08	0.213
Feeding rate (kg/min)	−0.03	0.083	0.756	−0.24	−0.21	0.248	−0.02	0.18	0.183	−0.09	0.13	0.520
AIC [2]	1508.2			2150.9			2055.5			1849.8		

[1] Dry matter intake. [2] Akaike's information criterion.

Significant differences could be detected in average and CV values of nutrient digestibility between animals with low mean and high mean feed intake, based on average DMI between week 2 and 15 of lactation of the cows during the trial period, for mean OMD, mean CPD, mean ADF and CV of NDF (**Table 4.11**). Mean OMD and CPD in early lactation were significantly higher for animals with mean comparatively low DMI. Mean ADFD and CV of NDFD in early lactation were significantly higher for animals with an average higher DMI.

Table 4.11. Mean and CV values of nutrient digestibility of early lactating dairy cows grouped in quartiles according to mean DMI observed between week 2 and 15 of lactation.

	Lower Quartile (<25%) **(DMI < 18.99 kg DMI/d, n = 7)**	**Intermediate Quartile** **(DMI between 19.00 and 20.93 kg DMI/d, n = 14)**	**Upper Quartile (>75%)** **(DMI > 20.94 kg DMI/d, n = 7)**	***p*-Value**
Mean OMD	737.08^{a}	727.17^{b}	726.16^{b}	0.032
CV OMD	0.03	0.02	0.03	>0.05
Mean CPD	638.98^{a}	631.20^{ab}	607.60^{b}	0.032
CV CPD	0.07	0.06	0.09	>0.05
Mean NDFD	600.85	578.72	583.77	> 0.05
CV NDFD	0.06^{a}	0.06^{a}	0.08^{b}	0.006
Mean ADFD	574.58^{ab}	561.61^{a}	587.49^{b}	0.021
CV ADFD	0.05	0.06	0.05	>0.05

$^{a\text{-}b}$ values with different superscripts within the same line are significantly different.

4.4. Discussion

Considerable variation was observed between individual animals and within animals over time when examining feed intake, intake behavior and nutrient digestibility. To be able to attribute the variation in the measured parameters to the differences between individual animals, possible confounding factors by breed, feed or environment were reduced to a minimum.

4.4.1. Intake Behavior and DMI

The mean values of dry matter intake and daily feeding time obtained in the current study were within the range, but at the lower end, of results which have been reported in other studies [29–31]. In the present study, daily DMI increased from 14.3 DMI per day in week 2 up to 22.1 in week 11. A similar increase in DMI during early lactation was described by Azizi et al. [31] and Park et al. [32]. In this context, the latter determined a parallel increase in ruminal capacity as a percentage of body weight of 31.2% during the first 90 days of lactation. DeVries et al. [33] observed an increase in daily mealtime and meal duration from period 1 (35 ± 16 DIM) to period 2 (57 ± 16 DIM) as well, but no changes between period 2 and 3 (94 ± 16 DIM). In contrast, Friggens et al. [34] found no significant effect of the stage of lactation on meal duration and meal size, and likewise, no significant effect of the stage of lactation on meal frequency.

However, mean values of meal frequency, meal duration, meal size and feeding rate in the current study were different to the results of other studies [31,35]. A possible explanation for the deviating values may be due to differences in the definition of meal criteria (meals/day). Miron et al. [35], who described an average of 14 meals per day and a meal duration of 15.9 min/meal, defined a meal as a visit to a trough that lasted at least 1 min and eating at least 0.2 kg of TMR. Based on a method developed by Tolkamp et al. [36] and DeVries et al. [33], Azizi et al. [31] calculated a meal criterion of 28.5 min on average. Variation in the results can be also attributed to differences in methodology (e.g., experimental procedures, diet composition, feeding level). Dado and Allen [30] defined a minimum of 7.5 min between events to define two eating periods. They reported by early lactating dairy cows (63 DIM) an average of 24.8 kg DM per day, 10.8 eating bouts per day (meal frequency) with a bout length of 31.1 min (meal duration), a daily feeding time of 314 min and a meal size of 2.5 kg. Because even the smallest amounts of ingested feed are digested, the definition of meal criteria should be examined more closely, especially in studies which focus on digestibility research.

DMI, meal duration, feeding time and meal size increased over the course of study. In contrast, daily meal frequency and feeding rate remained at the same level during study. The average feeding rate in the present study (93.4 g of DM/min) was lower than reported by others; for example, [37] 120.0 g of DM/min, but in agreement with the results shown by Beauchemin et al. [38] (90.0 g of DM/min), even though in that study the cows were housed in individual tie-stalls without competition between cows. In the current study, cows were housed together with non-experimental cows in a free-stall barn with an animal feeding place ratio of 2:1 and the potential for aggressive interactions and displacements from the feed bunk by other cows existed and, in contrast to Beauchemin et al. [38], no primiparous cows were used in the present study. Albright [7] described, that cows tend to consume more feed at a faster rate when they are fed in groups than when fed separately. In the present study, DMI was significantly influenced by variables of intake behavior. Therefore, intake behavior should be considered when developing strategies to increase feed intake. Grant and Albright [10] concluded that management factors, such as grouping strategy, feeding system design and apparatus, composition and physical characteristics of the feed being consumed, as well as social hierarchy and competition for food and water, are the main factors influencing the intake behavior of cattle, especially for cows with a lower standing in the hierarchy.

All parameters of intake behavior showed a substantial variation between dairy cows at the same stage of lactation and within individual dairy cows during the early lactation. An explanation for the variation between dairy cows at the same stage of lactation may be the different milk yields of the 28 cows. Dado and Allen [30] indicated that cows with higher milk yields achieved greater DMI by increasing meal size and decreasing eating time. Azizi et al. [31] also reported 20% less eating time and 28% more DMI in high-yielding dairy cows in comparison to below average yielding cows. Furthermore, higher correlations between intake behavior characteristics and dry matter intake (DMI) within milk yield groups than across all cows were reported [30,34]. Factors which potentially affect the frequency, size and rate of meals could be social interaction between cows [39], feed access [40], palatability and moisture content, as well as the possibility to sort feed, cow health and environmental temperature [39]. No differences, expect a significant lower DMI in second lactating dairy cows, were detected between cows in different parities. Between the categories of low, medium or high dry matter intake, no significant differences in intake behavior parameters and variation were detected, but cows with a higher DMI seemed to be more consistent in intake behavior, except the feeding rate. The high within- and between-cow variability in the current study and in other studies [31,33] indicated that different cows pursue different strategies for nutrient supply. Therefore, it seems even more important to create suitable conditions for the different eating habits to enable high feed intake for all individual cows. Thus, to allow all dairy cows to pursue their own feeding strategy and hence maximize feed intake, the management of young cows should unstintingly focus on designing cow’s individual conditions of feed intake. Especially strategies that avoid sorting of feed in heifers at young age, as well as ensuring a balanced nutrient supply via, e.g., TMR, should be considered [41]. However, also ensuring sufficient space and reducing stress by, e.g., avoiding overcrowding, can contribute to a more even feed intake [42]. The assumption of an almost uniform feed intake at the same stage of lactation is misleading and delusive [43].

4.4.2. Intake Behavior and Nutrient Digestibility

Besides feed intake, nutrient digestibility plays an important role for the available nutrient and energy in dairy cattle [9]. The mean between-cow CV in OM digestibility during the trial period was 0.03. In a recent meta-analysis by Guinguina et al. [44] and in the meta-analysis by

Cabezas-Garcia et al. [45], even lower values of 0.014 and 0.013 for between-cow CV in OMD were reported. The results demonstrate that there is little variance among early lactating dairy cows in their ability to digest organic matter of a given diet.

In contrast, the variation in digestibility of CP, NDF and ADF with CV values up to 0.08 between cows at the same stage of lactation was at a higher level. Together with the high intra-individual CV values up to 0.11 of CPD, NDFD and ADFD, the variation challenges the traditional approach of generalizing a uniform feed-digestibility value. By using iNDF240 instead of tabulated digestibility coefficients, it is possible to identify nutrient digestibility at animal level, and thus the level of nutrients available to individual animals, rather than only the feed level of total digestible nutrients (TDN). Therefore, digestibility assessed by iNDF240 from a random sample of fecal samples could be used to evaluate the digestibility of the diet, for example, after a change of the diet. Further research is needed to ascertain a robust sample size for determining digestibility within a herd.

The influence of intake behavior on nutrient digestibility is well described for beef cattle in conjunction with feed efficiency. Robinson and Oddy [46] found phenotypic correlations between feed efficiency and the time spent eating and the number of visits to the feeder of 0.64 and 0.51, respectively. Similar results were reported by Golden et al. [14] and Green et al. [47], who found that efficient cattle ate less and spent less time feeding then inefficient cattle given the same production level. In contrast, research on the influence of intake behavior on nutrient digestibility in dairy cows is rather rare. The presented results do not show any effect of variables of intake behavior on nutrient digestibility but an effect of week of lactation on CPD and ADFD and an effect of dry matter intake on OMD and CPD.

The amount of dry matter consumed and the passage rate are key drivers for the level of digestion, absorption and utilization of feed in the animal. Changes in one of the key drivers lead to changes in the other two [48]. Several researchers have shown that increased intake results in increased passage rates, which is associated with incomplete feed digestion and therefore decreased digestibility [49–51]. Colucci et al. [51,52] reported a close relationship ($R^2 = 0.86$) between the decrease in ruminal retention time and depression in digestibility in sheep and cows fed different diets. The effect of higher passage rate is especially distinct when the diet contains a high percentage of fiber. With increasing DMI and an increasing passage rate, fiber cannot be sufficiently digested [9]. The same happens in the first weeks after calving, when DMI increases daily. In the present study, DMI increased up to week 11 p.p. Park et al. [32] also reported a rapid increase in dry matter intake from 2.7 to 4.3% of body weight between day 6 and 34 postpartum and then a slower increase up to day 81. Parallel to the increase in dry matter intake, a strong increase in ruminal fill was also observed between days 48 and 62 p.p. [32].

Stafford [53] found a decrease in ruminal motility after day 60 p.p., which could reduce the digesta volume flowing from the rumen, increase the ruminal fill, decrease the passage rate and result in increasing digestibility [32]. Furthermore, the model predicted a positive influence of meal duration on OM and ADF to the amounts of 1.57 and 2.91 g/kg DM per extra minute of eating time per meal. A possible explanation for the positive influence of meal duration may be the increase in salivary secretion, which reduces the size of feed particles [54] and increases rumen fermentation and nutrient digestibility [55].

Significant differences were detected in mean and inter-individual CV values of nutrient digestibility between animals with a low and a high dry matter intake for OMD, CPD, ADF and CV of NDF. Mean OMD and CPD in early lactation was significantly higher for animals with a lower DMI. Mean ADFD and intra-individual CV of NDFD were significantly higher for animals having on average higher DMI during early lactation. Furthermore, the results indicate differences in fiber digestibility between animals, and challenge the assumption of uniform fiber digestibility for all animals fed the same diet.

