
New Approaches for the Quantification of Metabolic and Economic Trade-Offs Related to the Health and Longevity of Individual Dairy Cows

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1. Vorwort

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List of Abbreviations

AFC / iAFC – (individual) age at first calving
ALA – Alanine
aNDFom – Ash-free neutral detergent fibre
AT – Adipose tissue
BHB – β-hydroxybutyrate
BW – Body weight
C – Carbon
C_F – feed costs
C_H – costs of rearing a heifer
C_O – other (production) costs
CP – Crude protein
CS – Crude sugar
d_L – Ratio of adipose tissue mobilized
d_P – Ratio of protein tissue mobilized
deltaEBW – Daily changes in empty body weight
DIM – Days in milk
DIM_{LL}/ $iDIM_{LL}$ – (individual) days in milk at culling during last lactation
DM – Dry matter
DMI – Dry matter intake
d_{PL} / id_{PL} – (individual) days since first calving/of the productive life
E_M – Energy demand for maintenance
EB – Energy balance
EB – Energy balance
EBW – Empty BW
ECM – Energy-corrected milk
Ed – Apparent digestibility of GE
ED_N – Effective degradability of nitrogen
ED_P – Energy demand for production
ED_S – Effective degradability of starch
F% – Percentage of milk fat
FA – Fatty acids
FE – Feed efficiency
FL – Feeding level (level of intake relative to body weight)
Flref – Reference feeding level
g – Gram
gAA – NPA of glucogenic amino acids
GB – Glucose balance
GD – Glucose demand
GD_Brain – GD of the brain
GD_MG – GD of the mammary gland
GD_PT – GD of protein tissue
GD_RBC – GD of red blood cells
GE – Gross energy
GH – Growth hormone
GHR – GH-receptor
GLU – NPA of glucose
GLUT – Glucose transporter
GLY – Glycerol
GS – Supply of glucogenic carbon
HP – High profit cows
IGF-1 – Insulin-like growth factor 1
iIOLC – Individual income over service life cost
ILR – Irreversible loss rate

IOFC – Income over feed costs
IQR – Interquartile range
kg – Kilogram
L% – Percentages of milk lactose
LAC – L-lactate
LACdiet – NPA of dietary L-lactate
LACendo – Endogenous L-lactate
Lact / iLact – (individual) lactation number
LP – Low profit cows
LPS – Lipopolysaccharide
LY – Lactose yield
maxGB – Maximal GB (assuming no glucose consumption in tissues other than the mammary gland and maximal standard errors of the NPA of precursors)
MEC – Mammary epithelial cells
MG – Mammary gland
MJ – Megajoules
MP – Milk price
MY – Milk yield
MY_{Life} – Lifetime milk yield
MY_{LL} / iMY_{LL} – (individual) average daily milk yield during last lactation
MY_{PL} / iMY_{PL} – (individual) daily milk yield since first calving
N – Nitrogen
NDF – Neutral detergent fibre
NDFDint – NDF digestible in the intestines
NEB – Negative energy balance
NEFA – Non-esterified fatty acids
NEL – Net energy for lactation
NFkB – Nuclear factor kappa-B
NPA – Net portal appearances
OM – Organic matter
OMd – OM digestibility
P% – Percentages of milk protein
PC – Pyruvate carboxylase
PD – Production diseases
PDI – Protein truly digestible in the intestines
PEPCK – Phosphoenolpyruvatecarboxykinase
PF – Fermentation products of silages
PRO – NPA of propionate
PRO % - Percentage of propionate production of total VFA production in the rumen
PROD_VFA – Volatile fatty acid production
PT – Protein tissue
RBC – Red blood cells
R_C – Average revenue from carcass value per cow
RdNDF – NDF digestible in the rumen
RfOM – OM fermentable in the rumen
RL – Rumen liquid
ROS – Reactive oxygen species
SD – Standard deviation
ST – Starch
STdI – Starch digestible in the intestine
TDC – Total digestive contents
TG – Triglycerides
TLR-4 – Toll-like receptor 4
TRC – Total rumen contents
TW – Farms enrolled in the project “TierWirt”

UNIANOVA – Univariate linear regression model
VFA – Volatile fatty acids
ΔLIP – Adipose tissue mobilized
ΔLIPref – Reference level of adipose tissue mobilization
ΔPROT – Protein tissue mobilized
ΔPROTref – Reference level of protein tissue mobilization
ωp^2 – Partial omega square

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#1 Habel, Jonas; Sundrum, Albert (2020): Mismatch of Glucose Allocation between Different Life Functions in the Transition Period of Dairy Cows. *Animals: an open access journal from MDPI* 10 (6), p. 1028. DOI: 10.3390/ani10061028.

#2 Habel, Jonas; Chapoutot, Patrick; Koch, Christian; Sundrum, Albert (2022): Estimation of Individual Glucose Reserves in High-Yielding Dairy Cows. *Dairy* 3 (3), pp. 438–464. DOI: 10.3390/dairy3030033.

#3 Habel, Jonas; Sundrum, Albert (2023): Dairy Cows Are Limited in Their Ability to Increase Glucose Availability for Immune Function during Disease. *Animals* 13, 1034, DOI: 10.3390/ani13061034.

#4 Habel, Jonas; Uhlig, Verena; Hoischen-Taubner, Susanne; Schwabenbauer, Eva-Marie; Rumphorst, Theresa; Ebert, Lara et al. (2021): Income over service life cost – Estimation of individual profitability of dairy cows at time of death reveals farm-specific economic trade-offs. In *Livestock Science* 254, p. 104765. DOI: 10.1016/j.livsci.2021.104765.

Contribution to the Publications

 # 1 Conceptualization, together with Albert Sundrum
Investigation
Writing – original draft
Visualization

 # 2 Conceptualization, together with Albert Sundrum
Methodology, together with Patrick Chapoutot
Data curation, together with Christian Koch
Writing – original draft
Visualization

 # 3 Conceptualization, together with Albert Sundrum
Methodology
Data curation
Writing – original draft
Visualization

 # 4 Conceptualization, together with Susanne Hoischen Taubner, Eva Schwabenbauer, Theresa Rumphorst, Verena Uhlig, Detlev Möller & Albert Sundrum
Methodology
Data curation, together with Susanne Hoischen Taubner, Eva Schwabenbauer, Theresa Rumphorst, Verena Uhlig
Writing – original draft

2. Abstract

Background: In dairy cows, negative associations between the genetic merit for milk yield and fitness traits as well as a lack of success in lowering the prevalence of production diseases have been noticed over the past 30 years across different production methods, herd sizes and countries. On the other hand, individual farms and individual cows within a farm succeed in simultaneously achieving high performance, low prevalence and high profitability, while others cannot cope with these challenges. Differences in milk yield (MY), dry matter intake (DMI), and the degree of exposure to stressors determine the metabolic status and thus the susceptibility to disease. However, the same inter- and intraindividual differences also determine the profitability of a dairy cow's service life. Therefore, new approaches to quantify metabolic and economic trade-offs at the individual animal level were investigated in this thesis.

Material and Methods: Firstly, a comprehensive review addressing the current knowledge and open research questions related to the distribution of glucose in the metabolism of high-yielding dairy cows and the main influencing factors (stage of lactation, genetics, performance level) was prepared. Secondly, a mechanistic, conceptually driven whole-body model to determine the residual glucose available for non-quantifiable processes (immunoactivation) of individual cows was developed. Thirdly, this approach was used to calculate individual glucose balances (GB) of 417 lactations (298 cows) and to evaluate the effect of diagnosis on MY, DMI, GB and energy balance in the weeks before, at, and after diagnoses of inflammatory diseases in different stages of early lactation. Additionally, a methodology for the calculation of the income over service life cost (IOLC) of individual culled cows based on routine herd management data and farm-specific business sheets was established. The effect of different service life characteristics on IOLC was evaluated for 32 German dairy farms (4,962 culled cows).

Results: If high milk yields and immunoactivation occur simultaneously, whole-body glucose requirements exceed glucose supply. Because decades of selection for milk production affected the patterns of glucose partitioning to the favor of milk synthesis, it can be assumed that glucose shortage for immune cells contributes to peripartal immune dysfunction and the high incidence of production disease, particularly during early lactation. By merging individual cow performance and feeding data, individual GB can be estimated from digestive and endogenous fluxes of glucogenic carbon on the one hand, and the demand for glucogenic carbon imposed by major glucose-consuming organs on the other. When unaffected by disease, GB was close to zero in the first weeks of lactation. During disease, the decrease in DMI was proportionally higher than the decrease in MY, resulting in a decrease of GB in the week of diagnosis. Analysis of IOLC showed that the ability of cows to cope with disease is inherently linked to the economic viability of the dairy business, as three quarters of the cows were culled due to disease while the duration of the productive life had the greatest effect on cow profitability for most farms.

Conclusions: Low glucose reserves and a limited ability to increase glucose availability for immune cells during disease indicate that high performance and avoidance of production disease (and thus, a long productive lifespan) are difficult to achieve for individual cows. Since low productive lifespan is the main barrier to cow profitability, maximizing the reduction of feeding imbalances and environmental stressors is of key importance. Management measures to increase glucose availability for immune functions in diseased cows need to be investigated.

3. Zusammenfassung (Deutsch/German)

Hintergrund: Bei Milchkühen besteht ein negativer Zusammenhang zwischen dem genetischen Potential für Milchleistung auf der einen und Fitnessmerkmalen auf der anderen Seite. Zudem verharren die Prävalenzen von Produktionskrankheiten in der intensiven Milchviehhaltung seit Jahrzehnten auf einem hohen Niveau, unabhängig von Produktionsmethoden, Herdengrößen und Ländern. Andererseits gelingt es einzelnen Betrieben und einzelnen Kühen innerhalb eines Betriebes, gleichzeitig hohe Leistungen, niedrige Erkrankungsraten und eine hohe Rentabilität zu erzielen, während andere an diesen Herausforderungen scheitern. Die Unterschiede in Milchleistung (ML), Trockenmasseaufnahme (TMA) und im Grad der Belastung durch Stressoren entscheiden dabei über den Grad der metabolischen Herausforderung, und damit auch über die Anfälligkeit für Erkrankungen. Die gleichen inter- und intraindividuellen Unterschiede entscheiden auch über die Rentabilität einer Milchkuh. In der vorliegenden Arbeit wurden neue Ansätze zur Quantifizierung von metabolischen und ökonomischen Zielkonflikten auf Einzeltierebene untersucht.

Material/Methoden: Der aktuelle Wissensstand und offene Forschungsfragen zur Verteilung von Glukose im Stoffwechsel hochleistender Milchkühe und zu den wichtigsten Einflussfaktoren (Laktationsstadium, Genetik, Leistungsniveau) wurde in einem Review-Artikel zusammengefasst. Anschließend wurde ein mechanistisches Berechnungsmodell zur Bestimmung der für nicht quantifizierbare Prozesse (Immunaktivierung) verfügbaren Restglukose einzelner Kühe entwickelt. Dieses Modell wurde dann verwendet, um einzeltierliche Glukosesalden (GS) von 417 Laktationen (298 Kühe) zu berechnen. Ferner wurden die Dynamiken von ML, TMA, GS und Energiesaldo in den Wochen vor, während und nach der Diagnose von inflammatorischen Erkrankungen in verschiedenen Stadien der frühen Laktation untersucht. Für die ökonomische Bewertung von gemerzten Kühen wurde eine Methode zur Berechnung des einzeltierlichen Gewinns (IOLC) auf der Grundlage von Leistungs- und Fütterungsdaten der Kühe und den ökonomischen Daten aus Betriebszweigabrechnungen entwickelt. Der Einfluss verschiedener Leistungsmerkmale auf den IOLC wurde für 32 deutsche Milchviehbetriebe (4.962 Kühe) errechnet.

Ergebnisse: Durch die Selektion auf Milchleistung ist die Glukoseverteilung im Intermediärstoffwechsel zu Gunsten der Milchsynthese beeinflusst. Es kann davon ausgegangen werden, dass Glukose für Immunzellen bei gleichzeitig hohen Milchleistungen nur begrenzt verfügbar ist. Durch die Zusammenführung von Leistungs- und Fütterungsdaten auf Einzeltierebene kann der individuelle GS aus den digestiven und endogenen Flüssen von glukogenem Kohlenstoff auf der einen, und aus dem Bedarf an glukogenem Kohlenstoff der wichtigsten glukoseverbrauchenden Organe auf der anderen Seite geschätzt werden. Auch bei klinisch gesunden Kühen lag der GS in den ersten Wochen der Laktation nahe bei Null. Während einer Erkrankung war die Reduktion der TMA proportional höher als die Reduktion der ML, was zu einer Reduktion des GS in der Woche der Diagnose führte. Die Analyse des IOLC zeigte, dass das Überleben der Kuh eng mit dem wirtschaftlichen Überleben des Milchviehbetriebs verbunden ist, da drei Viertel der Kühe aufgrund von Krankheiten gemerzt wurden, während die Nutzungsdauer bei den meisten Betrieben den größten Effekt auf die Rentabilität hatte.