4.5. Conclusions

The results of this study show a large variation in intake behavior, feed intake and digestibility as well as interactions between these parameters in early lactating dairy cows. The greater the variation in intake behavior and DMI, the greater the variation in nutrient supply and the proportion of animals that are not adequately supplied with energy and nutrients. Because feed intake and feeding behavior appear to be subject to strong individual influence, it is necessary to consider the interaction at the individual animal level rather than inferring potential relationships via averaged group values in order to reduce the individual animal deficits, especially at the beginning of lactation. The described variation in feed intake behavior shows that animals of the same breed, at the same stage of lactation and in the same environment follow different strategies for nutrient and energy intake. The individuality of the feed intake behavior conflicts with herd-based efforts of predicting feed intake. To achieve a sustainable increase in feed intake, and hence nutrient availability, of individual animals, and thus of the herd in the long term, feed conditions must be created that allow for different intake strategies. This includes not only sufficient space at and good access to the feed table, but also a balanced diet and good feeding management. Technical developments that directly assess an individual animal's DMI should be further advanced to enable better future decisions regarding the supply situation of dairy cows. Only by individually increasing the availability or adjusting the output for each animal can long-term improvement in the health status of early lactating cows be achieved.

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4.7. Abbreviations

ADF	acid detergent fiber
ADFD	digestible acid detergent fiber
aNDFom	neutral detergent fiber assayed with heat-stable amylase and expressed exclusive of residual ash
ANOVA	analysis of variance
CP	crude protein
CPD	digestible crude protein
CV	coefficient of variation
CVAS	Cumberland Valley Analytical Services Inc.
DM	dry matter
DMI	dry matter intake
EE	ether extract
ESC	ethanol-soluble carbohydrates
GLM	generalized linear models

iNDF240	240 h in vitro indigestible neutral detergent fiber
LMM	linear mixed model
NDFD	digestible neutral detergent fiber
NEL	net energy for lactation
NIRS	near infrared reflectance spectroscopy
OM	organic matter
OMD	digestible organic matter
RIC	roughage Intake Control
RSE	relative standard error
SD	standard deviation
SP	soluble protein
TDN	total digestibly nutrient
TMR	totally mixed ration

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5. III. Balancing trade-offs in milk production by making use of animal individual energy balancing

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Abstract

Traditionally, energy supply of dairy cows is based on the average performance of the herd. Because this contradicts the great variation in requirements between individual animals, the objective of the present study was to quantify both the extent and consequences of variation in the relevant sub-variables used to calculate the energy balance (EB) on an individual animal basis. Total energy supply (TES) and requirements (TER) of 28 multiparous German Holstein dairy cows fed TMR with 7.0 MJ NEL were studied between the 2nd and 15th week after calving. TES, mainly influenced by DMI, increased from 100.1 (week 2) to 152.1 MJ NEL/d (week 15; $p < 0.01$). Weekly coefficients of variation (CV) ranged between 0.10 and 0.16 and were similar to CV of DMI (0.09 to 0.17). TER, as the sum of energy requirement for maintenance (body weight) and production (milk yield) decreased from 174.8 (week 2) to 164.5 MJ NEL/d (week 15; $p < 0.01$) and CV varied between 0.16 (week 2) and 0.07 (week 11). EB increased from -74.8 (week 2) to -12.4 MJ NEL/d (week 15; $p < 0.01$) and CV varied from 0.32 (week 3) to 1.01 (week 10). The results indicate that calculating EB on an individual animal basis is a prerequisite to identify animals with an increased risk of failing to cope with their energy situation, which cause failure costs that drain the profit of affected cows.

Keywords: variation; dairy cows; digestibility; energy supply; energy requirement; energy balance

5.1. Introduction

The average milk production per cow and year has continuously increased over the past decades (Hansen, 2000). The reasons are multifactorial and can be attributed not only to breeding for a high milk yield but also to improvements in feeding regimes and increased management efforts (VandeHaar and St-Pierre, 2006; Barkema et al., 2015). An increase in milk yield is equivalent with an increase in energy requirements for milk production. Daily requirements for glucose, amino acids and fatty acids during this period are, for example, respectively more than 2.7, 2.0 and 4.5 times higher than the uterine requirements during the last third of gestation (Bell, 1995). However, energy intake does not increase at the same rate, particularly in the first weeks after calving (NRC, 2001; Souza et al., 2019). The gap between energy requirements and supply causes a metabolic challenge for the dairy cow. Despite the increase in animal body mass and girth associated with breeding for higher milk yield, it is not possible for dairy cows to absorb the amount of energy from feed that is required for maintenance of body functions and milk production. In addition, with each increase in body size, body mass and milk yield, energy requirements continue to increase and the gap between energy requirements and supply continues to widen (Bauman and Currie, 1980; Hansen, 2000; Oltenacu and Broom, 2010). This inevitably leads to a massive energy deficit or negative energy balance (NEB) and an enhanced need for metabolic regulation by the cow (Sundrum, 2015). If sufficient energy can no longer be consumed to meet the demand, the body mobilizes body fat reserves, resulting in increased lipolysis (Sordillo and Raphael, 2013). The continually increasing hepatic uptake of non-esterified fatty acids (NEFA) from the blood can lead to the development of fatty liver which in turn can have far-reaching negative consequences on overall body functioning (Knight et al., 1999; Collard et al., 2000; Sundrum, 2015). An increase of NEB is associated with a decrease in reproductive performance (Butler and Smith, 1989; Nebel and McGilliard, 1993; Domecq et al., 1997) and claw health (Collard et al., 2000). Furthermore, additional health risks are created by the increase in milk yield because it leads to insufficient glucose content for the maintenance of the immune system due to the inability of intermediary metabolism to provide sufficient glucose to meet the simultaneous needs of mammary and immune cells (Habel and Sundrum, 2020). Metabolic disorders indicate an excessive overload of the adaptive capacity (Sundrum, 2015). This increases the risk of involuntary culling and of economic losses (Langford and Stott, 2012; Habel et al., 2021b). These losses are high in the case of a short useful lifespan since the costs incurred for rearing, keeping and feeding have usually not yet been recovered by the animal. Due to the economic implications, knowledge and control of the animal- individual energy balance is one of the most important tasks in dairy farming (van Soest et al., 2019).

Energy balance (EB) is calculated as the energy ingested through feed, ideally corrected for the animal-individual level of digestibility, minus the energy required to maintain body functions, milk production and pregnancy. Therefore, EB is a calculated variable consisting of several sub-variables. The sub-variables can vary over time within one animal and between individual animals of the same genotype or in the same stage of lactation due to reinforcing, but nevertheless partly opposing, biological processes (Beerda et al., 2007). For example, an increase in milk yield leads to an increase in feed intake (Roseler et al., 1997; Martin and Sauvant, 2002; Hristov et al., 2005) but feed digestibility decreases as feed intake increases (Thornton and Minson, 1973; Mertens and Grant, 2020). In the past, many studies have addressed the magnitude of energy deficit at the beginning of lactation and its effects on animal health. However, the implications of the variation within and between sub-variables on EB are often unknown or faded out when using prediction equations or table values to formulate feeding rations (Jorritsma et al., 2003). Therefore, the objective of the present study was to quantify the extent of intra- and interindividual variation in the relevant sub-variables and its effects on the intra- and interindividual variation of EB.

5.2. Materials and Methods

All procedures described in this study were performed according to the German Animal Welfare Act and approved by the local authority for animal welfare affairs (Landesuntersuchungsamt Rheinland-Pfalz; G 18-20-073) in Koblenz, Germany.

5.2.1. Animals, Housing and Diet

The study was carried out between April and October 2018 at the Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, Muenchweiler a.d. Alsenz, Germany. Twenty-eight German Holstein dairy cows, ranging from the second to eighth parity (mean = 2.9; SD = 1.3), and between the second and fifteenth week of lactation were used.

The cows were housed together with non-experimental cows in a free-stall barn with 60 cubicles and 30 feeding units. Cows had unlimited access to fresh water and were fed a total mixed ration (TMR) ad libitum (**Table 5.12**). The TMR was prepared in the morning and delivered twice daily (60% of total daily amount at 6 am and 40% of total daily amount at 11 am).

Table 5.12. Ingredient and chemical composition, and energy content of the total mixed ration.

Diet Composition (g/kg) ^{a}		**Chemical Composition** (g/kg) ^{b}		
			Mean	SD
Beet pressed pulp silage	188.3	Dry matter	402.0	13.8
Grass silage	97.0			
Grass hay	74.7	OM	931.4	3.8
Maize silage	259.6	CP	157.4	8.1
Concentrate	380.4	SP	63.2	7.3
		EE	43.0	2.4
		aNDFom	355.1	8.8
		ADF	219.3	4.9
		Lignin	28.3	1.2
		iNDF$_{240}$	86.4	4.5
		Starch	182.3	14.8
		ESC	63.1	3.4
		TDN^{c}	732.0	5.0
		Energy (MJ/kg DM)		
		NEL^{d}	7.0	0.0

[a,b] Diet and chemical composition reported on 105°C dry matter. Averaged values based on weekly conducted feed analysis; diet was offered as TMR. ADF, acid detergent fiber, expressed inclusive of residual ash; CP, crude protein; EE, ether extract; ESC, ethanol-soluble carbohydrates; iNDF$_{240}$, indigestible aNDFom; OM, organic matter; SD, standard deviation; SP, soluble protein.

[c] TDN (g/kg), Total digestibly nutrient values for TMR samples were calculated from the TDN value using Equations 2-5 by (NRC, 2001).

[d] NEL for TMR samples were calculated from the TDN value using Equations 2-3 by (NRC, 2001)

5.2.2. Data and Sample Collection

Individual feed intake was measured daily with Insentec B.V. (Marknesse, the Netherlands) RIC (Roughage Intake Control) automatic weighing system. Cows were identified using individual collar transponders which provided access to the feeding unit. TMR intake per visit was calculated from the differences in trough weight between start and end of the visit. The daily dry matter intake (DMI) per cow was calculated by adding the recorded daily amount of fresh matter intake and multiplying the result by the diet DM content and averaged over one week. Body weight of all cows was measured daily following the AM and PM milking using weighing scales and averaged over one week. Due to a technical error, no weights could be recorded over a period of 6 weeks. The missing data were linear interpolated using the 'linear trend at point' method in the Replace Missing Values command of SPSS 25.0 software (IBM Company Inc., Chicago, IL, USA). Cows were milked twice daily between 5.00 and 7.30 am and between 3.30 and 6.00 pm. The daily milk yield was recorded electronically via the herd management system Dairy Plan C21 (GEA Farm Technologies, Boenen, Germany). Milk aliquots from one evening and the next morning were taken biweekly and pooled for further analysis of milk fat, protein and lactose by infrared spectrophotometry using a MilkoScan FT6000 (Foss Analytical A/S, Hillerød, Denmark). TMR samples were taken daily within one hour of feed delivery. Daily samples were combined on a weekly basis with a representative TMR sample of 800 - 1000 g for determining the weekly dry matter content. Starting at the day of calving, individual cow fecal samples were collected weekly two hours after morning milking via rectal palpation. The samples were labelled and stored frozen at -20 °C until chemical analysis. Deviations in fecal content within a day, especially in aNDFom and iNDF240, were reduced by defining a fixed time of feeding and sampling which remained constant over the test period.