Schlussfolgerungen: Geringe Glukosereserven und die begrenzte Fähigkeit, die Glukoseverfügbarkeit für Immunzellen während einer Krankheit zu erhöhen, deuten darauf hin, dass gleichzeitig hohe

Leistungen und die Vermeidung von Produktionskrankheiten (und damit langer Nutzungsdauer) für einzelne Kühe nur schwer zu erreichen sind. Da eine niedrige Nutzungsdauer das Haupthindernis für die Rentabilität der Kühe ist, ist eine maximale Reduzierung von Fütterungsimbalancen und Umweltstressoren von zentraler Bedeutung. Es besteht Forschungsbedarf zu Managementmaßnahmen, die die Glukoseverfügbarkeit für Immunfunktionen von erkrankten Kühen erhöhen.

4. General Introduction

Dairy operations are characterized by complex socio-bio-economic interactions that are affected by the availability of resources, including land, buildings, machinery, the ability to invest, workforce, knowledge and legal guidelines. However, milk production is based on biological processes and the individual dairy cow is the central mean of dairy production. Although the attempt for mechanistic explanations of biological processes and for commercialized biological processes, such as livestock farming, is generally observed in western societies (Marechal et al. 2008; Wells and McLean 2013), concerns about a mechanistic view of systems that incorporate biological processes were raised for a long time (Wells and McLean 2013). In farm animal sciences, mechanistic explanations struggle not only with the hazardous nature of the occurrence of climatic or infectious challenges or the volatility of prices. They also neglect that, contrasting other means of production, the individual animal cannot be grasped by generalist approaches (Rollin 2015), but must be regarded in the first place in light of its intrinsic overall goal: self-sustainment via adaptation to a dynamically changing environment.

Unfortunately, the majority of cows fails to adapt to the stresses to which they are exposed. This is reflected by the fact that, nowadays, most cows are culled due to disease or infertility before they reach maximum productivity, while the incidence risk of culling attributed to poor production decreased since the mid-1980s (Compton et al. 2017). In animals that are high-producing, the nutritional status is of outmost importance for their health. Increasing longevity of high-producing cows thus requires increased emphasis on the individual nutritional status and in particular, the assessment of the individual level of nutrients available for self-sustaining life functions.

On the other hand, the longevity of dairy cows kept commercially must be considered from an economic point of view. For this purpose, farmers and extension commonly rely on herd averages or general recommendations, although the economic value of service life characteristics of an individual cow, such as longevity and productivity, depends on farm-specific costs and revenues.

4.1 Scientific Approaches to Production Diseases in Dairy Nutrition and Dairy Economics

Production diseases (PD) are multifactorial diseases of farm animals that are caused by or related to the production process itself (Jones et al. 2019; Maes et al. 2020; Sundrum 2020). In dairy cows, metabolic diseases such as ketosis and fatty liver occur mainly at the beginning of lactation and are a result of the cows attempt to metabolically adapt to the high nutritional demands imposed by the onset of milk synthesis in the mammary gland (Mulligan and Doherty

2008; Oltenacu and Broom 2010; Sundrum 2015). Various studies have shown that derailments of metabolic processes are closely related to the occurrence of PD’s, such as retained placenta, metritis, mastitis, displaced abomasum and lameness (Melendez et al. 2009; Ospina et al. 2010; Sordillo and Raphael 2013; Suthar et al. 2013). Although being addressed by public concerns, education, legislation, veterinarians, advisors and a vast amount of academic research, high prevalence of production diseases and high rates of involuntary culling persist in dairy farming (Suthar et al. 2013; Brunner et al. 2019; Krieger et al. 2017a; Hoedemaker et al. 2020). In fact, PD’s are responsible for ~75 % of all culling decisions (Ahlman et al. 2011; Bascom and Young 1998; Compton et al. 2017; Rilanto et al. 2020; Hoedemaker et al. 2020; Vries and Marcondes 2020). The ‚failure costs‘ associated with the occurrence of PD’s, including veterinary treatment, labor costs, discarded milk, decreased milk yield and in particular, replacement costs, are often underestimated (Huijps et al. 2008). In addition to barriers to the linear transfer of knowledge (Hoischen-Taubner et al. 2018), the lack of success in reducing the prevalence of PD’s suggests that traditional dogmas and accesses to the topic have to be questioned. In this regard, the search for universal points of actions seems to particularly contrast with the multifactorial character and farm-specificity of PD’s (Krieger et al. 2017b; Chantziaras et al. 2018; Blanco-Penedo et al. 2019).

In dairy nutrition and physiology, it is often assumed that genomic differences are able to explain why some cows stay healthy despite high milk yields while others fail to cope (Friggens and Newbold 2007; Baumgard et al. 2017; Koster et al. 2019; Fischer et al. 2018). Generally, antagonistic relationships between fitness-related traits, such as reproductive success, udder health or lameness on one hand, and the genetic merit for milk production on the other are well described (Ingvartsen et al. 2003; Knaus 2009; Oltenacu and Broom 2010). Yet, phenotypic correlations between the actual milk yield level and the occurrence of production diseases are weak (Veerkamp 2009) and indicate the difficulty in capturing the multifactorial character of PD’s. Accordingly, it has been stated that literature evaluation of the relationship between the performance and the incidence of production disease is unlikely to help solve the problem of PD (Ingvartsen et al. 2003; Mulligan and Doherty 2008), as numerous confounding factors impede a sound evaluation at both farm and cow level. In fact, cows experience very heterogeneous nutritional and environmental conditions emerging from the variation in both, supply with and demand for energy and essential nutrients. Continuous variation in the level of supply emerges from varying quality and composition of feedstuffs as well as from alterations in the feed intake capacity and digestibility (Owens et al. 2010; Guinguina et al. 2020; Rumphorst et al. 2022). As for the level of demands imposed by individual cows, inter- and intraindividual variation is related not only to variations in milk yield (Petitclerc et al. 2000), body weight (Dong et al. 2015) and pregnancy (Sguizzato et al. 2020), but also to the degree of exposure to metabolic, infectious or environmental stresses, as these stresses determine the demand of regulatory life functions, such as thermoregulation (Kadzere et al. 2002; Sammad et al. 2020) and immune defense (Kvidera et al. 2017). Despite this, knowledge on the level and variation of individual discrepancies between supply and demand is often restricted to the analysis of milk solids or the estimation of energy balance. Moreover, scientific and practical animal nutrition usually do not account for the requirement of self-sustaining life functions other than the basal metabolic rate, which is commonly termed the 'maintenance requirement' (Knap 2009). In fact, while the basic metabolic rate can be deduced from the metabolic body weight ($BW^{0.75h}$) and requirements for milk synthesis can be deduced from the amount of milk solids produced, the context-specificity of immune activation implies that the quantification of the corresponding requirements cannot easily be made (Colditz 2009). In light of the importance of glucose as an essential energy source and precursor for immune cell action (Newsholme et al. 1996; Rathmell et al. 2003; Maratou et al. 2007; Noleto et al. 2017), however, an assessment of individual glucose reserves of dairy cows left for immunoactivation is a promising measure for the dairy cow's ability to adapt to the stresses to which she is exposed.

With regard to dairy economics, the longevity of individual cows is inherently related to the survival of the farm because an individual cow can only contribute to farm profitability if she lives long enough to pay-back ‚her' costs of rearing together with ongoing production costs. However, the economically optimal length of the productive life depends upon various measures of efficiency, including reproductive and feed efficiency (Vries 2017; Vries and Marcondes 2020; Schuster et al. 2020). Similar to the evaluation of metabolic trade-offs, which is challenged by numerous external factors, the evaluation of economic potentials associated with different levels of productivity, health and longevity is confounded by large variations in the economic situation between farms. This heterogeneity has been related, among other things, to differences in milk prices and feed costs, which mask the mutual influences between the biological and the economic efficiency in cross-farm studies (Wolf 2010; Drews et al. 2018). Not surprisingly, the results of cross-farm analysis are contradictory. For instance, Hansson and Öhlmér (2008) did not observe significant effects of animal health practices on measures of economic efficiencies of Swedish dairy farms. Similarly, longevity and the gross margins of Dutch dairy farms were not significantly correlated in the study by Vredenberg et al. (2021). On the other hand, the studies of Bascom and Young (1998), Groenendaal et al. (2004) Vries (2017) and Schuster et al. (2020) clearly indicate that high mortality and morbidity rates, i.e. low longevity, is a major factor contributing to reduced profitability of dairy farms in different economic scenarios. In light of the complexity and individuality of bio-economic interactions in agricultural businesses, general recommendations may thus be illusive for individual farms (Jarvis and Valdes-Donoso 2018). In this regard, Teixeira de Melo et al. (2020) pointed out that case studies rather than large-number trials should be taken into account to describe such systemic interactions. Yet, even within the economic boundaries of a given dairy operation, the economic impact of management options cannot be readily determined. Firstly, identification of economic drivers at farm level is challenged by the volatility of prices over different financial years, which is expected to persist or even increase in the EU dairy sector (Smutka et al. 2015). Secondly, the involuntary nature of the majority of culling decisions (Compton et al. 2017) implies that herd averages of common bio-economic measures related to performance and longevity traits are seldom a result of the intended strategies. Hence, both cross-farm data as well as herd averages of one farm over different financial years are highly dependent on a variety of immutable factors and thus, are difficult to interpret.

The core of the present thesis therefore lies in the development of new methods for the quantification of metabolic and economic trade-offs related to the health and longevity of dairy cows at animal level. It is hypothesized, that not only metabolic trade-offs, which occur very obviously within a specific organism but are often dealt with at herd level, but also economic trade-offs, which are almost solely addressed at herd level, should be evaluated at animal level to advance their understanding.

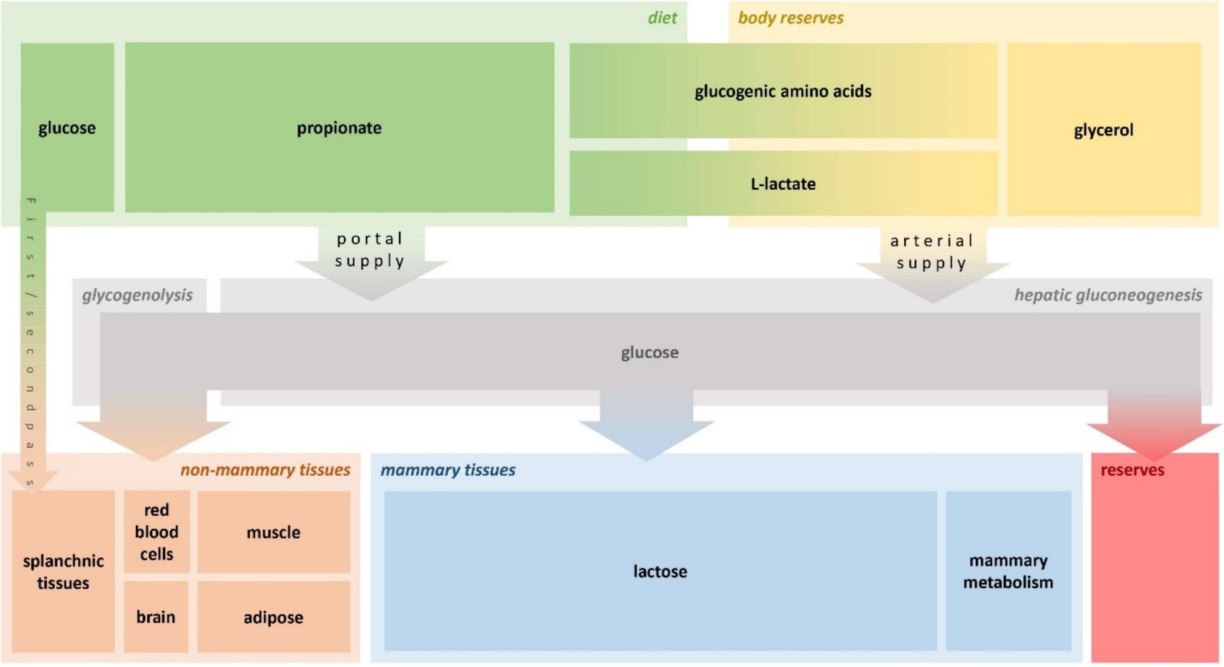
4.2 Quantification of Metabolic Trade-Offs between Productivity and Longevity