5.2.3. Chemical Analysis

Dry matter (DM) content was determined in a two-step process; thawed TMR and feces samples were first oven dried at 60 °C for 48 hours and ground to 1 mm particle size. This was followed by drying at 105 °C for 3 hours until constant weight was achieved. Organic matter (OM) was measured by ashing (550 °C) overnight. The dried and ground samples were submitted to Cumberland Valley Analytical Services Inc., Waynesboro, PA (CVAS) for chemical analysis.

All TMR samples were analyzed for DM, OM (method 942.05; (AOAC International, 2006), CP (method 990.03; (AOAC International, 2006), SP (Krishnamoorthy et al., 1982), EE (method 2003.05; (AOAC International, 2006)), aNDFom using α-amylase and expressed exclusive of residual ash (van Soest et al., 1991), ADF (expressed inclusive of residual ash, method 978.10; (AOAC International, 2006)), lignin (method 973.18; (AOAC International, 2006)), ethanol soluble carbohydrates (ESC), starch (Hall, 2009) and $iNDF_{240}$ (Raffrenato, 2011). Dried and ground fecal samples were split into two subsamples. One subsample was analyzed with near infrared reflectance spectroscopy (NIRS) described by Althaus et al. (Althaus et al., 2013). The analysis included DM, OM and CP. The other subsample was submitted to CVAS for determination of ADF, aNDFom and $iNDF_{240}$.

5.2.4. Calculations and Statistical Analysis

For comparison, DMI was predicted with the Gruber Model 5, which is a standard model for TMR without hay (Gruber et al., 2004). The prediction model is based on days in milk (DIM), the effect of country and breed, parity, body weight (BW) and milk yield (MY). In the present study, mean values of the animals in the different stages of lactation were used. The diet characteristics used in the model were the proportion of concentrate in the mixed ration (cDMI%; %concentrate of the mixed ration; DM/day) and the net energy (NE) value of forage (NELf; MJNE/kg DM) (Jensen et al., 2015). Organic matter digestibility was calculated from $iNDF_{240}$ as internal marker and nutrient concentrations in TMR and feces using the following equation by (Schalla et al., 2012):

$$\text{Organic matter digestibility (g/kg)} = 100 - \{100 \times (\text{TMR iNDF}_{240} / \text{fecal iNDF}_{240}) \times [\text{fecal OM content (\% of DM)}/\text{TMR OM content (\% of DM)}]\} \quad (1)$$

The energy corrected milk yield (ECM) was calculated as follows (Spiekers and Potthast, 2004):

$$\text{ECM (kg/day)} = \text{milk yield (kg/d)} \cdot \{([0.38 \cdot \text{fat (\%)} + 0.21\ \text{protein (\%)}] + 1.05)/3.28\} \quad (2)$$

ECM was calculated with the daily milk yield and the fat and protein content from the milk check. The daily ECM was averaged over one week. EB was calculated from the total energy supply (TES), the energy requirement for maintenance (ERM) and the energy requirement for production (ERP) of ECM as proposed by GfE (2001). Energy requirement for the production of 1 kg ECM was calculated with 3.28 MJ NEL (GfE, 2001):

$$\text{TES (MJ NEL/d)} = \text{DMI (kg)} \times \text{MJ NEL / kg DM} \quad (3)$$

$$\text{ERM } (MJ\ \text{NEL/d}) = 0.293 \times BW\ 0.75\ (kg) \quad (4)$$

$$\text{ERP } (MJ\ \text{NEL/d}) = \text{ECM (kg)} \times 3.28\ \text{(MJ NEL)} \quad (5)$$

$$\text{TER (MJ NEL/d)} = \text{ERM (MJ NEL/d)} + \text{ERP (MJ NEL/d)} \quad (6)$$

$$\text{EB (MJ NEL/d)} = \text{TES (MJ NEL/d)} - \text{ERM (MJ NEL/d)} - \text{ERP (MJ NEL/d)} \quad (7)$$

Data was analyzed using the SPSS 25.0 software (IBM Company Inc., Chicago, IL, USA). The mean, range, standard deviation (SD) and coefficient of variation (CV), calculated as ratio of standard deviation to mean, were calculated. Each variable was checked for normal distribution by a histogram and a Q-Q plot. Changes of each parameter for all cows over the course of the study are shown by box plots which represent the median, interquartile range and extreme cases of individual variables. The individual comparisons were performed using post-hoc pairwise comparisons, with the Sidak correction applied, to determine which of the comparisons were significant. When the Mauchly's test indicated that the assumption of sphericity had been violated, the degrees of freedom were corrected using Greenhouse-Geisser estimates if the estimate was lower than 0.75 or the Huynh-Feld estimate if the estimate was greater than 0.75 (Girden, 2003). The effect sizes for main effects and interactions were determined by Partial eta squared (η^2) values.

Partial eta squared (η^2) values were classified as small (0.01 to 0.059), moderate (0.06 to 0.137) and large (> 0.137). Differences were considered significant at a level of $P \leq 0.05$, and a tendency was considered at $0.05 < P \leq 0.10$.

Cows were retrospectively grouped in quartiles based on average energy balance between week 2 and 15 of lactation. The formation of groups using such quartiles has the advantage of separating cows with high differences in EB more precisely. Groups were defined as low, intermediate and upper quartile of mean energy balance. Performance data was analyzed using ANOVA, with quartiles as fixed factor. The individual quartile comparisons were performed using post-hoc pairwise comparisons, with the Sidak correction applied. Further, linear regressions were performed to analyze the impact of variation in the different sub-variables on the variation of EB.

5.3. Results

5.3.1. Variation of energy supply parameter

The energy content of the offered total mixed rations during the trial period was constant at 7,0 ± 0.0 MJ NEL. DMI significantly increased with a linear ($P < 0.001$; $\eta 2 = 0.75$) and quadratic ($P < 0.001$; $\eta 2 = 0.677$) trend from a mean value of 14.3 ± 2.3 kg DMI per day in week 2 up to 22.1 ± 2.3 kg in week 11. Thereafter, DMI varied until the 15th week of lactation between 21.2 und 21.7 kg DMI. Weekly CV values of DMI ranged between 0.09 (week 9) and 0.17 (week 2). Mean individual cow DMI (**Figure 5.10**) between week 2 and 15 of lactation ranged between 16.8 ± 2.5 kg and 24.5 ± 1.3 kg DMI per day and was significantly different between animals ($P = 0.00$; $\eta 2 = 0.669$). The CV of DMI for individual animals over the trial period ranged between 0.05 and 0.25.

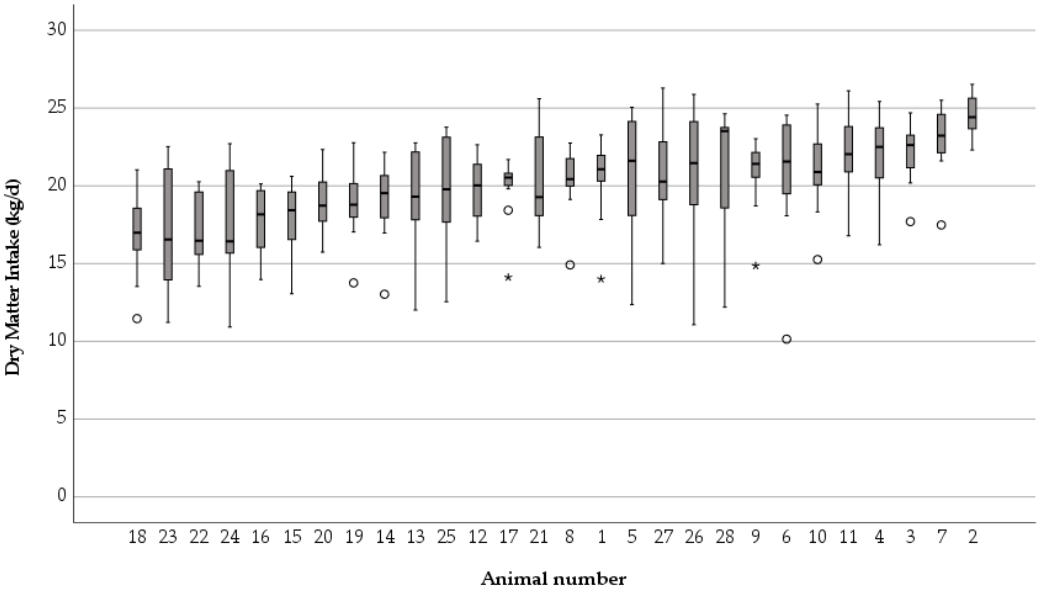

Figure 5.10. Dry matter intake (kg/day) measured between week 2 and 15 postpartum (p.p.). In each subfigure, the boxplots respectively highlight the median, upper and lower quartiles of each week. Box whiskers extend to the most extreme nonoutlier data points above and below the box; data points are considered outliers if they lie more than 1.5 times the interquartile range from the edge of the box. Small circles = outliers (1.5 x interquartile range (IQR)); asterisks = extreme values (3 x IQR).

By comparing the predicted DMI with the measured DMI, differences up to 6.7 kg DMI/d could be detected (**Table 5.13**). In addition, the measured DMI showed a high variation with CV values up to 0.15 and wide ranges between minimum (min) and maximum (max) DMI during the different stages of lactation.

Table 5.13. Predicted DMI vs. measured DMI during different stage of early lactation (n = 28)

	up to 4th week of lactation	up to 8th week of lactation	up to12th week of lactation	up to 15th week of lactation
Intercept	2.274	2.274	2.274	2.274
country x breed	HF h (GER+AT)[d]	HF h (GER+AT)	HF h (GER+AT)	HF h (GER+AT)
lactation	2-3	2-3	2-3	2-3
DIM[a]	28	56	84	105
BW[b]	667.16	663.40	664.13	663.33
MY[c]	42.66	47.50	46.82	44.72
Concentrate proportion	38.10	38.10	38.10	38.10
NEL value of forage	6.80	6.80	6.80	6.80
Predicted DMI[e]	**23.49**	**25.52**	**26.01**	**25.77**
Mean measured DMI	16.79	20.13	21.72	21.39
SD measured DMI	2.60	2.51	2.34	2.71
CV measured DMI	0.15	0.12	0.11	0.13
Mean max DMI	22.12	24.89	25.79	25.77
Mean min DMI	11.27	15.23	16.94	15.07
Difference mean predicted and measured DMI	6.70	5.39	4.29	4.38

[a] DIM, days in milk

[b] BW, body weight

[c] MY, milk yield

[d] HF h (GER+AT), Holstein Frisian breed on a high management level, in Germany GER) and Austria (AT)

[e] DMI, dry matter intake

Organic matter digestibility (OMD) during the trial period varied between 738.6 g/kg (week 2) and 725.1 g/kg (week 8). Weekly CV values of OMD ranged between 0.02 and 0.03. Mean individual cow OMD ranged between 712.6 ± 17.7 and 744.2 ± 16.5 g OM/ kg DM and it was also significantly different between animals ($P = 0.00$; $\eta 2 = 0.275$). The CV of individual animal OMD ranged between 0.02 and 0.04. Total energy supply (TES) increased with a linear ($P < 0.001$; $\eta 2 = 0.753$) and quadratic ($P < 0.001$; $\eta 2 = 0.674$) trend from a mean value of 100.1 ± 15.8 MJ NEL / day in week 2 up to 152.1 ± 15.4 MJ NEL / day in week 15. Weekly CV values of TES ranged between 0.10 (week 9, 11 and 15) and 0.16 (week 2, 3, 14).

Mean individual cow TES (**Figure 5.11**) varied between 117.3 ± 17.9 and 171.1 ± 10.1 MJ NEL per day (P = 0.00; η2 = 0.669) and the CV ranged between 0.06 and 0.25.

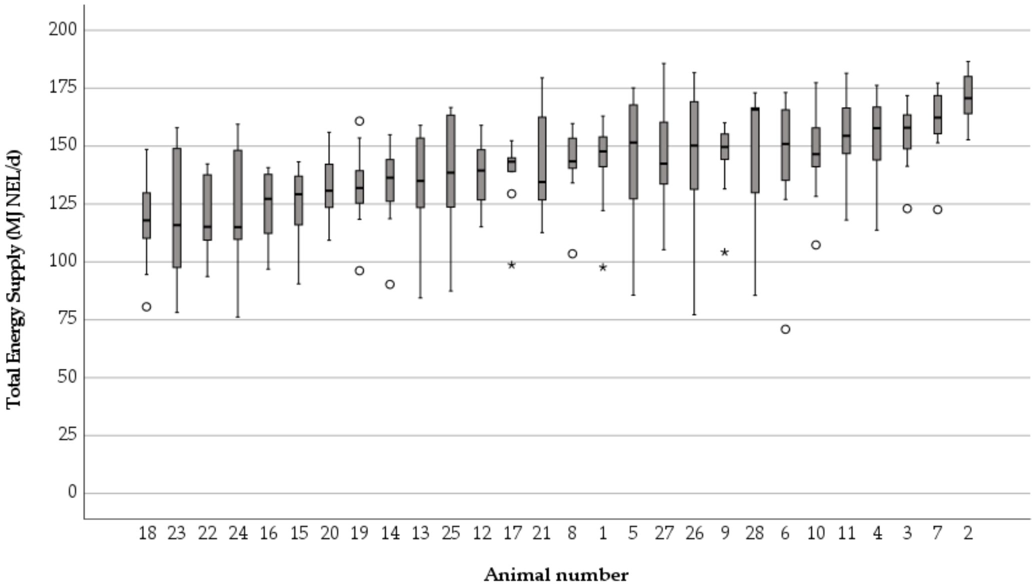

Figure 5.11. Total energy supply (MJ NEL/d) calculated between week 2 and 15 postpartum (p.p.). In each subfigure, the boxplots respectively highlight the median, upper and lower quartiles of each week. Box whiskers extend to the most extreme nonoutlier data points are considered outliers if they lie more than 1.5 times the interquartile range from the edge of the box. Small circles = outliers (1.5 x interquartile range (IQR)); asterisks = extreme values (3 x IQR).

5.3.2. Variation of energy requirement parameters

Body weight decreased from 669.7 ± 58.8 kg in week 2 to 659.4 ± 64.1 kg in week 12 and was not significantly different over the trial period. Mean individual cow BW during the trial period ranged between 593.7 ± 11.5 and 818.3 ± 19.7 kg and the CV varied between 0.01 and 0.05. ECM reached a mean value of 42.9 kg ECM and significantly increased with a linear (P < 0.001; η2 = 0.628) trend from a mean value of 43.3 ± 8.8 kg ECM per day in week 2 up to 44.6 ± 7.1 kg in week 4. Thereafter, ECM varied until the 15th week of lactation between 40.3 und 43.9 kg. Weekly CV values of ECM ranged between 0.1 (week 11) and 0.2 (week 2). Mean individual cow ECM between week 2 and 15 of lactation ranged between 32.1 ± 6.4 kg and 53.4 ± 3.4 kg ECM per day (P = 0.00; η2 = 0.740) and the CV of ECM ranged between individual animals from 0.04 to 0.2.

Energy requirement for maintenance (ERM) varied between 38.5 ± 2.6 (week 2) and 38.0 ± 2.8 MJ NEL (week 12). Mean individual ERM ranged between 34.3 ± 0.3 and 44.8 ± 0.8 MJ NEL and CV values varied between 0.01 and 0.04.

Energy requirement for production (ERP) decreased in a linear trend ($P < 0.001$; $\eta2 = 0.633$) from 140.8 ± 21.8 (week 3) to 126.2 ± 14.5 MJ NEL in week 15. Mean individual ERP varied between 100.6 ± 20.0 and 167.6 ± 10.8 MJ NEL between individual cows and CV values ranged between 0.04 and 0.2 (**Figure 5.12**).

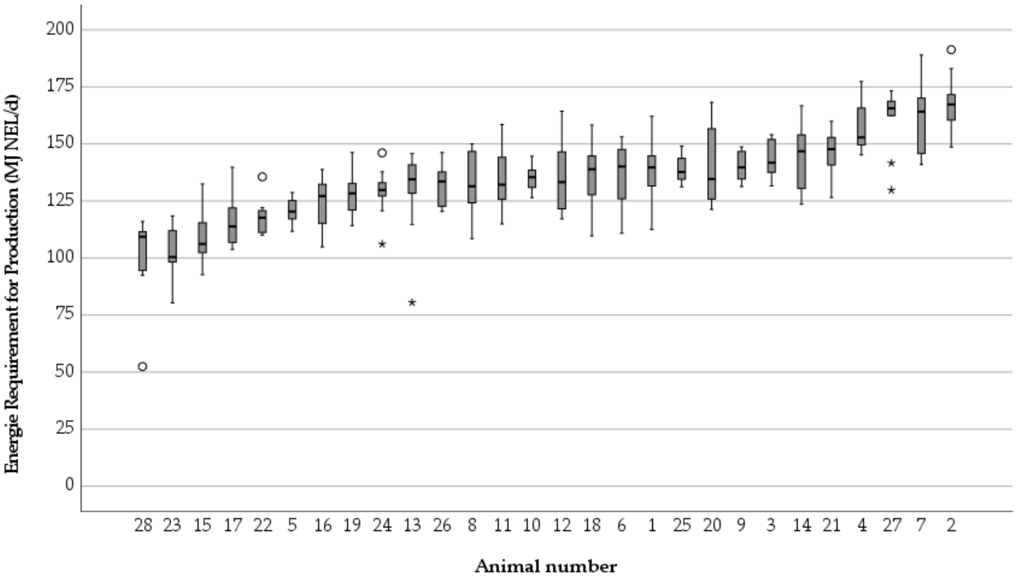

Figure 5.12. Energy requirement for production (MJ NEL/d) calculated between week 2 and 15 postpartum (p.p.). In each subfigure, the boxplots respectively highlight the median, upper and lower quartiles of each week. Box whiskers extend to the most extreme nonoutlier data points are considered outliers if they lie more than 1.5 times the interquartile range from the edge of the box. Small circles = outliers (1.5 x interquartile range (IQR)); asterisks = extreme values (3 x IQR).

Total energy requirement (TER) had a mean of 172,6 MJ NEL/d and decreased in the same way as ERP ($P < 0.001$; $\eta2 = 0.314$) from 174.8 ± 28.0 (week 2) to 164.5 ± 14.4 MJ NEL/d in week 15. CV varied between 0.16 in week 2 and 0.07 in week 11. Mean individual TER varied between 140.9 ± 19.7 and 205,2 ± 10.7 MJ NEL/d between individual cows (**Figure 5.13**). CV values ranged between 0.03 and 0.14. The share of ERM in the TER was 22.4 % on average and varied between a 16.3 % at minimum and 44.0 % at maximum.

In contrast, the share of ERP in TER averaged 77.6 % with a minimum of 56 % and a maximum of 83 %. On average, TER was 1.3 times higher than TES. The minimum TER was 0.9 times and the maximum 2.4 times above the TES. Linear regression showed a strong positive trend for CV of ERP (0.727, P < 0.01), indicating that the variation in TER is essentially influenced by the variation in ERP. Variation in ERM showed no significant effect on variation in TER (-0.057, P = 0.610).

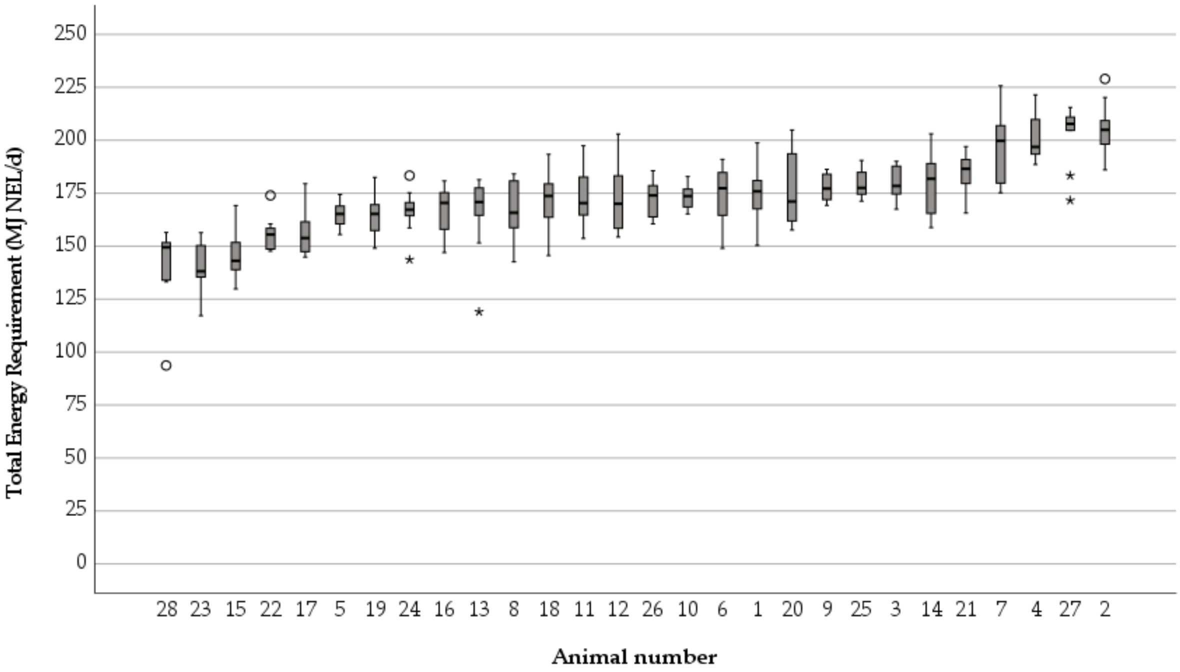

Figure 5.13. Total energy requirement (MJ NEL/d) calculated between week 2 and 15 postpartum (p.p.). In each subfigure, the boxplots respectively highlight the median, upper and lower quartiles of each week. Box whiskers extend to the most extreme nonoutlier data points are considered outliers if they lie more than 1.5 times the interquartile range from the edge of the box. Small circles = outliers (1.5 x interquartile range (IQR)); asterisks = extreme values (3 x IQR).