According to resource allocation theory, trade-offs between different tissues and life functions within the organism, such as reproduction, production, growth and self-sustaining life functions emerge in cases of limited nutrient supply (Glazier 2009). The performance-oriented selection of animals took advantage of the biological principle according to which mammals prioritize nutrient partitioning towards the mammary gland after calving to ensure the nourishment of the offspring. The genetic progress in milk yield, however, is not accompanied by a sufficiently large genetic progress in feed intake capacity (Veerkamp and Koenen 1999; Hristov et al. 2005; Veerkamp 2009). Also, digestive (Ledinek et al., 2019) as well as hepatic efficiencies (Loncke et al., 2020) do not increase proportionally to increases in milk production. Additionally, the energy density of ruminant diets is limited by rumen health concerns (Zebeli et al. 2015). Thus, the gap between demand and supply, commonly expressed as the difference between energy or nutrient intake and demand, i.e., the energy or nutrient balance, widens as the trend to increase milk yield and body mass continues (Rumphorst et al. 2022). Over the last 25 years, several empirical relationships between the degree of nutritional stress and the occurrence of production diseases have been characterized (e.g., Collard et al. 2000; Drackley et al. 2005; Sordillo and Raphael 2013). Dysregulated immune cell action is thought to be a key factor for the susceptibility to disease and several links exists between the level of metabolic stress and disturbances to immune cell population and function (for reviews see, e.g., Esposito et al. (2014), Minuti et al. (2020)). For instance, levels of IgG and IgM antibodies are decreased (Herr et al. 2011; Kimura et al. 2002; Nonnecke et al. 2003) and the function of neutrophils, including phagocytosis capacity and their ability to fight bacteria, is impaired in periparturient dairy cows (Kehrli 1989; Hoeben et al. 2000; Rinaldi et al. 2008). Moreover, high plasma concentrations of NEFA and ketone bodies as well as the degree of negative energy balance are associated with reduced function and proliferation of various immune cell populations (Holtenius et al. 2004; Lacetera et al. 2005; Scalia et al. 2006; Wathes et al. 2009; Ster et al. 2012; Cheng et al. 2021). On the other hand, severe nutritional stress also contributes to the inflammatory-like condition experienced by dairy cows around calving, which is characterized by elevated levels of pro-inflammatory cytokines (Mann et al. 2018; Mezzetti et al. 2020). Because the inflammatory-like status of postpartum dairy cows eventually aggravates nutrient deficiency (Trevisi et al. 2010; Mezzetti et al. 2020), distinguishing between the effects of the metabolic status and glucose metabolism on immune function on one hand and the effects of inflammation on the metabolic status on the other, is difficult (van den Bossche et al. 2017).

Contrasting the high number of publications addressing the quality of the interactions between the level of plasma metabolites and immune dysfunction or the occurrence of disease, quantitative approaches addressing the specific demand imposed by regulatory life functions and in particular, by the immune system, have not been a major topic of interest in animal nutrition so far. Yet, an adequate supply of immune cells with essential nutrients is a fundamental prerequisite to enable the cow to cope with the challenges she is exposed to. The immune system plays a major role not only for pathogen elimination but is part of the coordinated reaction of the organism to all kinds of stressors, including metabolic stress (Colditz 2009). The energy- and nutrient-demanding processes of immune activation encompass the general elevation of metabolic rates, increased turnover rates of the leukocyte pool, the energy needed to fuel the synthesis of acute phase proteins and immunoglobulins and the costs associated with reparation of damaged tissues (Colditz 2009).

Glucose is the most important metabolite not only for lactose synthesis in mammary epithelial cells but also for the synthesis of immunometabolites, phagocytosis and the production of reactive oxygen species in immune cells (Barghouthi et al. 1995; Pithon-Curi et al. 2004; van den Bossche et al. 2017). Kvidera et al. (2017) showed that a fully activated immune system of dairy cows exposed to lipopolysaccharide challenges may need up to 3 kg of glucose per day. Assuming an energy content of glucose of 16 KJ / g, this equals ~ 48 MJ. This amounts to almost 1.5 x the basal metabolic rate (i.e., the maintenance requirement) of a dairy cow of 600 kg and is equal to the amount of glucose required for the synthesis of ~40 kg of milk. Besides mammary and immune cells, splanchnic tissues, muscle tissue, red blood cells and the brain are known to rely on glucose as a fuel (Reynolds 2005), while the adipose tissue uses glucose during anabolism only (Smith and Crouse 1984). Moreover, the uterus/fetus require glucose during (late) pregnancy (Oddy et al. 1984). With regard to glucose supply, hepatic gluconeogenesis is the major source of glucose in ruminants, while only a low amount of glucose is directly absorbed from the gut, even if large amounts of starch are fed. Current knowledge on precursor supply for hepatic gluconeogenesis has been summarized by Aschenbach et al. (2010) and Larsen and Kristensen (2013). In brief, major precursors include propionate derived from carbohydrate digestion in the rumen, glucogenic amino acids from intestinal protein degradation and dietary L-lactate as well as glucogenic amino acids and glycerol derived from the mobilization of protein and adipose tissue, respectively, and L-lactate derived from cori-cycling of glucogenic carbon in muscle tissues. A schematic representation of major flows of glucogenic carbon, excluding the demand imposed by immune cells, is displayed in Figure 1.

Figure 3.1. Schematic representation of the flow of glucogenic carbon in lactating ruminants. Reserves indicate the amount of glucose that is potentially available for immune cells.



Maximal gluconeogenic capacity is limited by the supply with precursors as well as by liver health. Data on the hepatic glucose production in dairy cows reveals a maximum of 4 to 5 kg per day (Aschenbach et al. 2010; Larsen and Kristensen 2013; Loncke et al. 2020). With regard to the quantities described above, the supply of sufficient glucose to all tissues is at risk in the case of high milk yields and simultaneous occurrence of immune challenges. However, assessing glucose shortage for immune cells by means of plasma concentrations of glucose, is barely possible. This is not only due to tight homeostatic control in lactation stages other than the immediate postpartum period (Herdt 2000) but also due to the interactions between (neuro-) endocrine and immune-cell-mediated regulation of glucose metabolism: Similar to the metabolic effects associated with the changes in the somatotropic axis around calving, the inflammatory condition postpartum also enhances gluconeogenesis, hypoinsulinemia and insulin insensitivity (Kyriakis and Avruch 2012; Horst et al. 2019). Due to the high number of lactocytes and the strong increase in the number of non-insulin dependent glucose transporter in the mammary gland after calving (Komatsu et al. 2005; Zhao and Keating 2007; Gross et al. 2015), the strong increase in endogenous glucose production is even surpassed by mammary withdrawal of glucose. With dry matter intake lacking behind, this in fact leads to a breakdown in glucose homeostasis. A drop in plasma glucose in the first days after parturition is well described in high-producing dairy cows (Ingvartsen and Friggens 2005; Graber et al. 2012). However, decreases in plasma glucose concentrations were shown to be not proportional to the level of energy deficit but strongly influenced by the stage of lactation (Bjerre-Harpøth et al. 2012). It is thus difficult to differentiate between the effects of inflammation on the metabolic status and vice versa. Consequently, the results of studies investigating the association between plasma glucose levels and the occurrence of disease are somewhat confusing. For instance, Galvão et al. (2010) and Senosy et al. (2012) found lower glucose levels to be associated with the occurrence of uterine disease, while the drop in plasma glucose 3 days after calving was shown to be less pronounced in cows developing metritis in the study of Bicalho et al. (2017). Higher levels of plasma glucose in diseased compared to healthy cows might thus indicate a higher level of immunoactivation rather than increased overall availability of glucose, which primarily depends on the mammary gland’s ability to withdraw glucose.

Other approaches for the evaluation of glucose balance in dairy cows have been presented as well. Reynolds (2005) compiled previous works on several aspects of glucose balance, including the mammary glucose requirements, glucose absorption from starch digestion and the role of endogenous precursors in dairy cows. In the study of Bo-fei Sun et al. (2020), the authors used a method developed by Fu Cong et al. (2014). This is based on the combination of the average contents of metabolizable glucose in feedstuffs and the individual deficiency in dry matter intake to calculate glucose balance of dairy cows. Guo et al. (2007) and Omari et al. (2019, 2020) estimated the intake of metabolizable glucose rather roughly from the dry matter intake, but specifically estimated the glucose demands imposed by the mammary gland, the uterus and “maintenance functions” within the cow to obtain glucose balance. While mammary glucose requirement has often been estimated using an equation reported by Elliot (1976) based on the data of Annison et al. (1974), more recently published articles investigated the regulation of mammary glucose metabolism in greater detail (Lemosquet et al. 2009; Galindo et al. 2011). Data on the fluxes of different glucogenic precursors for gluconeogenesis, including propionate, amino acids and L-Lactate originating from the digestion of feedstuffs as well as glycerol, alanine and L-Lactate originating from tissue mobilization and muscle metabolism were recently reviewed (Aschenbach et al. 2010; Larsen and Kristensen 2013). However, with nutritional databases becoming more exhaustive, more emphasize has been given to the quantification of single glucogenic nutrients in digestive processes as well as in the intermediary metabolism. In particular, findings compiled in the INRA feeding system for ruminants (INRA 2018) and in the publications of Loncke et al. (2009, 2010, 2011), Nozière et al. (2011) and Martineau et al. (2014), who presented regression equations for the estimation of ruminal and portal fluxes of glucose, propionate, L-lactate and amino acids, enabled a precise estimation of glucogenic supplies from the digestion of different rations and feedstuffs. Additionally, the dynamics of tissue mobilization and L-Lactate-recycling, which provide endogenous precursors for gluconeogenesis, have been described intensively (Larsen and Kristensen 2013; INRA 2018; Daniel et al. 2018).

Based on these findings, the following questions, which form the basis of the physiological part of the present thesis, emerged:

1) How do dairy cows deal with glucose shortages during early lactation?
2) How can the individual daily supply with and the demand for glucogenic nutrients of dairy cows, i.e., the glucose balance, be assessed from animal and feed data?
3) How much glucose reserves are available in the intermediary metabolism during early lactation?
4) How does glucose balance vary before, during, and after disease?

In a first step, the metabolic trade-offs between reproductive and productive life functions on one side, and self-sustaining life functions, such as immune defense, on the other, were evaluated with a special focus on the patterns of glucose allocation during early lactation by means of a review article (publication #1).

The second and third question was addressed by developing a methodology for the assessment of daily glucose demands of major glucose-consuming tissues (except for the immune system) and for the assessment of the daily supply with glucogenic nutrients originating from feed intake and from endogenous sources. This allowed for the calculation of daily individual glucose balances of dairy cows indicating their level of glucose reserves available in case of immunoactivation (publication #2).

Finally, the methodology was applied to a larger dataset including health records to address the fourth question (publication #3).

4.3 Quantification of Economic Trade-Offs Related to Biological Efficiencies of Dairy Cows

A comprehensive depiction of the economic situation of a dairy business requires balancing the production input, i.e., the sum of resources such as labor, feed, material, energy, etc.

needed and the production output such as milk, carcasses, and – from a more holistic perspective – animal health and welfare as well as the ecologic sustainability of a farm. Each dairy farm can choose from a mix of input factors as well as from different management strategies that aim at profitably and efficiently combining these factors. Cross-sectional studies investigating the economic impact of technical, management or financial efficiencies are thus a great topic of interest in dairy research. In this regard, significant economic effects were shown to be related to e.g. the farm size (Stankov et al. 2015; Macdonald et al. 2017), the milking system (Bijl et al. 2007; Hansen et al. 2019), the education and age of human resources (Byma and Tauer 2010; Hansen and Greve 2015), participation in herd health programs (Derks et al. 2014) or the debt use of a farm (Gloy and LaDue 2002; Schorr and Lips 2019; Ma et al. 2020)

The farm-specific economic impact of biological efficiencies related to milk production, reproduction, health or longevity, however, depends on the farm-specific levels of milk price, heifer costs, feed costs and other production costs. The amount of milk sold per cow has been shown to be among the most significant variables contributing to the economic success in several cross-farm studies (e.g., Johnson and El-Osta 1998; Gloy et al. 2002; Hansson and Öhlmér 2008; Stankov et al. 2015). On the other hand, high-yielding cows require progressively greater marginal increases in nutrient supply (Vandehaar and St-Pierre 2006; Moallem 2016; Bach et al. 2020). Accordingly, feed costs, which account for up to 50% of the total cost of milk production in high producing dairy herds, are negatively related to measures of financial profitability as well (Hemme et al. 2014; Evink and Endres 2017; Hardie et al. 2014; Johnson and El-Osta 1998).

In dairy practice and the scientific literature, these trade-offs are often captured by measures such as income over feed costs, marginal feed costs or the milk to feed price ratio (Wieck and Heckelei 2007; Atzori et al. 2013; Shoemaker et al. 2019). In addition to these partial output measures, which are obviously important to monitor single factors, such as feed efficiency, however, long-term economic strategies should aim at integrating all input variables (full cost approach) as well as all output variables to identify the most significant economic drivers for the respective farm. While feed costs and milk revenues are central parts of dairy branch budgets, costs related to the health and longevity are often hidden in the aggregated form of business sheets, although they have been shown to play an important role for farm economics (Liang et al. 2017; Mostert et al. 2018; van Soest et al. 2019). Apart from veterinary bills, failure costs related to disease events also emerge from lower milk revenues due to lower performance and discarded milk, reduced fertility and increased labor costs. With 10 to ~ 20 % of all cows dying on farm and ~ 70% of culling being due to disease (Compton et al. 2017; Hoedemaker et al. 2020), increased replacement costs are a major cost item contributing to the monetary losses related to the prevalence of PD’s. The economic implications of replacement encompass not only the costs of rearing a heifer but also the losses in milk yield following premature culling and the genetic opportunity costs associated with the increase in genetic merit of a replacing heifer. Vries (2017) analyzed asset replacement theory with regard to dairy cows and described that although the genetic improvement is accelerating, economic cow culling should not be driven by the increase in genetic merit but by cow depreciation. With carcass values being well below heifer costs, annual cow depreciation costs decrease if longevity increases. Moreover, an older herd – if healthy – is able to produce more milk (and calves) as average lactational milk yields increase with parity, at least until the 4th lactation (Cabezas-Garcia et al. 2021). Accordingly, maximum profitability of cows was supposed to be reached not before the 5th (Vries 2017), 6th (Wangler et al. 2006; Horn et al. 2012) or 7th lactation (Missfeldt 2015) in different production systems. Yet, the average length of the productive life is 2 to 4 years and 2.5 to 3.5 lactations in countries with intensified milk production (Vries and Marcondes 2020; Hoedemaker et al. 2020).

Not only due to the volatility of external factors, including prices and national policies, but also due to the heterogeneity of accounting and management strategies of dairy farms, however, finding a common ground for the economic evaluation of biological efficiencies seems difficult. For instance, Barkema et al. (2015) supposed that declining milk prices may force farmers to reduce costs associated with disease curation or prevention, such as labor costs associated with individual animal care or costs for the participation in herd health programs, which would negatively affect the health, welfare and longevity, and in the long-term, performance of the herd. In organic production, subsidies, higher milk prices as well as lower total feed costs following increased self-sufficiency may outweigh the effects of lower milk yields and/or costs associated with disease curation and prevention (McBride and Greene 2009; Naglova and Vlasicova 2016; Nehring et al. 2021).

Keeping in mind that the processes responsible for biological efficiencies occur within an organism, the individual cow could be regarded as both the basic biological and the basic economic unit of the dairy production. In fact, individual cows can only contribute to the (economic) survival of the farm or dairy branch, if their revenues from milk sold (and, if applicable, calf sales and slaughter) override their costs of rearing, feeding and other production costs required to keep the cow in the herd. This requires, however, the breakdown of branch budgets from herd-level to animal level and a sound distribution of all costs and revenues between different levels of cow performance in various aspects of their service life, including measures of productivity and longevity. Because only a full cost approach at the individual animal level overcomes limitations in the interpretability of partial output- and partial efficiency measures, a methodology for the quantification of the economic results of individual culled cows was developed. With regard to the limited applicability of generalized economic recommendations to individual, heterogenous dairy businesses, the economic variation emerging from differences in the service life characteristics between cows living and producing within the same economic boundaries of a specific farm may be better suited to pinpoint farm-specific drivers of profit or loss.

Therefore, the following research questions underly the economic part of the present thesis and are addressed in publication #4.

1) How can dairy branch budgets and herd data be combined at animal level to account for monetary differences associated with the duration of rearing, the duration of the productive life, the level of production and the circumstances of culling?
2) How does the profit of individual culled dairy cows vary between dairy businesses and production methods in Germany?
3) Which service life characteristics have the greatest effect on individual cow's profit or loss in different economic circumstances?

5. Publication #1 - Mismatch of Glucose Allocation between Different Life Functions in the Transition Period of Dairy Cows

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Keywords

glucose metabolism; immune dysfunction; production diseases; lactational physiology; trade-offs

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Simple Summary

The reasons for the development of diseases in the transition period of dairy cows are manifold and highly farm- and cow-specific. Nevertheless, links exist between the degree of negative energy balance (NEB) and disease susceptibility, which suggest a mutual adverse relationship between immune and productive functions. Glucose is the most essential fuel and precursor for both immune cells and mammary epithelial cells (MEC). While the delivery of glucose by the intermediary metabolism is not always able to keep up with whole-body demands, trade-offs between mammary and immune cells emerge. The prioritization of mammary supply during early lactation is a physiologic principle in all mammals. In contrast, tremendous increases in milk yield and the specific demand for glucose in high-yielding dairy cows resulting from decades of selection for milk production override the evolutionary principles of nutrient partitioning. Therefore, high-producing dairy cows face an increased risk of glucose shortages in their immune cells, particularly during early lactation.

Abstract

Immune cell functions such as phagocytosis and synthesis of immunometabolites, as well as immune cell survival, proliferation and differentiation, largely depend on an adequate availability of glucose by immune cells. During inflammation, the glucose demands of the immune system may increase to amounts similar to those required for high milk yields. Similar metabolic pathways are involved in the adaptation to both lactation and inflammation, including changes in the somatotropic axis and glucocorticoid response, as well as adipokine and cytokine release. They affect (i) cell growth, proliferation and activation, which determines the metabolic activity and thus the glucose demand of the respective cells; (ii) the overall availability of glucose through intake, mobilization and gluconeogenesis; and (iii) glucose uptake and utilization by different tissues. Metabolic adaptation to inflammation and milk synthesis is interconnected. An increased demand of one life function has an impact on the supply and utilization of glucose by competing life functions, including glucose receptor expression, blood flow and oxidation characteristics. In cows with high genetic merits for milk production, changes in the somatotropic axis affecting carbohydrate and lipid metabolism as well as immune functions are profound. The ability to cut down milk synthesis during periods when whole-body demand exceeds the supply is limited. Excessive mobilization and allocation of glucose to the mammary gland are likely to contribute considerably to peripartal immune dysfunction.

1. Introduction

Animal welfare concerns, as well as the economic implications of the high prevalence of production diseases [1–3] and increasing rates of involuntary culling in dairy farming [4], emphasize the need to address possible conflicts of aims between these impacts and the level of milk production. Many scientific investigations assume that individual genomic and/or metabolomic differences are able to explain why some cows are both high producing and healthy while others fail to cope [5–8]. These approaches try to identify cows that are more efficient in digestion, absorption, synthesis and mammary utilization of nutrients. However, they disregard the limitations in the capacity to deal with deficiencies in a highly heterogeneous and dynamically changing environment that is elusive to deterministic approaches. They disregard that immune functionality is fundamental to ensure health, longevity and productivity of dairy cows, as it is not only essential for pathogen elimination but is part of the coordinated reaction of the organism to all kinds of stressors.

After parturition, high-producing dairy cows generally enter a negative energy balance (NEB), because their level of dry matter intake (DMI) does not meet the demands imposed by the onset of milk production [9]. Consequently, they mobilize body tissue to overcome this shortage. Excessive mobilization can lead to a hypercatabolic response described as metabolic stress, associated with the occurrence of subclinical and clinical diseases [10]. The overall energy budget of an organism includes various energy sources, metabolic pathways and interactions between subsystems of nutrient trafficking that make it difficult to evaluate the consequences of sustained overall NEB on metabolic disorders and health. In fact, plasma concentrations of single metabolites vary substantially between individual cows with similar status of NEB in early lactation [11–14]. Thus, it has been emphasized that we must move “from joules to moles of molecules or groups of molecules” to advance animal nutrition concepts [15].

In dairy cows, the amount of glucose required to fuel milk production outreaches by far energy expenditures of other life functions like reproduction or maintenance [16]. Besides being a precursor for the synthesis of lactose, which is the osmotic regulator of milk volume [17,18], glucose-derived carbon is also found in milk fat and protein [19,20]. Moreover, reduction of NADP+ through pentose phosphate pathway as well as the production of ATP, which are required for the synthetic processes depend on the availability of glucose. During peak lactation, mammary epithelial cells are able to retrieve up to 2.7 kg of glucose per day from the plasma pool at a milk level of 40 kg [16]. On the other hand, cells of the innate and adaptive immune system rely largely on the uptake of glucose and the storage of glycogen, because glucose supports proliferation, survival and differentiation as well as essential functions like phagocytosis and reactive oxygen species (ROS) production [21]. Recent data about dairy cows exposed to lipopolysaccharide (LPS) challenges showed that a fully activated immune system needs 2.5 to 3.1 kg of glucose per day [22]. In other words, immunoactivities in dairy cows can amount to degrees of glucose demands similar to those required for high milk yields. In contrast to monogastric species, ruminants cover their glucose demand almost exclusively through hepatic gluconeogenesis, which seems limited to about 3 kg of glucose at a milk yield level of ~40 kg/day [23]. These considerations and the quantities at hand clearly indicate a competitive situation between milk production and immune defense and give rise to the question of how allocation of nutrients between different tissues and life functions is regulated when essential nutrients become scarce.

2. Resource Allocation between Maintenance and Productive Life Functions in Early Lactating High-Producing Dairy Cows

2.1. Resource Allocation Theory

According to the resource allocation theory [24], resources including energy and essential nutrients must be partitioned between all life functions. Common differentiations of life functions that an animal must fuel include productive processes such as growth, gestation and lactation as well as maintenance functions. However, definitions of maintenance and the requirements of regulatory systems are essentially conceptual, of a qualitative nature and are also imprecise and contradictory throughout the scientific literature [25,26]. However, activities like thermoregulation and immune function are known to impose high demands [27].

As for the relationship between productive and other life functions in dairy cows it was hypothesized that cows with high genetic merit for milk production cover their demand by an increase in feed intake, while cows with low genetic merit—if they do consume more feed—accumulate body reserves [28]. Consequently, maintenance requirements would be unaffected by milk yield, and differences in milk yield could be due to differences in the efficiency of energy and nutrient utilization—a phenomenon that was described as “dilution of maintenance” [28]. Although correlations between yield and intake ranging from 0.46 to 0.65 [29] indicate the strong relationship between these variables, they reveal as well that the increase in feed intake does not keep pace with the increased demand imposed by an increased number of lactocytes in the mammary gland. Accordingly, increases in body weight associated with selection for milk production have been shown to increase maintenance requirements as well [30]. While energy expenditures by visceral organs represent 0.4-fold of the maintenance requirements for non-productive adults, they increase to 1.2-fold for lactating ruminants [31]. Moreover, increased energy and nutrient demands for milk synthesis may also lead to time constraints since eating and rumination time must be traded off against all other activities. In fact, there is little sign that high genetic merit cows digest feed more efficiently than low genetic merit cows. It has been shown decades ago that digestive efficiency decreases as feed intake increases [32]. Accordingly, the nonlinear character of the relationship between milk yield and body weight suggests an optimum body weight beyond which the feed-efficiency decreases in specialized breeds [33]. Loncke et al. (2020) recently showed a similar pattern for the efficiency of hepatic glucose synthesis (conversion rate of precursors to glucose), which decreases at high levels of precursor supply [34]. As a result, high-producing dairy cows show increased rates of energy mobilization from body tissues to bridge the gap between the supply of energy from feedstuffs and the energy needed to support milk production along with all other energy-demanding life functions [35].

2.2. Homeorhetic and Allostatic Control of Nutrient Partitioning

Because most organs and tissues lack autonomy to control their nutrient access, the organism needs prioritization rules that coordinate nutrient partitioning between different life functions in all situations where demand exceeds supply. In this regard, different concepts of regulation have been established and adapted to dairy cow physiology. In the concept of homeorhesis, nutrient partitioning is described as a function of biological needs that alternates cyclically between storing and mobilization of energy from body tissues and the associated prioritization of reproductive and productive functions [9]. Following this approach, it is a fundamental biological principle that after parturition, mammalian organisms prioritize mammary tissues to provide an adequate supply to the neonate. Dairy production is based on, and takes advantage of, this principle through a performance-oriented selection of animals.

However, environmental and nutritional stressors associated with varying quality and quantity of feedstuffs, social stress, climate variability and extremes, poor hygiene, technical failure, etc. also affect supply and demand of nutrients independent of the physiological state [27,36]. In this regard, it has been emphasized that systematically reviewing the literature from epidemiological studies is unlikely to support understanding of the effects of metabolic imbalances of each cow in her specific genetic and environmental circumstance since intricate biological correlations—besides within- and between-herd confounding effects—exist [37].