5.3.3. Variation of energy balance during early lactation

The values of the calculated energy balance (EB) increased with a linear (P < 0.001; η2 = 0.895) and quadratic (P < 0.001; η2 = 0.584) trend from -74.8 ± 24.9 MJ NEL/d in week 2 to -12.4 ± 12.2 MJ NEL/d in week 15 (**Figure 5.14**). CV values varied between 0.32 in week 3 and 1.01 in week 10.

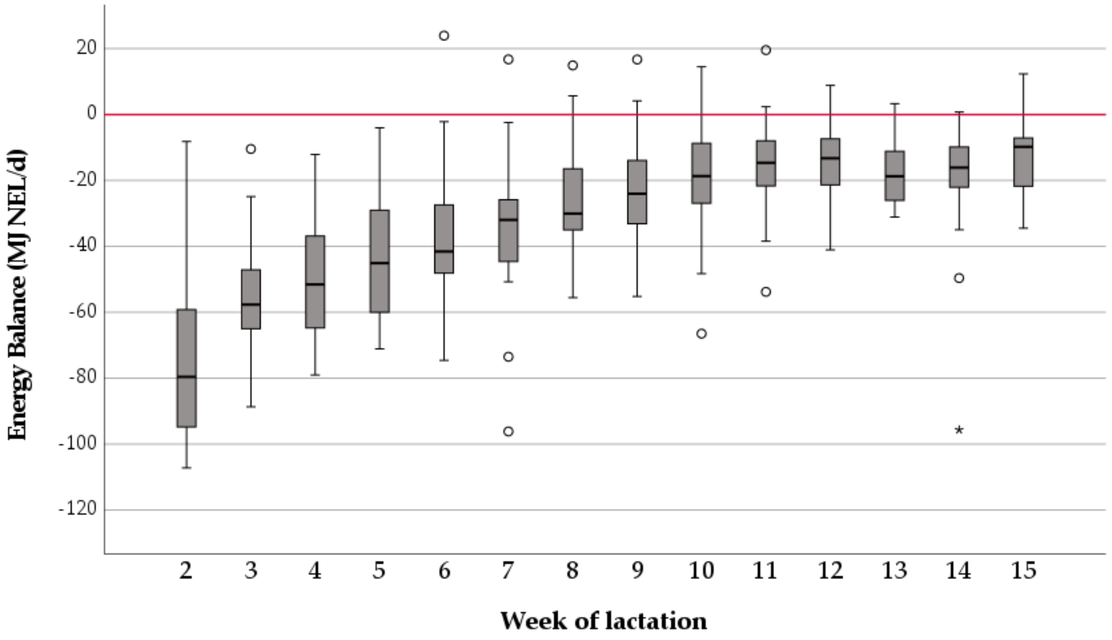

Figure 5.14. Energy balance (MJ NEL/d) of dairy cows (n = 28) between week 2 and 15 postpartum (p.p.). In each subfigure, the boxplots respectively highlight the median, upper and lower quartiles of each week. Box whiskers extend to the most extreme nonoutlier data points are considered outliers if they lie more than 1.5 times the interquartile range from the edge of the box. Small circles = outliers (1.5 x interquartile range (IQR)); asterisks = extreme values (3 x IQR).

Mean individual cow EB ranged between -56,4 ± 13.6 kg and 5.2 ± 14.0 MJ NEL/d ($P = 0.00$; $\eta 2 = 0.675$) as shown in **Figure 5.15**. The CV of EB during trial period ranged from 0.24 and 2.68 between individual animals.

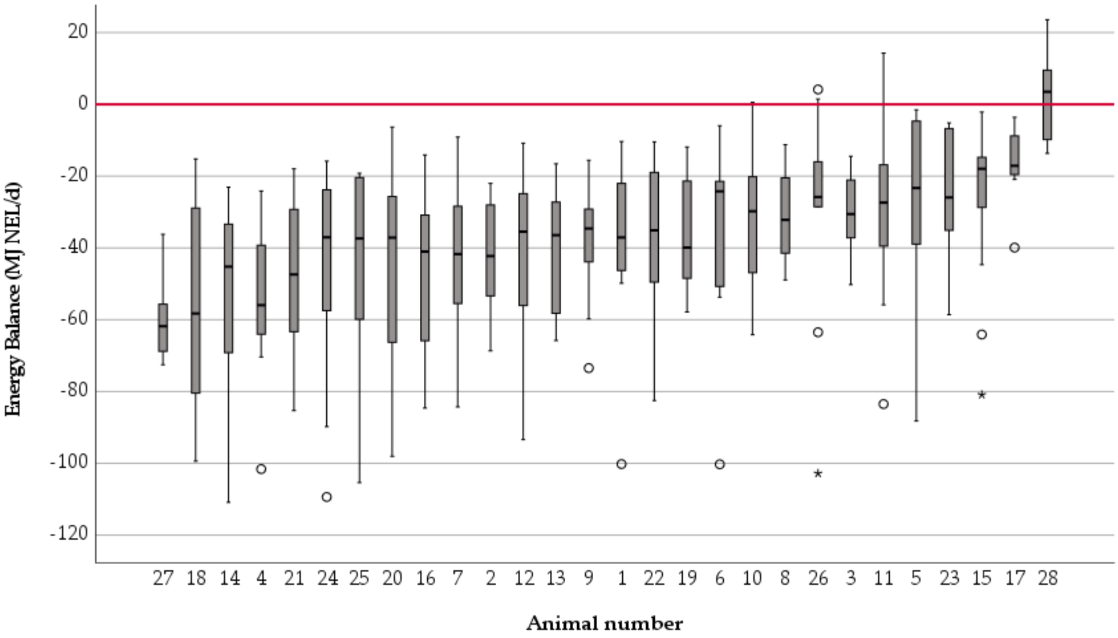

Figure 5.15. Energy balance (MJ NEL/d) calculated between week 2 and 15 postpartum (p.p.). In each subfigure, the boxplots respectively highlight the median, upper and lower quartiles of each week. Box whiskers extend to the most extreme nonoutlier data points are considered outliers if they lie more than 1.5 times the interquartile range from the edge of the box. Small circles = outliers (1.5 x interquartile range (IQR)); asterisks = extreme values (3 x IQR).

Other parameters to describe the extent of NEB in early lactation were the timepoint and the value of the nadir. 71% (n = 20) of the dairy cows reached the nadir in week 2 and 3 with a mean value of -82 MJ NEL/d. 25% (n = 7) reached the nadir between week 4 and 7 with a mean value of – 49 MJ NEL/d. One cow achieved the nadir in week 10 with a value of -13 MJ NEL/d. These values also show a large variation in the course of energy balance in early lactation.

To further analyze the cows with different energy balances, the cows were retrospectively grouped in quartiles based on average energy balance between week 2 and 15 of lactation. Cows with the lowest mean energy balance between week 2 and 15 of lactation reached higher values than cows in the other quartiles in mean milk yield ($P < 0.05$), milk fat content, fat: protein ratio ($P < 0.01$), ECM yield ($P < 0.01$), energy requirement for production ($P < 0.05$) and total energy requirement ($P < 0.05$) but also reached the lowest mean DMI, the lowest energy supply and the lowest energy balance ($P < 0.001$). Milk protein content was highest in the upper quartile ($P < 0.01$) (**Table 5.14**).

Table 5.14. Mean, SD, min and max values of early lactating dairy cows grouped in quartiles according to mean energy balance observed between week 2 and 15 of lactation.

	Quartile of mean energy balance value during early lactation												
	Lower quartile (< 25%)				Intermediate quartile				Upper quartile (> 75%)				
	mean	SD	min	max	mean	SD	min	max	mean	SD	min	max	P
Lact no	3.2	0.8	2.0	4.0	2.9	1.6	2.0	8.0	3.0	1.1	2.0	5.0	
Milk yield (kg/d)	47.8^{a}	5.2	35.0	56.5	46.7^{ab}	5.9	25.4	61.0	41.9^{b}	4.8	19.0	50.4	*
Milk fat (%)	3.9	0.9	2.5	6.2	3.7	0.7	2.1	5.8	3.6	0.7	2.4	6.4	
Milk protein (%)	3.0^{b}	0.3	2.6	3.7	2.9^{b}	0.2	2.5	3.9	3.2^{a}	0.2	2.8	3.9	**
Ratio fat:protein	1.3^{a}	0.3	0.9	2.4	1.2^{ab}	0.2	0.8	1.7	1.1^{b}	0.2	0.7	1.7	**
ECM (kg/d)	45.8^{a}	5.2	34.0	56.5	43.5^{ab}	6.0	25.6	60.8	39.4^{b}	5.6	16.7	50.4	*
Body weight (kg/d)	669.1	76.1	574.5	801.4	653.7	43.6	565.6	781.2	682.1	69.2	604.5	841.9	
DMI (kg/d)	19.2	3.2	10.9	26.3	20.2	3.3	10.1	26.5	20.6	3.0	12.2	26.1	
OMD(g/kg)	731.0	21.3	681.9	774.8	728.5	19.6	667.3	786.6	727.2	19.0	679.2	787.6	
TES (MJ NEL/d)	134.4	22.9	76.2	185.7	141.6	23.0	70.9	186.6	144.0	20.7	85.6	181.5	
ES as a multiple of ERmain	3.2	0.5	1.9	4.1	3.4	0.6	1.7	4.7	3.4	0.5	1.8	4.3	
ERmain (MJ NEL/d)	38.5	3.3	34.4	44.1	37.9	1.9	34.0	43.3	38.7	3.3	33.7	45.8	
ERpro (MJ NEL/d)	143.7^{a}	16.3	106.1	177.4	136.3^{ab}	18.9	80.4	191.3	123.5^{b}	17.8	52.5	158.5	*
TER (MJ NEL/d)	182.2^{a}	18.1	143.7	221.5	174.2^{ab}	18.6	117.2	229.0	162.2^{b}	17.0	93.7	197.5	*
EB (MJ NEL/d)	-47.6^{c}	26.0	-107.2	-7.1	-32.8^{b}	21.5	-101.4	10.7	-18.2^{a}	21.7	-84.5	23.9	***

Linear regression between sub-variables and EB showed a strong positive trend for CV of ECM ($P < 0.01$), indicating that the variation in energy balance is essentially influenced by the variation in milk yield. Variation in DMI or OMD showed no significant effect on variation in EB (**Table 5.15**).

Table 5.15. The effect of variation in sub-variables on variation in energy balance (EB) (n=28).

Variable	Estimate	SE[a]	P
Intercept	-1.498	0.631	0.026
CV DMI[b]	0.382	2.297	0.869
CV OMD[c]	-9.015	18.202	0.625
CV ECM[d]	12.444	3.468	0.001
R^2	0.375		

[a] Standard error

[b] Coefficient of variation of dry matter intake

[c] Coefficient of variation of organic matter digestibility

[d] Coefficient of variation of energy corrected milk

5.4. Discussion

In the conventional approach, energy balance is often deduced from a modeled curve for an average cow in a group or herd (DeVries and Veerkamp, 2000), with individual animals in a group or herd usually deviating from this virtual cow. However, neither deviations from the average nor variations in sub-variables and the corresponding EB between individual animals and over time are usually unconsidered (Jorritsma et al., 2003).