The concept of allostasis goes beyond the concepts of homeostasis and homeorhesis by assuming dynamic set points emerging from the integration of both the requirements of different tissues at different physiological states and current nutritional, social and housing conditions [38]. In dairy cows, various genotype x environment interactions have been described, including a reduced ability to adapt to unfavorable conditions (plasticity) in cows with high genetic merit for milk production [39]. This suggests that the process of adaptation to such conditions imposes a certain demand for energy itself, which has been described qualitatively as the “allostatic load” of the animal [40]. If the allostatic load becomes too big (allostatic overload), the capacity to cope with additional stressors is reduced and the animal enters a pre-pathological state [41]. However, by considering maintenance functions as costs, they must be traded off against productivity goals. Following this approach, resource availability for immune cells provided through a balanced resource allocation is a prerequisite for an organism to be able to cope with internal and external stressors.

3. Glucose Metabolism to Fuel Milk Synthesis and Immune Functions in Dairy Cows

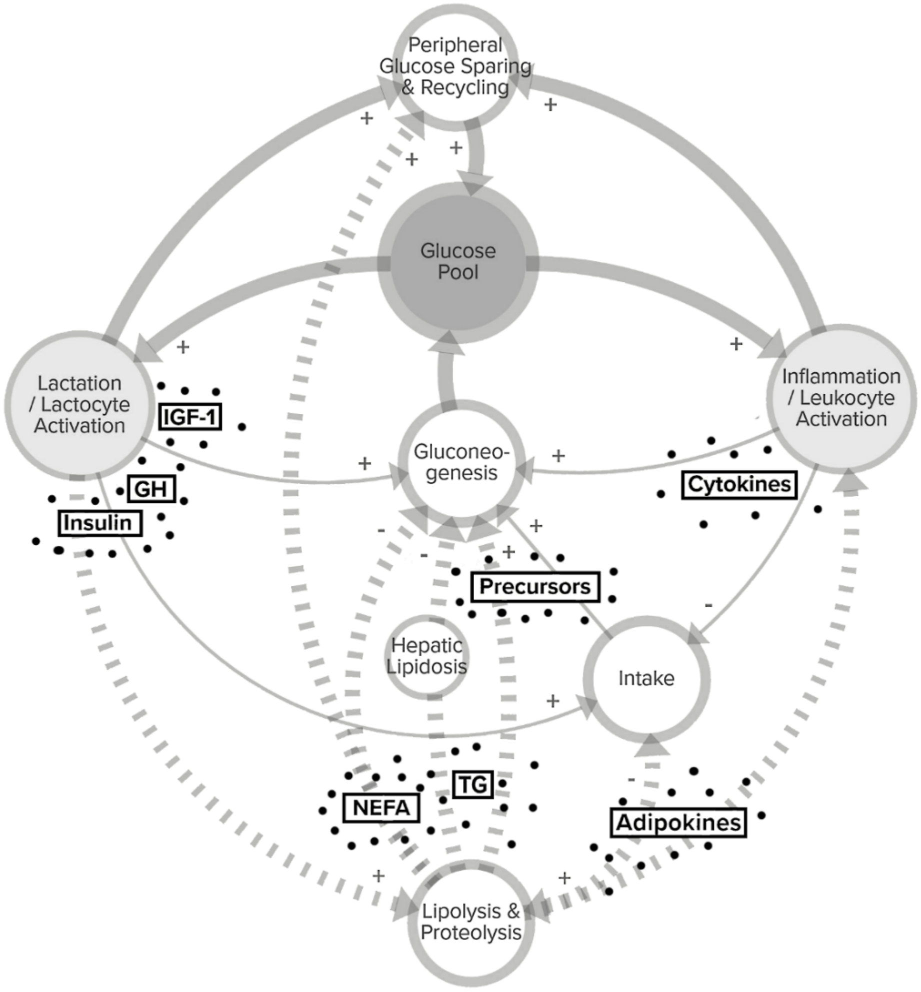
Proinflammatory signaling promotes similar metabolic adaptations, as does the lactational “reprogramming” with the aim of maximizing glucose availability to the respective cell types (Figure 4.1). These changes are mediated by a network of hormones and affect both mobilization and allocation, including specific regulation of blood flow and receptor expression patterns in peripheral, mammary and immune tissues. In the following chapters, the metabolic processes associated with the onset of lactation (Section 3.1) and those occurring during inflammation (Section 3.2) are discussed separately. Subsequently, trade-offs for glucose between MEC and immune cells in dairy cows are evaluated in Section 4.

3.1. Adaptation to Lactation

Morphological changes required for mammary growth, morphogenesis, and milk synthesis are created during gestation, with ductal elongation and lobulo-alveolar development being mediated through high plasma concentration of prolactin, growth hormone (GH) and gonadotropic steroids progesterone and estrogen before parturition [42,43]. Subsequently, high concentrations of GH are known to stimulate galactopoiesis, while prolactin and Insulin-like growth factor 1 (IGF-1) are involved in establishing and maintaining milk synthesis through their effect on epithelial cell differentiation and survival [44,45].

To meet the sudden increase in demand during early lactation, further alterations in the endocrine setup support dairy cows to metabolically adapt to lactation. Besides the above-mentioned hormones, insulin, thyroid hormones, glucocorticoids and the gonadotropic axis are main effectors of the new catabolic physiology and plasma concentrations of these hormones typically fluctuate at the transition from a pregnant to a lactating physiology [46]. Especially changes in the somatotropic axis, i.e., decreased pancreatic secretion of insulin and reduced GH-receptor (GHR) expression in the liver [47] are thought to be major levers of the new catabolic physiology [48–50]. Subsequently, hepatic GH resistance and hypoinsulinemia mitigate stimulating effects on hepatic IGF-1 production [51]—a condition that has been described as the “uncoupling of the somatotropic axis” [48].

Figure 4.1. Schematic representation of metabolic pathways related to the glucose balance of dairy cows during lactation and inflammation. Milk synthesis and immune defense rely on an adequate supply with glucose, as it is an important energy source and precursor for lactocytes and leukocytes. To increase overall glucose availability to the respective cell type, inflammatory signals (cytokines) as well as peripartal fluctuation of hormones associated with the somatotropic axis such as growth hormone (GH), insulin and insulin-like growth factor 1 (IGF-1) enhance (+) the rate of gluconeogenesis and affect the level of intake (solid lines, thin), and increase the mobilization of body reserves (dashed arrows). Lipolysis and proteolysis provide endogenous glucose precursors such as alanine and glycerol as well as alternative energy sources like non-esterified fatty acids (NEFA) that help spare glucose in peripheral tissues, where insulin sensitivity is reduced. Because glucose uptake is non-dependent on insulin in both leukocytes and lactocytes, trade-offs for glucose allocation (solid arrows, bold) may arise in situations where inflammation and lactation impose high demands. Limitations may also arise from negative effects (-) of adipokines and cytokines on the hypothalamic regulation of intake and from hepatic accumulation of triglycerides (TG) and NEFA when lipolysis is excessive.



Insulin signaling is particularly essential for a successful adaptation to lactation by affecting the rate of lipolysis, the rate of uptake and transport of glucose and fatty acids to different tissues, and the expression of key enzymes at the metabolic crossroads of glucose and fatty acid metabolism [52,53]. A main effect of peripartal hypoinsulinemia is the reduction in insulin's antilipolytic properties, which facilitates the mobilization of nutrients from body reserves by increasing the rates of lipolysis and proteolysis [54]. These processes are accompanied by increased rates of gluconeogenesis, reflected by an increased mRNA amount of the important rate-limiting enzymes pyruvate carboxylase (PC) and phosphoenolpyruvatecarboxykinase (PEPCK) postpartum [55]. Precursors for gluconeogenesis include rumen-derived volatile fatty acids, mainly propionate and, to a lesser extent, circulating C3-bodies like glycerol, alanine and lactate from intermediary metabolism [23]. Increased levels of circulating non-esterified fatty acids (NEFA) resulting from adipose tissue remodeling are taken up proportionally to their plasma level by the liver. Together with a simultaneous lack of oxaloacetate, which is highly used for gluconeogenesis, increased NEFA lead to an accumulation of acetyl-CoA in the liver. Subsequently, hepatocytes are forced to switch acetyl-CoA utilization from complete (Krebs cycle) towards incomplete oxidation (ketogenesis) and/or to re-esterification with subsequent storage of triglyceride in the liver [56]. Besides negative effects of hepatic TG accumulation on general hepatic function, increased levels of β-hydroxybutyrate (BHB) were shown to impair gluconeogenic capacity [57,58]. In cultured bovine hepatocytes, increasing levels of NEFA gradually decrease mRNA levels and catalytic activity of PC and PEPCK [59]. Thus, glucose balance is challenged severely when lipolysis becomes excessive. Although the usefulness of plasma glucose as an indicator of a cow's metabolic status is particularly contentious due to the tight regulation of glucose homeostasis [60], hypoglycemia is associated with the onset of ketosis, higher first test-day milk production and milk production at 100 days in milk [61].

Moreover, allocation patterns that regulate the flow of nutrients between different tissues within the organism change according to the new dominant physiological state of lactation. Again, these changes are related to the 'uncoupled' somatotropic axis and in particular, to the phenomenon of reduced insulin sensitivity in peripheral tissues of postpartum dairy cows [62–65]. Because glucose receptors prevailing in the mammary gland are mostly non-dependent on insulin while muscle and adipose cells are highly insulin-responsive cell types [66], reduced peripheral insulin sensitivity favors the glucose supply of lactocytes. Simultaneously, mRNA encoding insulin-independent glucose transporter (GLUT) with the highest affinity to glucose (GLUT1) increases strongly in mammary tissues at the onset of milk synthesis [67]. As lactation advances, the mammary gland becomes more insulin-sensitive and insulin-dependent while glucose uptake via GLUT4 increases [68]. In contrast, GLUT1 decreases about 6-fold in mRNA and protein levels in adipose tissue of early lactating cows compared with dried off or late lactating cows [67]. However, the extraction of great amounts of glucose from circulation is promoted primarily through a greater blood flow to the mammary gland, which was found to be stimulated by the characteristic endocrine regulation of lactation [69,70]. In particular, thyroxine is thought to enhance mammary nutrient extraction by increased heart rate and subsequent increases in blood flow [71]. This was identified decades ago as a main determinant of quantitative udder metabolism [16,72]. In more recent studies, blood sampling techniques comparing glucose concentrations from the jugular and mammary vein showed a lower jugular/mammary quotient for glucose concentration in dry and low-yielding cows, while revealing significantly higher levels in high yielding cows [73].

In peripheral tissues, modest reductions [74,75] or no significant changes [67] in the expression of insulin-dependent GLUT 4 in peripheral tissues have been reported in early lactating dairy cows. However, these tissues are aligned to save glucose during that period by shifting their glucose metabolism from complete oxidation towards lactate production. Accordingly, irreversible losses of glucose excluding the loss in milk lactose decreases significantly in the first days after parturition [76]. Together with alanine and glycerol derived from muscle resp. adipose tissue, lactate can recirculate to the liver, where it is supposed to have a higher proportional contribution to gluconeogenesis during early lactation [23].

In summary, a complex endocrine network develops to increase glucose availability to the mammary gland. If precursor supply or hepatic synthetic capacity are inadequate, the sudden increase in mammary demand for glucose at the onset of lactation is the main driver of the hypermetabolic reaction that affects a variety of metabolic pathways, tissues and organs within the organism.

3.2. Adaptation to Inflammation

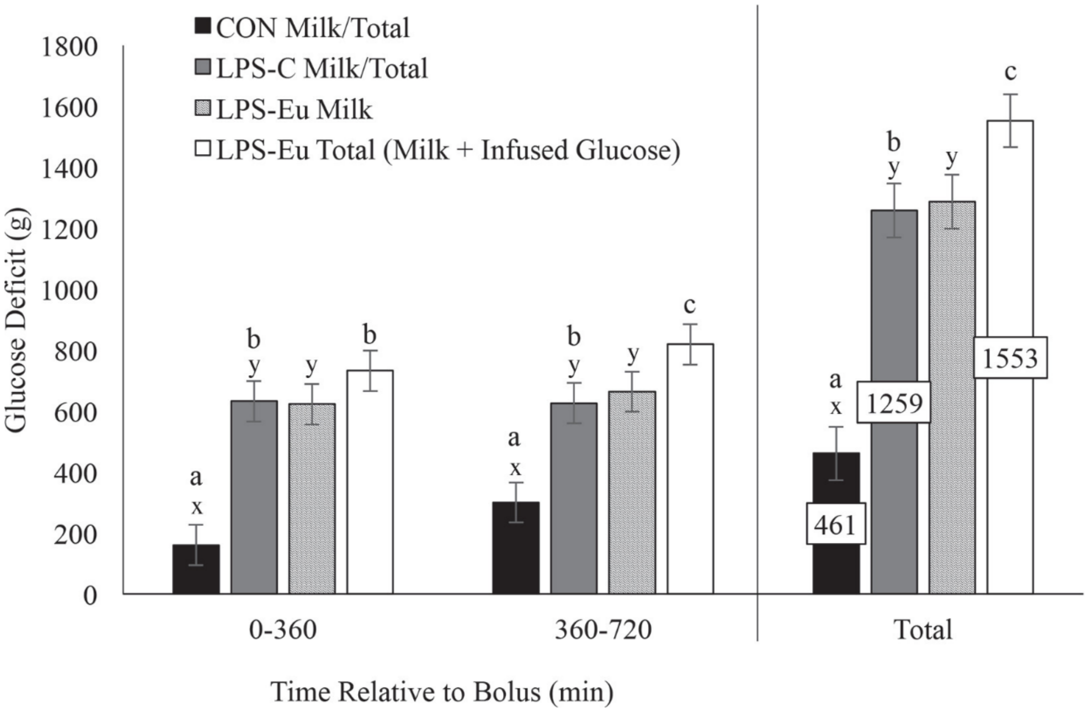
Immune cell activity and inflammation are not only essential for pathogen elimination but are part of the coordinated reaction of the organism to all kinds of stressors, including infective and non-infective, metabolic and environmental stressors. After parturition, dairy cows experience an inflammatory-like status, which is systemically linked to the inherent stress of parturition, social and nutritional changes and the endotoxin-releasing processes of ruminal adaptation and uterine tissue reorganization [77,78]. The response is characterized by a marked increase in plasma concentration of positive acute phase proteins [79]. Their plasma level has been associated with the occurrence of retained placenta, other diseases and impaired reproductive and productive performance during early lactation [80,81]. However, the necessity of some degree of “physiological inflammation” is illustrated by the action of anti-inflammatory drugs that inhibit the synthesis of prostaglandins required to expel the placenta [82]. Following administration of an anti-inflammatory drug after calving, dairy cows have an increased risk of retained placenta (2.5-fold) and metritis (1.5-fold) [83].

Whatever the origin of inflammation, the accumulation of proinflammatory processes implies a supply with energy that adequately meets the requirements of immune response. Qualitatively, the costs of immune activation include (1) a general elevation of metabolic rates due to a rise in body temperature, (2) reduced nutrient availability following anorexic effects of proinflammatory signaling, (3) the precursors and energy needed to fuel the synthesis of acute-phase proteins and immunoglobulins, (4) altered priorities for nutrient utilization in other tissues, (5) the costs associated with the repair of damaged tissues and (6) increased turnover rates of the leukocyte pool [27]. Although an almost infinite number of possible combinations between metabolic and environmental stressors make it impossible to estimate the current degree and duration of inflammation and immunoactivation and to determine the energy demand of immune cells, some quantification has been performed. For instance, it has been shown that the demand for oxygen, glucose and glutamine increases two- to three-fold during lymphocyte activation [84]. By examining the effect of an infection with nematode larvae on the energy requirement of merino sheep, it was estimated that infection increased the requirement for metabolizable energy by 28% [85]. Even more impressively, Kvidera and colleagues combined an intravenous LPS challenge, a euglycemic clamp and measurement of milk yield reduction in cows of parity 2 or 3 that were at 69 ± 7 days in milk to calculate the demand of a fully activated immune system. The authors estimated that dairy cows may require up to 3.1 kg of glucose per day to mount an acute inflammatory response (Figure 4.2) [22].

In fact, cells of the innate and adaptive immunity rely largely on the uptake of glucose and the storage of glycogen, because glucose supports proliferation, survival and differentiation as well as essential functions like phagocytosis and ROS production [21]. Moreover, an activation of apoptotic pathways in response to limited glucose uptake in cultured hematopoietic cells was reported [86]. In dairy cows, reduced glycogen concentrations in circulating neutrophils at calving indicate a depletion of glucose depots during this challenging period and are associated with the occurrence of subclinical endometritis and metritis [87]. Although immune cells are able to use alternative energy sources like glutamine and ketone bodies to some extent [88–90], the importance of glucose as their main fuel was corroborated by Noleto et al., who found that supplying increasing amounts of glutamine in the absence of glucose was not sufficient to raise the inflammatory response to LPS in endometrial monocytes and macrophages of dairy cows, whereas supplying more glucose was able to increase inflammation in the absence of glutamine [91].

Figure 4.2. Milk or total glucose deficit from zero to 360, 360 to 720, and accumulated over 720 min in cows administered a bolus of saline (CON), lipopolysaccharide (LPS-C), or lipopolysaccharide accompanied with a euglycemic clamp (LPS-Eu). Different letters (x,y) represent differences between milk glucose deficits ($p \leq 0.05$). Different letters (a–c) represent differences between total glucose deficits ($p \leq 0.05$; total glucose deficit = milk glucose deficit in CON and LPS-C cows; total deficit = milk glucose

deficit + infused glucose in LPS-Eu cows). Results are expressed as least square means ± standard error of means. Reprinted from Kvidera et al. (2017), Copyright (2017) with permission from Elsevier.



Not surprisingly, leukocytes trigger a number of metabolic pathways that increase the glucose supply to these cells while reducing consumption of glucose by other tissues. First references describing the link between inflammation and insulins actions date far back [92]. By now it is clear that the interplay between proinflammatory and insulin signaling is common to all the mammals [93]. In dairy cows, the effect of continuous and increasing LPS-infusion on whole-body insulin-resistance has recently been demonstrated [94]. T-cells were shown to shift glucose transporter expression from insulin-dependent GLUT4 towards GLUT1 and GLUT3, which are non-dependent on insulin, to maintain glucose disposal during activation [95–97]. Inflammatory pathways also promote the transcription of gluconeogenic genes via toll-like receptor 4 (TLR-4) [98]. Macrophages and neutrophils undergo a metabolic switch from oxidative phosphorylation towards glycolysis during activation, thereby increasing their demand for glucose as well as their lactate production [99]. Metabolic reactions to the alterations induced by proinflammatory cytokines further encompass increased rates of lipolysis and proteolysis, that could provide energy for leukocyte functions as well as substrates for gluconeogenesis [100,101]. However, the inflammation-mediated metabolic reprogramming appears very similar to the reprogramming mediated by lactation, both aiming at a maximum supply of glucose for the respective cell functions. On a systemic level, this includes increased rates of gluconeogenesis and reduced glucose consumption in peripheral tissues.

4. Trade-Offs for Glucose between Lactocytes and Leukocytes

All mammals favor the supply of nutrients to the mammary gland during early lactation. In contrast, the increases in milk yield and the specific demand for glucose required for high milk yields override evolutionary principles of nutrient partitioning [102]. Because nutritional supply is limited through various factors, e.g., percentage of concentrate in the diet, time to eat, turnover rates in the rumen as well as the synthetic capacity of the liver during this period, most high-producing dairy cows experience a period of glucose shortage. In such situations, trade-offs for glucose between MEC and immune cells are unavoidable as they both rely on this essential substrate. Therefore, a special focus on the allocation dynamics of glucose is necessary during periods when both lactation and inflammation impose high demands.

4.1. Peripartal Immune Dysfunction

The phenomenon of reduced immune cell competence is well established in peripartal dairy cows. It is broadly characterized by a dysfunction of PMN, macrophages and lymphocytes, including an impairment of viability, survival, phagocytosis and respiratory burst capacity [103]. Studying gene expression profiles in the bovine mammary gland during stage I and II of lactogenesis, it was found that most of the genes associated with immune response were downregulated at the end of gestation [104]. This is in line with the interpretation of Goff and Horst, who suggested that neutrophil phagocytosis and lymphocyte proliferation begin to be impaired around three weeks before parturition [105]. Moreover, significant changes in lymphocyte subsets occur. Overall number and proliferation of circulating lymphocytes are reduced, while mammary cell number and proliferation peaks around calving [43,106–108]. Accordingly, altered immune functions during the dry period are associated with the development of metabolic disease during early lactation [106]. Around parturition, elevated levels of glucocorticoids and decreased plasma levels of estrogens and progesterone also affect immune response through altered MHC-expression, cytokine production, diapedesis capacity and viability of immune cells [109–111]. In summary, the mammary gland prepares for lactation not only by improving functionality but also by suppression of competitive functions, allowing more resources to be used for milk synthesis [104].

4.2. Metabolic Stress and the Immune System

The aforementioned relationships suggest that the substantial but transient suppression of immune functions before parturition is related to the physiological adaptation to lactation. However, not only cell number and proliferation, but also functionality of immune cells is impaired strongest when MEC start the abundant synthetic activity of lactogenesis as was demonstrated by the transient loss of expression of vascular factors and antimicrobial chemokines [104,112–114]. Accordingly, mastectomized cows had a shorter and less marked immune suppression, including less impairment of oxidative burst capacity and faster recovery of myeloperoxidase activity in neutrophils at calving compared to non-mastectomized cows [115,116]. This indicates that some immunosuppressive effects may be independent from the endocrine changes associated with parturition but related directly to the capacity to synthesize milk. In fact, various effects of severe NEB on immunosuppression have been published and many of them are related to the effects of adipose-tissue remodeling on key molecules involved in glucose and lipid metabolism [117]. For instance, high plasma levels of BHB are negatively correlated with DNA replication and repair in leukocytes [118]. Plasma concentration of NEFA correlate with increased hepatic expression of mRNA encoding proinflammatory cytokines and acute-phase proteins [119]. Increased hepatic uptake of NEFA may also result in increased production of reactive oxygen species (ROS) that carry out important tasks of immune defense by facilitating the destruction of pathogens and enhancing the proinflammatory cascade at physiological plasma concentrations [120]. They can affect the integrity of immune cells, which are very susceptible to peroxidation due to high concentrations of polyunsaturated fatty acids in their membranes. Additionally, ROS generated during inflammation have been proposed to play a role in mediating insulin resistance [121]. If NEFA mobilization and ROS production is excessive, host tissues may fail to mitigate the negative effects of ROS by activation of antioxidant pathways, resulting in severe tissue damage [122]. Leukocyte function is also affected by the shift in fatty acid profile resulting from lipomobilization [123]. Altered concentrations of adipokines postpartum mitigate stimulating effects on chemotaxis and phagocytosis of neutrophils, proliferation of native T-cells and the secretion of cytokines as well as anti-inflammatory effects [124,125], presumably via activation of TLR-4 and nuclear factor kappa-B (NFkB) [74,126].

Accordingly, cows with severe NEB have a reduced ability to clear uterine infection postpartum. The active uterine inflammatory response in these cows was associated with impaired local insulin-receptor signaling [127]. In the mammary gland of lactating dairy cows subjected to a dietary-induced NEB, expression of genes related to proinflammatory signaling via NFkB (AKT1, IRAK1, MAPK9 and TRAF6), IL-8 (e.g., CXCR1/R2) and chemokine signaling (e.g., SOCS2) were downregulated [128].

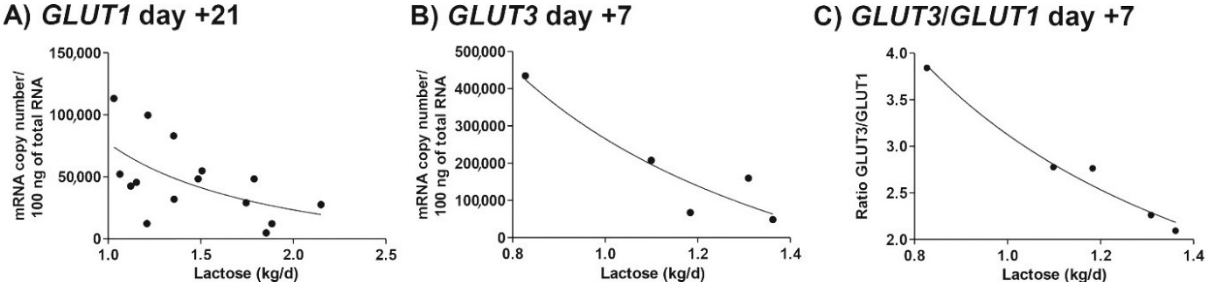
Nevertheless, experimentally induced negative energy balance in advanced lactation was repeatedly shown to be unable to cause alterations of inflammation and immune cell function that are as severe as those occurring during early lactation [129–131]. With regard to the importance of glucose for immune cells, a possible mechanism associated with different reactions to similar NEB could be an increased glucose availability during late lactation, as it was demonstrated that late-lactation induced NEB evoked less severe proportional decreases in plasma glucose concentrations, compared to early-lactation NEB [132,133].