5.4.1. Variation in energy supply variables

Feed intake, defined as DMI per day, is the most important component of nutrient and energy intake in dairy cows. During the study, DMI increased from a mean of 14.3 ± 2.3 kg in week 2 to 22.1 ± 2.3 kg in week 11. This course was similar to the course described by Kessel et al. (2008). The mean between-cow CV in DMI during the trial period was 0.13, with a range between 0.09 (week 9) and 0.17 (week 2). The mean CV was similar to the between-cow CV value of 0.14 we estimated from the meta data set of Huhtanen et al. (2015) and slightly lower than the CV value of 0.18 we estimated from the meta data set of Cabezas-Garcia et al. (2017) and the CV of 0.20 between day 5 and 100 of lactation reported in Collard et al. (2000).

Besides the large variation between dairy cows, there was a large variation in DMI with CV values between 0.05 and 0.25 within individual cows during early lactation. This makes an accurate prediction of DMI for individual animals nearly impossible. Although the variation in DMI has been often described, it has not yet been considered in the assessment of energy balance. The large variations in DMI can be explained by interactions between a variety of influencing factors.

Besides the factors of feed composition, environment and management, which were the same for all animals in this study, DMI is affected by the number of meals consumed per day, the length of meals and the rate of eating during meals as well as by different mechanisms which may affect total daily DMI between cows (Dado and Allen, 1994; Grant and Albright, 2000). These can be roughly divided into physical (e.g. rumen filling), endocrine (e.g. gut peptides) and metabolic (e.g. oxidation of fuels) regulatory mechanisms that additively decide when to start or stop a meal (Dulphy and Demarquilly, 1994; Allen and Piantoni, 2013). However, social interaction and possible social stress due to ranking as well as health status and milk yield also influence DMI levels (Dado and Allen, 1994; Allen and Piantoni, 2013).

Differences of up to 6.7 kg DMI/d were found when comparing predicted DMI to measured DMI (**Table 5.13**). Although the accuracy of the Gruber model is high compared to other estimating equations (Jensen et al., 2015), the results once again show that estimating animal-specific feed intake based on herd average values is not very reliable. This applies even more given the dynamic developments within individual animals during early lactation.

Despite the difference in the feeding ration compared to other studies, the mean value of OM (729 g/kg DM) was only slightly lower than the mean value of Cabezas-Garcia et al. (2017), who reported a mean value for OM of 740 g/kg DM and very similar to the OM digestibility value of 728 g/kg presented in a meta-analysis by Huhtanen et al. (2010) and also to the TDN value of the TMR (732 g/kg). The mean between-cow CV of OMD during the trial period was 0.03, with a range between 0.02 and 0.03. In recent meta-analyses, lower CV values of OMD of 0.014 (Guinguina et al., 2020) and 0.013 (Cabezas-Garcia et al., 2017) were reported. The results demonstrate that there is comparably little variability among early-lactating dairy cows in their ability to digest a given diet. The individual cow CV was also low with a range between 0.02 and 0.04.

To facilitate comparability with the common method of EB calculation, total energy supply (TES) was calculated by multiplying DMI with the NEL value calculated with the TDN value of the TMR instead of the animal individual OMD. This is the reason why similar variations in TES and DMI were observed within and between animals.

During the first weeks after calving, when variations in DMI and TES between cows are especially relatively large, DMI and TES values gained from estimation formulars lack accuracy. Only by focusing on the individual animal and considering the animal-specific variation is it possible to obtain a more accurate assessment of the energy supply of the animals. However, suitable technologies, especially for recording animal specific DMI, are not currently available in practice. At the same time, several projects are working on a way to measure feed intake (Bezen et al., 2020). The above-mentioned alternative calculations by means of estimation equations are currently not accurate enough at the individual animal level and require a high additional effort, which is in practice not accepted due to the limited possibilities of direct intervention, e.g., if the DMI is too low.

5.4.2. Variation in energy requirement variables

Due to the early stage of lactation and the fact that only multi-lactating cows were considered in this study, only energy requirements for maintenance and performance and not for growth or pregnancy were considered with regard to energy output. Compared to our study, Poncheki et al. (2015) reported a similar mean body weight for multiparous dairy cows at calving of 676.7 kg but a higher loss in body weight of up to more than 50 kg. Gross et al. (2011) also described a greater body weight loss of around 56 ± 4 kg in the first weeks after calving, which is a response to NEB. Due to the comparatively small changes in body weight, the maintenance requirements only fluctuated moderately between 38.5 ± 2.6 (week 2) and 38.0 ± 2.8 MJ NEL (week 12) but high variation with CV values between 0.01 and 0.04 could be detected. Being only an average of 24% of total energy requirements, maintenance energy requirements played a rather subordinate role in the variables influencing the energy balance. Furthermore, they can be well determined, and changes can be readily identified in practice by regular animal weighing.

Milk production was high with a mean value of 45.9 ± 6.2 kg/d and was similar to values in other experiments in which cows were fed a diet with 160 g/ kg CP and 7.0 MJ NEL/kg DM during early lactation (Komaragiri and Erdman, 1997). Azizi et al. (2009) also reported similarly high ECM yields during early lactation in multiparous dairy cows with a mean of 44.5 kg/d with a similar level of energy concentration in the rations (6.99 MJ NEL/kg DM). Weekly CV values of EM ranged between 0.1 (week 11) and 0.2 (week 2). Besides the large variation between dairy cows, there was a large variation in ECM with CV values between 0.05 and 0.25 within individual cows during early lactation, which also resulted in a large variation in energy requirement for production. Furthermore, the variation in ECM showed a significant influence on the variation in EB. DeVries and Veerkamp (2000) and McParland et al. (2015) also reported a negative genetic correlation (-0.29) between milk yield and predicted EB. In contrast to the energy requirement for maintenance, the energy requirement for production is much higher. It accounts for nearly 78% of total energy requirement, and is thus critical to the level of energy balance. The level and variation of energy requirements for maintenance and production resulted in a wide variation of total energy requirements between animals, especially at the beginning of lactation.

However, total energy requirements of individual animals also varied during the experimental period with CV values ranging from 0.03 to 0.14. At the minimum, TER averaged 140.9 ± 19.7 and at the maximum, 205.2 ± 10.7 MJ NEL/d. This range shows that it is almost impossible to compare energy requirements of animals kept and fed under exactly the same conditions and at the same stage of lactation. But because McNamara (2015) also describes a variation in energy requirements for cows kept under similar conditions, the observed variation between individual cows can be considered as usual in herds. Because of the huge variation and the importance of the amount of energy requirement, it is necessary to control the sub-variables of energy requirement. To control energy requirement for production, milk quantity and quality records can already be used in practice to regularly collect data, map lactation curves and identify changes in energy requirement. In addition to recording live weight and calculating the energy requirement for maintenance, this data also allows a comparatively good estimate of energy requirements for production. However, because of a lack of suitable processing and linking of the data, as well as suitable animal-specific instructions for action, the step of calculating energy requirements is currently still mostly omitted in practice. The potential further development of on-farm technical hardware and software a first could be done to record the level of animal-specific energy supply and reconcile it with the demand.

5.4.3. Variation in energy balance

Due to the exact recording of the sub-variables and the realizable comparison of supply and demand, it was possible to map the animal-specific energy balance during lactation. The values of the calculated energy balance (EB) increased during the experimental period from -74.8 ± 24.9 MJ NEL/d in week 2 to -12.4 ± 12.2 MJ NEL/d in week 15. Variation in the variables resulted in an increase in the CV of EB. The CV varied from 0.32 at week 3 to 1.01 at week 10. Mean individual cow EB ranged from -56.4 ± 13.6 kg to +5.2 ± 14.0 MJ NEL/d (P = 0.00; η2 = 0.675). The CV of EB during the experimental period ranged from 0.24 to 2.68 between animals. This corresponds with results obtained by Beerda et al. (2007), Kessel et al. (2008) and Jorritsma et al. (2003), who reported a large energy deficit and a large variation between animals during this period. 71 % of the trial cows reached the nadir in week 2 and 3 with a mean value of -82 MJ NEL/d. These results are similar to the course observed by DeVries et al. (1999) and Jorritsma et al. (2003), who described a timeframe between 2.5- and 12-days p.p. in which most cows reached the lowest of all EB values. Furthermore, the study revealed a wide variation in the depth of the nadir between individuals. In a study investigating the onset of luteal activity in association with NEB, DeVries and Veerkamp (2000) also reported a wide variation in the depth of the nadir between individuals. In a further study, the balance between energy supply and energy requirement was on average attained at approximately week 10 p.p. (72 d p.p.) (1999). Other studies reported an achievement of positive EB on average in week 6 p.p. (day 41.5 (DeVries and Veerkamp, 2000) and 45 (Grummer and Rastani, 2003)), with a range of one to 15 weeks (7 and 105 days), respectively and a standard deviation of 3 weeks (21 days). In the present study only 25 % (n = 7) reached a positive energy balance by week 15 p.p., indicating a comparably long period of NEB. A common EB pattern during early lactation is a negative start after calving followed by a steady increase and then a decrease after return to positive values and stabilization after 17 to 21 weeks p.p. (1999).

In order to confirm and to establish a continuation of progression, it is necessary to choose a period beyond the 15th week of lactation for future studies. Nevertheless, it is self-explanatory that the longer the duration of NEB, the greater the difficulty for a cow to metabolically adapt (Kessel et al., 2008).

Between cows under standardized conditions, a large variation in numerous parameters was also observed in other dairy related areas. For example, Kessel et al. (2008) described remarkable differences in the concentrations of metabolites and hormones during the first weeks after calving. This indicates that the ability to adapt to the metabolic challenge varies greatly between individual animals. Similar results were shown by the comparison of the three groups formed retrospectively based on average EB (**Table 5.14**). Animals with a particularly large energy deficit in the first weeks after calving showed the highest milk yield, the highest milk fat content, the lowest milk protein content, the highest fat:protein ratio and the highest ECM performance. High milk fat content and low milk protein content are the result of postpartum body fat mobilization caused by the energy deficit. The resulting elevated fat:protein ratio can be used as a first indicator of a negative energy balance. If the fat:protein quotient rises above 1.3 in the first weeks after calving, an increased risk of ketosis can be assumed (Grieve et al., 1986; Heuer et al., 1999). Overall, these animals showed the lowest energy intake, caused by the lowest DMI and at the same time, the highest energy requirement and the highest OMD. A reason for the highest OMD could be the deficient situation caused by low DMI, where digestion is more effective because of a lower passage rate (Thornton and Minson, 1973; Mertens and Grant, 2020).

With the help of energy balancing, it is possible to address several components and thus get a comprehensive overview of the energy situation of the animals. This is highly relevant especially with regard to the close link between negative EB and the occurrence of production diseases. To achieve a long-term reduction in production diseases through early detection of animals at particularly high risk, knowledge of individual energy and nutrient availability as well as the corresponding variables and the variation within and between dairy cows is of great importance, especially in early lactation. The high variation in the sub-variables of the energy balance between and within cows during early lactation indicates the necessity to monitor the available nutrient and energy amount on an individual animal basis. In addition to monitoring, there is also a necessity for continuous adjustment of demand and supply (Sundrum, 2020). Only when the extent of NEB and the variation between individual animals is known can target oriented options for action be taken.