4.3. Competition for Glucose between MEC and Immune Cells

As addressed previously, the usefulness of plasma glucose as an indicator of a cow's metabolic status is unsure due to the tight regulation of glucose homeostasis [60]. However, Graber and colleagues differentiated metabolically robust or vulnerable cows based on the occurrence of various metabolic and (re)productive disorders in previous lactations and identified plasma glucose as the only variable explaining the differences between those groups at both time points investigated (3 weeks before and 4 weeks after parturition) [12]. In another study, plasma concentrations of glucose and insulin during lactation were found to be the single most important predictors related to the development of disease, explaining 36% of the between-cow variability in energy-corrected milk [14].

Generally, dietary energy supply affects glucose oxidation and transport in leukocytes in ruminants [134,135] and provide hints regarding the special competition for this essential metabolite. Inversely, elevated plasma concentrations of the acute phase protein haptoglobin are associated with remarkable decreases in milk yield [81,136]. Anti-inflammatory treatments substantially increase lactational milk yield [137,138]. This demonstrates that inflammation has some kind of regulatory potential of on mammary glucose extraction. Still, the question 'how nutrient partitioning is regulated when resources become scarce' remains. In this regard, it was speculated that decreases in monocyte GLUT1 protein and mRNA expression after calving are due to lactogenesis [135]. In fact, Eger et al. demonstrated a direct negative correlation between lactose yield and overall expression of GLUT1 and GLUT3 as well as a decrease in GLUT3/GLUT1 ratio of monocytes with increasing lactose yield (Figure 4.3.A-C) [96]. On the other hand, downregulation of some GLUT isoforms in the mammary gland was observed following LPS-induced mastitis during mid lactation [139]. However, mRNA abundance of mammary GLUT1 transporter, which is the most important one for lactose synthesis [140], does not decrease in cows submitted to a hyperinsulinemic–hypoglycemic clamp, not even when these cows were submitted to an additional intramammary LPS challenge [139]. As described above (Section 3.1), mammary extraction of glucose from the plasma pool is likely to not be limited by GLUT expression of these cells and the plasma concentration of glucose but rather depends on the rate of local blood flow resulting from the metabolic activity of the gland. In contrast to mammary epithelial cells, circulating immune cells rely on the rate of GLUT expression and increases in types of GLUT that are insulin-independent to cover their glucose demand.

Figure 4.3. Peripartal monocyte glucose transporter expression is correlated with lactose production. glucose transporter 1 (GLUT1) (A) and GLUT3 (B) mRNA expression as well as the GLUT3/GLUT1 ratio (C) at d + 7 and d + 21 relative to parturition were correlated with milk production data of wk 1 (n = 5) and wk 3 (n = 15) of lactation, respectively, using Pearson (GLUT1 and GLUT3/GLUT1) or Spearman (GLUT3) correlation. Significant correlations with lactose production (at least $p < 0.05$, $r > -0.50$) were followed by nonlinear regression analysis (inverse model: $Y = B0 + B1/X$). (A) GLUT1 d + 21 (R2 = 0.314, B0 = −30,557, B1 = 107,794), (B) GLUT3 d + 7 (R2 = 0.871, B0 = −495,507, B1 = 761,265), and (C) GLUT3/GLUT1 ratio d + 7 (R2 = 0.975, B0 = −0.432, B1 = 3.549). Reprinted from Eger et al. (2016), Copyright (2016) with permission from Elsevier.



Decreases in lactose yield as well as decreases in the mRNA-abundance of the ALA-subunit of lactose synthase were reported in hypoglycemic cows [139,141]. Similarly, reduced lactose content in milk following intramammary infection was reported [142,143] and could be a mechanism to save glucose for immune functions or/and to reduce substrate for bacterial growth during infection. On the other hand, Kreipe et al. showed that fat and protein percentages increased in hypoglycemic cows while energy-corrected milk did not differ significantly between hypoglycemic and control animals [141]. Thus, the extraction of glucose by the mammary gland might be unchanged during hypoglycemia, whereas glucose partitioning within the mammary gland is shifted from lactose synthesis towards glycolysis and pentose phosphate pathway to support protein and fat synthesis as was detected in bovine MEC exposed to various levels of glucose [144].

In fact, high-producing dairy cows were shown to be unable to reduce milk synthesis during early lactation in particular, while being able to reduce milk synthesis during induced energy deficiency at 100 days in milk, even if induced NEB was more severe compared to early-lactation NEB [132]. Accordingly, milk yield reductions following infusion of 100 µg LPS were found to be more pronounced in late lactation compared to early lactation [145,146]. Milk production of cows challenged with intramammary infusion of 30 cfu [147], 1 × 104 cfu of diluted E. coli per quarter or 1000 µg LPS [148] decreased to low levels. In contrast, milk yield was unaffected by chronic and exponentially increasing intravenous infusions of LPS (0.017–0.148 µg/kg of body weight per hour from day 1 to 7) in a recently conducted study [149]. Daily subcutaneous injection of 3 µg/kg body weight of bovine tumor necrosis factor-α during the first week of lactation decreased milk yield only slightly (33.7 to 28.4 kg at highest dose) [149].

Osmotic TNFα pumps releasing 14 µg/kg body weight over 7 days implanted in adipose tissue in late lactation cows had no effect on milk yield [150].

Further evidence for an antagonistic, yet dysbalanced relationship between metabolic pathways involved in adaptation to lactation and adaptation to inflammation is derived from studies examining the effect of the characteristic endocrine alterations required for high milk yields. Compared to low or medium genetic merit cows, high genetic merit cows show lower plasma concentrations of glucose, insulin and IGF-1, as well as higher plasma concentrations of GH [151,152], while insulin resistance is increased [63,153–155]. As described above, hypoinsulinemia favors glucose uptake in both immune cells and MEC because these cells are not dependent on insulin whereas glucose uptake to insulin-dependent cells like adipose and muscle cells is reduced [67]. However, hypoinsulinemia also mitigates stimulating effects of insulin on the rate of glucose utilization and phagocytosis in immune cells [156,157]. Moreover, increased GH-resistance is associated to selection for milk production and might contribute to the dysbalanced allocation of resources between MEC and immune cells in dairy cows. While GH exerts its mammogenic and galactopoietic effects directly in the mammary gland, either through GHR or through mammary IGF-1 production [158,159], many of the immune-stimulating effects attributed to GH are mediated indirectly through induction of hepatic IGF-1 production [160]. However, IGF-1 production in the liver is blunted through hepatic GH-resistance during early lactation [47]. Interestingly, it was shown that different breeds selected for milk production (Holstein-Friesian and Guernsey) showed similar decreases in GHR1A mRNA expression [161], whereas a comparison between Holstein-Friesian and beef cattle revealed decreases in the expression of GHR1A in dairy cows only [162].

5. Management of High-Producing Dairy Cows that Risk Glucose Shortage

The management of high-producing dairy cows should aim for a maximal reduction in metabolic and environmental stress to reduce the energy demand of regulatory systems. Although the specific demand of an activated immune system is difficult to assess, tendencies may be estimated from plasma levels of inflammatory markers, as it has been recently suggested by Trevisi and Minuti [163]. Moreover, the amount of residual glucose left for life functions other than milk synthesis may be estimated by consideration of the glucose demand of quantifiable processes like milk synthesis on one hand and the amount of glucose supply from precursors (derived from feedstuffs and body tissue mobilization) and the hepatic gluconeogenic potential on the other.

Furthermore, dry off feeding and heifer management should be optimized, as it was repeatedly shown that nutrition during these life stages affects availability and allocation of nutrients during early lactation [164]. For instance, overfeeding cows by 50% of predicted requirements decreased postpartum plasma glucose and insulin while increasing glucagon, BHB, and NEFA concentrations after calving compared with cows fed a balanced energy diet during the dry period [165]. During lactation, feeding should be more adapted to meet the need of individual cows in their specific physiological and environmental condition. In particular, supply with glucogenic precursors should be optimized. Although Lucy et al. demonstrated the key role of glucose by showing that infusions of substantial daily doses of glucose (8500 to 1500 g/day) into early postpartum cows were able to completely reverse the hypercatabolic reaction (significant increases in blood concentrations of insulin and IGF-1 along with significant decreases in the concentrations of NEFA and BHB) [166], feeding glucogenic diets is unlikely to significantly reverse lactational energy partitioning, although controversial results can be found in the literature [167–169]. Certainly, nutritional interventions are limited through, e.g., careful use of grain in the diet, time to eat, rumen volume and liver function. There are reasonable doubts whether dairy feeding regimes can further optimize the supply with precursors and the potential of gluconeogenesis and thus increase total glucose availability. For the sake of animal health and welfare and the economic implications of production diseases, dairy farmers should consider a modest but precise reduction on the other side of the equation, i.e., apply management measures that decrease glucose output via milk during periods when dairy cows are challenged simultaneously by both high yields and infectious or non-infectious stressors. Possible management tools include a reduced milking frequency at the onset of lactation [170–172]. In fact, it has been demonstrated that reduced milking frequency reduces both milk yield and inflammation simultaneously [173]. Moreover, instead of implementing general strategies for a very heterogeneous target group, dairy cows should be assessed individually according to their status of NEB. Dairy cows with a high NEB should be allocated to a risk group and dealt with appropriately. In the long term, breeding should be redefined to include increased selection for persistence, lifetime performance and longevity while reducing emphasis on selection for milk yield and early-lactation performance in particular.

6. Conclusions

NEB is commonly thought to identify metabolically instable situations associated with increased risk of disease in dairy cows. However, overall energy balance disregards the reliance of immune cells on glucose as their essential metabolite and synthetic precursor. Although both proinflammatory signaling and lactational reprogramming promote several similar metabolic pathways with the aim of maximizing glucose availability to the respective cell types (lactocytes or leukocytes), adaptation to lactation clearly shifts nutrient partitioning to the favor of the mammary gland. Adaptations are mediated primarily by hormones of the somatotropic axis and affect both mobilization and allocation, including specific regulation of blood flow and receptor expression patterns in peripheral, mammary and immune tissues. Additionally, decades of performance-oriented selection of dairy cows enhanced these patterns substantially by increasing the amount of mammary epithelial cells as well as the metabolic and endocrine setup required to support the demand of these cells. Due to the central role of glucose for milk production and immune cell function, glucose balance is especially submitted to competitive allocation dynamics and is at risk of being overstressed in the early postpartum, high-producing dairy cow, as indicated by reduced responsiveness of lactose synthesis and milk yield to energy or glucose restriction, or other stressors. Therefore, we hypothesize that the uncoupling of the somatotropic axis in cows with high genetic merit for milk production implies, at least in part, an uncoupling of the mammary gland from life function trade-offs. To address possible impacts of glucose shortage on the immune defense, research should focus on the dynamics of glucose supply and demand of immune cells in high producing dairy cows during different periods of lactation.

Author Contributions

Conceptualization, J.H. and A.S.; investigation, J.H.; writing—original draft preparation, J.H.; writing—review and editing, A.S.; visualization, J.H.; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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6. Publication #2 - Estimation of Individual Glucose Reserves in High-yielding Dairy Cows

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Abstract

Glucose plays a central role in numerous physiological processes in dairy cows related to immune defense and milk production. A lack of glucose impairs both objectives, although to different degrees. A method for the estimation of glucose balance (GB) in dairy cows was developed to assess glucose reserves in the intermediary metabolism. Digestive fluxes of glucogenic carbon were individually estimated via the Systool Web application based on data on body weight (BW), dry matter intake (DMI), and chemical analyses of feedstuffs. Fluxes of endogenous precursors glycerol, alanine and L-lactate and the glucose demand imposed by major glucose-consuming organs were deduced from BW, lactose yield and lactation stage. Daily GB was calculated for 201 lactations (1 to 105 days in milk) of 157 cows fed isoenergetic rations. Individual DMI, BW and milk yield were assessed on a daily basis. The results showed that the GB varied greatly between cows and lactation stages. In the first week of lactation, average daily GB reached levels close to zero (3.2 ± 13.5 mol C) and increased as lactation progressed. Most cows risk substantial shortages of glucose during the first weeks of lactation. In face of the specific role of glucose for the functional capability of the immune system, the assessment of glucose reserves is a promising measure for the identification of cows at risk of impaired immunocompetence.