5.4.4. Strategies to deal with intra- and interindividual variation

Due to the high animal-individual variability, the predominant approach of working with average values needs to be reconsidered. By starting with assumed data on feed intake, milk yield and body weight of an average cow and then deriving a generalized energy situation for all animals in the corresponding herd or group, assessing the existing differences between individual animals in a herd, evaluating the degree of individual energy imbalances and inducing health improvement of animals at the beginning of lactation is impossible. Although prediction equations are admittedly of limited robustness due to intra- and interindividual variation at the animal level (Jorritsma et al., 2003), using estimation formulas to determine requirements and supply on an individual animal basis can be a first starting point.

The results of the estimation formulas provide a benchmark and allow a first approximation of the energy status of individual animals during lactation under the respective conditions. Comparison of the actual values with the target values reveals the energy discrepancy, the magnitude of which gives an indication of the health risk associated with it for the dairy cows (Sundrum, 2015; Maltz, 2020). The extent of the described intra- and interindividual variation also applies to the variation in the ability to cope with the discrepancies. The goal of more precisely establishing the real energy situation of each individual cow could be readily approached from the demand side by integrating continuous measurement of body weight using scales and regular recording of milk quantities and ingredients on the farm. Continuous monitoring would enable identification of deviations from reference values, trends and animals at risk and provide essential hints for early intervention to prevent excessive metabolic challenges.

To improve the assessment of nutrient and energy intake, future research should focus on the development of on-farm technical hardware and software solutions to record the level of animal-specific energy supply and reconcile it with the demand. Obvious approaches to recording DMI which have not yet been sufficiently used in agricultural practice would be weighing systems that record either the amount eaten by weighing the amount of feed available, or weighing the animal before and after the meal (Bikker et al., 2014; Ruuska et al., 2016; Carpinelli et al., 2019), or camera-based deep learning approaches to record cow-individual feed intake (Bezen et al., 2020).

However, since such adequate solutions for animal-specific feeding are not implemented in practice, appropriate individual animal nutritional strategies need to be developed. In order to reduce the rate of animals in a group with insufficient energy supply, the establishment of feeding groups based on the nutrient and energy demand is one approach among others (St-Pierre and Thraen, 1999). In this context, McGilliard et al. (1983) suggested grouping cows by energy and protein requirements using clustering algorithms rather than by milk yield, as is traditionally done. Such grouping can reduce within-group variation and increase differentiation of across-group variation. The more homogeneous the group, the more likely the individual energy and nutrient needs of the animals can be met. Yet for reasons of labor saving or structural constraints, feeding groups are often not established, resulting in a TMR with the same energy levels being fed across multiple groups or the entire herd (Sniffen et al., 1993). However, because of individual animal variation in energy requirements and energy balance, it is often not possible to adequately supply each animal in the group. The discrepancy increases the further an individual cow deviates in milk yield and feed intake from that of the average cow (Bach and Cabrera, 2017). The resulting nutrient and energy imbalances turn out to be so severe that the associated risk of overtaxing adaptability and causing the development of early diseases outweighs the economic benefits of unit feeding. Assigning animals to feeding groups based on their individual nutrient and energy needs allows for more needs-based feeding and can thereby help limit NEB.

An approach to support cows with a strong NEB is the individual feeding of highly digestible concentrates using a computerized feeding system that recognizes cows and dispenses specific mixes at timed intervals throughout the day (VandeHaar et al., 2016). However, as the proportion of concentrate increases, the amount of fiber in the feed decreases. This leads to a reduction in saliva production and a decrease in rumination time, which results in a decrease in rumen pH (Nocek, 1997). A drop in pH due to the accumulation of organic acids in the rumen can result in subacute rumen acidosis (SARA). In addition to reduced feed intake, which can be caused by SARA, increased concentrate intake also results in increased release of histamine and other endotoxins into the blood which can contribute to dilatation of blood vessels and thus damage the network of blood vessels in the hoof (Boosman et al., 1989; Kleen and Cannizzo, 2012). Not only the existing possible health risks due to reduced ruminant health but also the additional administration of concentrates to increase energy intake should be well considered in light of rising concentrate prices and the additional economic burden this imposes.

Another less frequently discussed potential management measure to improve NEB in early lactation is to temporarily reduce milking frequency, for example, to once-daily milking at the beginning of lactation (Bar-Pelled et al., 1995; Stelwagen et al., 2013; Lacasse et al., 2018). Lower milking frequency at the beginning of lactation reduces energy loss for milk production. This results in less metabolic disturbance and a reduction in immunosuppression without negative effects on the rest of lactation (Lacasse et al., 2018). Monetary losses due to reduction in deliverable milk are to be expected with this method but these should be evaluated against the reduced risks of adaptive overload and disease (Riley et al., 2018). Better physical and immunological states, which increase general health and useful lifespans, can contribute to significant cost savings and reduce failure costs due to disease (Habel et al., 2021b).

However, in the long term, breeding objectives must be modified to focus on a more consistent lactation curve, lifetime performance and longevity rather than milk yield and peak performance. The results of this study show the urgency of a change because a quarter of the cows reached the nadir of EB only at week 4-7. The concentration on breeding for increased milk yield has simultaneously resulted in lower energy balances and continued focus on performance improvement can be expected to have serious consequences for cow metabolism, animal health and failure costs (McParland et al., 2015; Sundrum, 2015).

The additional financial costs or reduced revenues associated with the described options for limiting NEB may initially appear to be counteractive to attempts at improving the economic situation on dairy farms. However, the higher economic losses due to premature retirements and increased disease rates need to be recognized (Habel et al., 2021b), and every possibility which could reduce these cost items and stabilize milk production in the long term should be considered. Furthermore, increasing social pressure in the areas of animal welfare must also be considered, which seems to be well founded due to high health problems and resulting early cullings due to an overload of the adaptation mechanism.

Only by knowing the extent of the negative energy balance, and the sub-variables behind it, is it possible to recognize risk animals on time and initiate measures to support animal-specific regulation and thus avoid economic losses due to reduced animal health. Therefore, the described approaches to limit animal-specific NEB are worthwhile despite the partial inaccuracies or the possibilities of intervention being only limited (DeVries and Veerkamp, 2000; Sundrum, 2015).

5.5. Conclusions

The results of the present study showed a large intra- and interindividual variation in the sub-variables of energy requirements, energy supply and, finally, in energy balance during early lactation. When farm management is challenged to balance the trade-offs in dairy production between animal needs and economic demands, the results indicate the need to monitor available nutrient and energy levels on an individual animal basis and to continuously adjust energy supply. To date, little consideration has been given to the variation in energy supply between and within individual animals over a period of time because of the difficulty in collecting the information needed to calculate EB under practical conditions. The variation between individual animals as well as the extent of the negative energy balance show that the current herd-based approach to assessing the energy situation is not sufficiently target-oriented for determining the animal-specific requirements and enabling a supply that meets the requirements. In order to achieve economically fundamental long-term reductions of production diseases and a consequent containment of useful lifespans by early identification of animals with a particularly high risk of production diseases, knowledge of the individual energy and nutrient availability, corresponding sub-variables and the variation within and between dairy cows is essential, especially in early lactation. The focus on individual animal care pays off by reducing health and economic risks and thus represents a good investment, regardless of the individual farm situation.

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5.7. Abbreviations

ADF	acid detergent fiber
ADFD	digestible acid detergent fiber
aNDFom	neutral detergent fiber assayed with heat-stable amylase and expressed exclusive of residual ash
ANOVA	analysis of variance
CP	crude protein
CPD	digestible crude protein
CV	coefficient of variation
CVAS	Cumberland Valley Analytical Services Inc.
DM	dry matter
DMI	dry matter intake
EB	energy balance
EE	ether extract
ESC	ethanol-soluble carbohydrates
GLM	generalized linear models
iNDF240	240 h in vitro indigestible neutral detergent fiber
IQR	interquartile range
LMM	linear mixed model
NDFD	digestible neutral detergent fiber
NEFA	non-esterified fatty acids
NEB	negative Energy balance
NEL	net energy for lactation
NIRS	near infrared reflectance spectroscopy
OM	organic matter
OMD	digestible organic matter
RIC	roughage Intake Control
RSE	relative standard error
SD	standard deviation

SP	soluble protein
TDN	total digestibly nutrient
TER	total energy requirement
TES	total energy supply
TMR	totally mixed ration

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6. General Discussion

One aim of this thesis was to identify intra- and interindividual variation in nutrient and energy availability in dairy cows during early lactation. For the study, 28 multiparous German Holstein dairy cows were selected and their animal-specific data, including feed intake and digestibility, were collected between weeks 2 and 11 of lactation. Calving spanned the period between February and July 2018. All animals were kept in the same research environment and received the same ration throughout the experimental period. To be able to attribute the variation in the parameters studied to the differences between individual animals, possible influencing factors caused by breed, feed or environment were reduced as much as possible. Slight variations in the chemical composition of the feed during the experimental period were caused by the ensiling process from the field to the feeding stage. Based on weekly feed analyses, deviations were detected promptly and compensated for by adjusting the ration as needed.

With the help of the internal marker iNDF240, it was possible to determine the animal-specific nutrient digestibility over a longer period under comparable on-farm conditions. The proven high correlation with the results of the previous gold standard method of total collection (TC), confirm good suitability of the marker for the determination of digestibility (Schalla et al., 2012; Lee and Hristov, 2013; Morris et al., 2018). In addition, the marker allows a statement about the digestibility of different nutrient fractions when the feed and fecal ingredients are appropriately fractionated. Due to the high accuracy, the wide range of possible applications and the comparatively simple determination (rectal fecal sampling + laboratory analysis), iNDF240 can be recommended as a marker for the determination of digestibility in agricultural practice. Data, collected using feeding weighing troughs and iNDF240, showed a wide variation in feed intake and nutrient digestibility between individual animals at the same stage of lactation and within individual animals during the weeks after calving. The enormous variation with CV values of feed intake up to 0.14 and differences of up to 12.4 kg DMI /day between animals at the same stage of lactation show that there are animals that deviate particularly strongly from the rest of the animals. This situation is especially critical against the background of adequate nutrient and energy supply. Other studies also confirm a similarly large variation with CV values up to 0.18 between animals (Huhtanen et al., 2015; Cabezas-Garcia et al., 2017).

However, due to the high additional workload associated with an individual animal approach to determine nutrient and energy supply (Sniffen et al., 1993), and to the lack of perspective in dealing with knowledge about variation, the fact of variation is given little consideration in current dairy cattle management. In recent years, the number of dairy farms has decreased but the number of animals per farm has increased (FAO, 2010, 2019). Keeping animals in groups, although undoubtedly representing a major advance in work efficiency, make individual animal consideration increasingly difficult. In fact animal-specific control of fresh cows is finding its way into modern herd management (Heuwieser et al., 2010) but with major focus on health status and performance development. However, apart from the basic genetic makeup of the cow, the development of health and performance is primarily determined by the cow's nutrient and energy balance (Sundrum, 2015; Habel and Sundrum, 2020).

Especially in early lactation, the abrupt change from a non-lactating metabolic state to a state of exponentially increasing milk production presents the cow with a massive metabolic challenge. Due to the massive energy demand for milk production, the main task of the animal during this phase is to ensure an adequate supply of nutrients and energy. If this cannot be ensured, the demand will exceed the supply and a deficiency situation will result. The lack of nutrients and energy has been widely described as a trigger for various diseases and disorders of the cow (Esposito et al., 2014). Despite the negative influence and the consequences of this deficiency situation, little or no attention has been paid to it on an individual animal basis. Although ketone bodies or NEFAS are measured on an individual animal basis and are now part of good professional practice (Krogh et al., 2020), these values only provide information about the degree of physical stress on the animal. However, this approach cannot be used to investigate the cause which each parameter contributes to the animal's overload.

The goal of feeding management and ration calculation is to provide nutrition that meets the animal's needs (NRC, 2001) while being efficient and practical in terms of labor economics at the same time. Due to the ever-increasing herd sizes and the keeping of animals in groups, feeding management has diverted from an individual to a group-feeding approach (Maltz et al., 2013). In this approach, a TMR is calculated for a defined group of cows with the aim of formulating a diet for a fictive cow which represents the average of the group. In addition, the feed allocation occurs for all cows together (Sniffen et al., 1993; Maltz et al., 2013; Sundrum, 2020). It is assumed that animals having a high demand for energy accordingly consume more of the ration and that animals with a lower demand accordingly consume less (Maltz et al., 2013).

The results of the present study confirm considerable variation among animals in feed intake. However, knowledge of feed intake alone does not allow inference of the amount of nutrients and energy available to the animal. Only by combining the amount of feed intake, the ingredients of the feed, and the level of animal-specific digestibility, a conclusion could be made about nutrient and energy availability. In addition to the wide variation in feed intake, considerable variation in nutrient digestibility between dairy cows was observed, with CV values up to 0.10 for crude protein digestibility (CPD). Other studies confirmed variations of this magnitude (Cabezas-Garcia et al., 2017; Guinguina et al., 2020). Based on the knowledge of feed intake, chemical composition and nutrient digestibility, it was possible to make individual calculations about the amount of nutrients and energy available to each animal in each week of lactation. It could be shown that there are considerable variations in the supply situation of the animals. When converting the available amount of nutrients and energy per kg ECM, it was noticed that not even all animals absorb the required amount of energy that would be necessary to produce 1 kg ECM. Therefore, the question arises as to how target-oriented the current approach of managing increasing milk yields by aggregation and calculation with average values, estimation formulas and herd-based tabulated values actually is in attempting to feed dairy cows according to their needs.

In addition, it was shown that the variation in feed intake is essentially responsible for the variation in nutrient availability. To capture possible reasons for the large variation in feed intake and in digestibility, animal-specific feeding behavior was analyzed in more detail. Within the framework of this analysis, the observation period was extended to the 15th week of lactation. Large variations in meal frequency, meal duration, total feeding time, meal size, and feeding rate were shown between animals at the same lactation stage. Azizi et al. (2009) also reported a large variation between dairy cows within these parameters. A significant influence on the level of feed intake was demonstrated for the parameters meal frequency, meal duration and meal size. In addition, longitudinal data collection allowed the representation of within-animal variation in feeding behavior during early lactation, which revealed significant variation. Animals with a high mean feed intake during early lactation tended to be more stable in their feeding behavior. Furthermore, the calculations showed that any variation in only one parameter of feeding behavior had a significant effect on the variation in other parameters of feeding behavior. These results emphasize the important significance of stable feeding management and optimal conditions in early lactation which have also been described in other studies (Proudfoot et al., 2009). If there are changes in only one parameter due to irregularities, it directly affects the variation in other parameters of feeding behavior. In addition to the relationship between feed intake and feeding behavior, the interaction of the above parameters with digestibility was also investigated. The presented results did not show any effect of variables of intake behavior on nutrient digestibility but an effect of week of lactation on CPD and ADFD and an effect of dry matter intake on OMD and CPD. The influences of meal frequency or meal size on digestibility described by Llonch et al. (2018) or Allen (1997) could not be shown in this study. The large variation and the shown interaction between the factors, which are certainly to be assumed as even larger under less standardized conditions in the field, show that it is necessary to not only continuously check feed intake but also digestibility.

However, knowledge about availability alone is not sufficient to identify animals that are in a particularly deficient situation. It needs to be compared with the demand (Habel and Sundrum, 2020). Therefore, a further objective of this study was to compare the individual animal energy requirements and the individual animal energy supply and to calculate the energy balance at the individual animal level and, based on this, to map the energy situation of the animals in early lactation. The energy balance (EB) is the energy ingested via feed, in the best case corrected for animal-specific digestibility, minus the energy required to maintain body functions, milk production and pregnancy. EB is thus a calculated, aggregated quantity composed of several sub-variables and subject to their variation.

The aforementioned sub-variables can vary, sometimes considerably, over time and between individual animals of the same genotype or at the same lactation stage due to sometimes opposing but also reinforcing trajectories (Beerda et al., 2007). For example, an increase in milk yield also leads to an increase in feed intake (Roseler et al., 1997; Martin and Sauvant, 2002; Hristov et al., 2005), whereas feed digestibility decreases with increasing feed intake (Thornton and Minson, 1973; Mertens and Grant, 2020). However, the effects of variation within sub-variables on EB and its fluctuations are often unknown when energy status is calculated based on average animals using prediction equations or tabulated values (Jorritsma et al., 2003). Only by knowing the magnitude of the sub-variables, the negative energy balance and the variation between individual animals over time is it possible to identify risk animals and highlight the need for action to support animal-specific regulation (DeVries and Veerkamp, 2000; Sundrum, 2015).

It has been shown that variation in the various sub-variables that are included in the calculation of energy balance does not result in a state of energetic equilibrium as conventionally assumed. On the contrary, it results in an increase of variation, and consequently in a much larger energy deficit. In addition to the large variation between animals at the same stage of lactation, a large animal-specific variation was observed. Animals that were in a particularly deficient position differed significantly from animals with a lower energy deficit, especially in milk yield, milk constituents and energy requirements. These animals were at particularly high risk of being overwhelmed in their metabolic regulatory challenge and thus more susceptible to disease (Butler and Smith, 1989; Nebel and McGilliard, 1993; Domecq et al., 1997). Due to the large variation of the energy balance caused by the interaction existing between the sub-variables, it is almost impossible to predict the extent of the animal-individual energy balance.

The differentiation of animals and, based on this, the identification of animals at risk is only possible by looking at the individual aspects of each animal and by combining the data into the energy balance. The approach of the calculation of the individual animal energy balance offers the possibility of an approximation to the energetic situation of the individual animal. Based on the EB, the animals of a herd can be compared and ranked. By looking at the sub-variables, it is then possible to identify the variables in which individual animals deviate particularly strongly and to take measures to reduce the variation. The approach of animal-individual energy balancing offers considerable potential in early disease detection and thus can be a starting point for long-term health improvement of fresh cows. Future research should initially focus on further development of feasible solutions for quantifying animal-specific feed intake and feeding behavior under practical conditions. Conceivable approaches would be sensor-based systems on or in the animal, as well as weighing systems that record the amount eaten either by weighing the amount of feed available or weighing the animal before and after the meal (Bikker et al., 2014; Ruuska et al., 2016; Carpinelli et al., 2019).

In another step, the measured data on feed intake must be integrated into the existing pool of milk, body condition, health, pedigree data etc. With such data, an animal- and day-specific energy balance could then be calculated and continuously monitored. Data collection and linking, as well as the calculation of the animal-specific energy balance, however, do not directly contribute to the improvement of the animal-specific situation.

The data only provides a starting point for targeted management decisions that can support the animal in its regulatory work.

In addition to the targeted allocation of feeding groups based on requirements (St-Pierre and Thraen, 1999; Bach and Cabrera, 2017), which can contribute to reducing variation in the first step, a targeted allocation of concentrates represents a possible starting point for direct individual animal support. In addition to reduced feed intake, which can be caused by sub-acute ruminal acidosis (SARA), increased concentrate intake also results in an increased release of histamine and other endotoxins into the blood which can contribute to dilatation of blood vessels and thus damage to the network of blood vessels in the hoof (Boosman et al., 1989; Kleen and Cannizzo, 2012). Therefore, supplemental concentrates to increase energy intake should be given with great caution. Another less frequently discussed potential management measure to improve NEB in early lactation is to temporarily reduce milking frequency to, for example, once daily milking at the beginning of lactation (Bar-Pelled et al., 1995; Stelwagen et al., 2013; Lacasse et al., 2018). Lower milking frequency reduces energy loss for milk production and results in improved body condition and reproductive performance without compromising animal welfare. A reduction in inflammation has also been observed (Riley et al., 2018).

The extent of energetic stress, the high prevalence of production diseases (Krieger et al., 2017; Hoedemarker et al., 2020) and the high rate of early culling (Habel et al., 2021a), all indicate an urgent need for action. However, the magnitude becomes apparent only by viewing on an animal-by-animal basis as the current approach of generalization does not bring any progress and should therefore be critically questioned. Maintaining the focus of breeding objectives on further increasing milk yield and ever higher peak yields should be critically questioned as an increasingly lower cost-benefit ratio can be expected due to severe consequences for the cow's metabolism and health (McParland et al., 2015; Sundrum, 2015). Future research could provide an answer to the question to what extent the presented approach of animal-specific energy balancing and the subsequent identification of variation and causes of variation as well as the subsequent targeted derivation of management measures to limit variation can influence and improve animal health in early lactation in the long term.

7. General Conclusion

This work has shown that there is considerable variation in feed intake, nutrient digestibility and consequently also in nutrient and energy availability and balance within and between individual animals. The degree of variation contradicts the predominant approach of the allocation model and the approach of making use of average values. To determine the animal-specific digestibility of different nutrient groups under farm conditions, the internal marker iNDF240 was identified as a suitable tool. Furthermore, parameters of feeding behavior were identified as influencing factors on feed intake as well as nutrient digestibility. In particular, variation in feeding behavior had a significant negative effect on the level of average feed intake. The predominant approach of assuming a predicted uniform feed intake for all animals at the same stage of lactation based on a fictive average cow or working with tabulated values for determination of nutrient digestibility do not allow identification of individual animals that may be in a critical situation due to their low nutrient and energy availability. Looking only at the supply side does not provide a complete picture of the overall energy situation of the individual animal. Only by comparing the individual animal energy demand with the individual animal energy supply offers the option to identify potentially severe deficient situations, which go beyond previous assumptions, as well as the variation within and between individual animals. The approach of individual animal energy balancing offers a possibility to assess the individual animal energy situation over time and in comparison, to the rest of the herd and to identify individual animals with a particularly pronounced negative energy balance. By looking at the individual parameters used to calculate the energy balance, possible causes for the extent of the deficit can be identified and options for action can be derived. Despite the higher effort compared to the conventional approach of aggregation and averaging, animal-specific energy balancing can be seen as a starting point for sustainable improvement of animal health. To achieve fundamental economical long-term reductions of production diseases by early identification of animals with a particularly high risk of production diseases, knowledge of the individual energy and nutrient availability, corresponding sub-variables and the variation within and between dairy cows is essential, especially in early lactation. The focus on individual animal care pays off by reducing health and economic risks and thus promises a good investment, particularly on high yielding dairy farms.

8. References

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