1. Introduction

In contrast to monogastric animals, ruminants cover large proportions of their glucose demand through hepatic gluconeogenesis, while only small amounts are absorbed directly from the gut [1]. Major digestive precursors for gluconeogenesis include propionate, L-lactate and glucogenic amino acids [2,3]. In the event of shortage, precursors such as glycerol and alanine from the mobilization of adipose (lipolysis) and muscle tissue (proteolysis) as well as the increased recycling of lactate from muscle tissue can provide additional glucogenic carbon (C). In lactating animals, high amounts of glucose are required as a precursor for milk lactose [4] as well as for nicotinamide adenine dinucleotide phosphate reduction during the synthesis of milk fatty acids and milk proteins [5,6]. Furthermore, glucose is an essential fuel for self-sustaining life functions, such as neurological and immune functions. In the event of an infection, the immune system is a top consumer of glucose, inter alia required for the accelerated processes of immune cell proliferation and differentiation, phagocytosis, and the production of reactive oxygen species [7]. Accordingly, a strong activation of immune functions is associated with a drop in the concentration of plasma glucose, e.g., following the injection of endotoxins [8] or immunostimulants [9]. Kvidera et al. [8] showed that during the 12h following the injection of lipopolysaccharide (LPS), the glucose demand of the immune system of dairy cows may increase up to 1.5 kg. This demand is similar to the amount of glucose required for the synthesis of ~40 kg of milk per day. With regard to the metabolic priority for milk production during early lactation [10], shortages in the availability of glucose in the intermediary metabolism may thus compromise not only milk production but also immune cell function, such as phagocytosis and the synthesis of immunometabolites, and may be related to the occurrence of production diseases [11].

In the past, various models of glucose metabolism in dairy cows have been developed to study metabolic adaptations during the periparturient period [12] as well as the interrelationships between glucose metabolism and health parameters, such as fertility [13] and ketogenesis [14]. In those models, digestive processes leading to the absorption of glucogenic metabolites from the gut were not considered in detail. However, detailed aspects of ruminant digestion, such as ruminal propionate production as well as starch and protein digestion, have been modelled since then, both in connection with ruminant feed formulation software for dairy cows [15–17] and as a part of research models including regulatory subsystems [18,19]. Following the claim “from joules to moles”, the calculation of digestive nutrient fluxes from nutrient composition of feedstuffs and rations fed was recently brought forward in the INRA feeding system for ruminants [20]. This enables the cow- and ration-specific analysis of digestive nutrient fluxes. Moreover, regression equations for the prediction of the net portal appearances (NPA) of glucose and glucose precursors from these nutrient fluxes have been developed [21].

Further research on quantitative glucose metabolism encompassed tracer studies on the rate of disappearance of glucose in peripheral tissues [22,23]. In protein tissue (PT), the ratio of glucose being oxidized or converted to L-lactate, a precursor for gluconeogenesis [2], is affected by the stage of lactation. Moreover, the dynamics of adipose tissue (AT) and PT mobilization and their share in providing glucose precursors glycerol and alanine [24] as well as the demands caused by major glucose-consuming organs (mammary gland, muscle tissue, red blood cells and the brain) can be deduced from animal data such as the lactose yield and changes in BW [25–27].

While comprehensive research has been conducted in the different subareas of intermediary metabolism, knowledge has so far not been operationalized by integrating input and output values into a coherent whole-body model for a glucose balance sheet on a daily basis. Therefore, the aim of this study was to develop a herd-data-based method of assessing the glucose balance (GB) of dairy cows during early lactation. In order to identify the extent as well as the inter- and intraindividual variations in GB over the course of early lactation (1 to 105 days in milk), key parameters such as the level and timepoint of the lowest GB (nadir), the contribution of precursors to the overall glucose supply and the contribution of glucose-consuming tissues to overall glucose demand have been assessed and evaluated for a sample of 157 dairy cows (201 lactations).

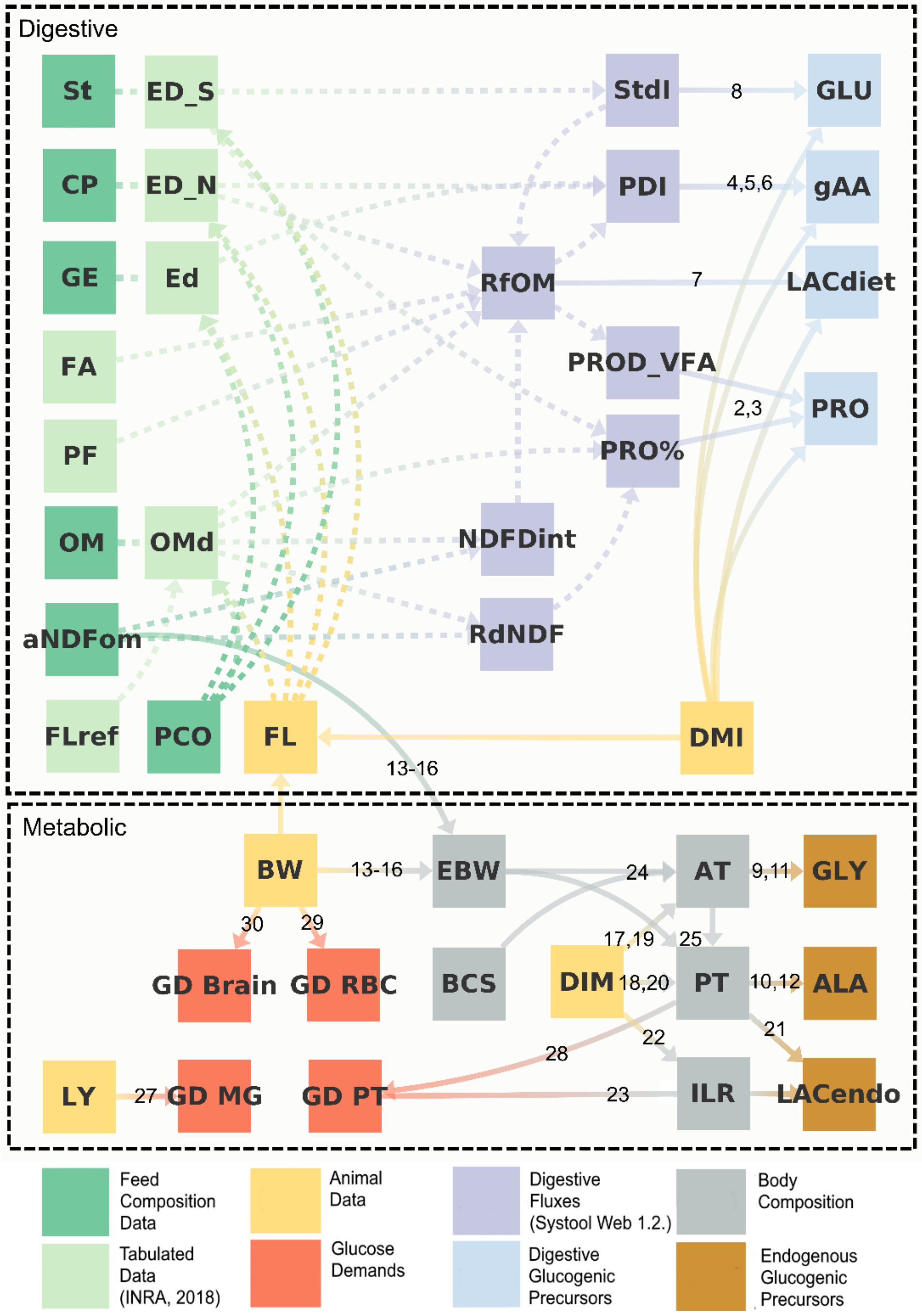
2. Materials and Methods

2.1. Model

2.1.1 General/Overview

The mechanistic, conceptually driven whole-body model of dairy cow GB described in this paper was initially a research model, but it has prospects for practical use in dairy farming. The main input variables are milk yield (MY) and milk composition, BW, DMI, ration composition and chemical analyses of feedstuffs. The structure of the calculations integrates the main pathways of the absorption of glucose and other glucogenic C (propionate, L-lactate, amino acids) from the rumen and the duodenum, the supply of the endogenous precursors glycerol and alanine (during periods when body reserves are mobilized), the recycling of glucogenic C through L-lactate, and the demands of the main glucose-consuming tissues. The input subsystem, i.e., the calculation of glucose supply, is based on data-driven models that predict the portal flows of glucose and glucogenic precursors according to Loncke et al. [21] and Martineau et al. [28] as well as estimates of the release of glucogenic C from AT and PT [24,29]. Further, estimates of the proportions of glucose oxidation and L-lactate production in PT, and thus the recycling of L-lactate from PT for hepatic gluconeogenesis are included according to the research by Larsen and Kristensen [2]. The output sub-system encompasses the estimation of the daily glucose demand of major glucose-consuming tissues, including the mammary gland (MG), PT, the brain and red blood cells (RBC), derived from previously published research in the field [22,27,30–34] "Mol of glucogenic C per day" (mol C/d) was used as a general unit for the calculation of in- and output fluxes and GB. The main pathways included in the model are illustrated in Figure 5.1.

Figure 5.1. The digestive and metabolic pathways for the calculation of glucose balance. Dashed arrows indicate pathways integrated in the Systool Web 1.2. application (for details see Chapoutot et al., 2015 and/or https://systool.fr, accessed on 22 April 2022). Solid arrows and small numbers refer to the equations given in Table 5.1. ALA: alanine; aNDFom: ash-free neutral detergent fibre; AT: adipose tissue; BW: body weight; CP: crude protein; CS: crude sugar; DIM: days in milk; EBW: empty BW; Ed: apparent digestibility of GE; ED_N: effective degradability of nitrogen; ED_S: effective degradability of starch; FA: fatty acids; FL: level of intake relative to body weight; FLref: reference feeding level; gAA: NPA of glucogenic amino acids; GD_Brain: GD of the brain; GD_MG: glucose demand (GD) of the mammary gland; GD_PT: GD of protein tissue; GD_RBC: GD of red blood cells; GE: gross energy; GLU: NPA of glucose; GLY: glycerol; ILR: irreversible loss rate; LACdiet: NPA of dietary L-lactate; LACendo: endogenous L-lactate; LY: lactose yield; NDFDint: neutral detergent fibre (NDF) digestible in the intestines; OM: organic matter; OMd: OM digestibility; PDI the protein truly digestible in the intestines; PF: fermentation products of silages; PRO %: the percentage of propionate production of total VFA production in the rumen; PRO: net portal appearance (NPA) of propionate; PROD_VFA: volatile fatty acid production; PT: protein tissue; RdNDF: NDF digestible in the rumen; RfOM: OM fermentable in the rumen; ST: starch; StdI: starch digestible in the intestine



2.1.2. Digestive and Portal Flows of Glucogenic Nutrients

The major part of circulating glucose derives from hepatic gluconeogenesis. Metabolites from both the feed and the intermediary metabolism serve as precursors. Propionate, which is produced from long-chain carbohydrates by rumen bacteria is the main glucogenic precursor, supplying between 40 and 90 % of total C for gluconeogenesis [1,35]. Other precursors that derive from the digestion of feedstuffs encompass glucogenic amino-acids absorbed from the gut as well as L-lactate, which is synthesized by rumen microbes, particularly when a large amount of starch is fed [1]. However, the availability of these exogenous precursors of gluconeogenesis depends on the rates of transfer from the ruminal or duodenal lumen to the portal vein, as propionate is used or modified by first-pass metabolism within the rumen epithelium [36,37]. In contrast, amino acids serve as an energy source throughout the digestive tract [38,39].

In contrast with monogastric animals, only small amounts of total glucose availability in ruminants originate from glucose absorbed directly from the gut. Even in dairy cows fed high-concentrate diets, glucose absorption is limited, primarily by the rate of starch hydrolysis in the gut [40–42]. In addition to the utilization of luminal glucose within the duodenal epithelium, the portal-drained viscera may use 0.3 to 2.2 g/d/kgBW of glucose from arterial supply [43,44].

Based on a series of scientific papers reporting regression equations for the main digestive and portal flows of glucogenic precursors [21,28,37,45], the net portal appearance (NPAs) of propionate (PRO), glucose (GLU), L-lactate (LAC) and glucogenic amino acids (gAA) were estimated based on the concentrations of ruminal fermentable organic matter (RfOM), rumen propionate production, the truly digestible protein in the intestines (PDI), the digestible starch in the intestines (STdI) and organic matter digestibility (OMd). The estimations are based on the work of Loncke and colleagues [21,28,37,45], who established response equations through meta-analysis of studies involving multicatheterized ruminants. These equations were compiled in the FLORA database and include a diversity of experimental factors (species, physiological state, nutritional regimes). The digestive flows of RfOM, rumen propionate production, PDI, STdI and OMd, as well as contents of ruminal digestible NDF (RdNDF) are estimated from the nutrient compositions of feedstuffs calculated with the Systool Web application [46].

For the equations applied in this study, contents of six moles of glucogenic C per mole of glucose, three moles of glucogenic C per mole of propionate as well as per mole of L-lactate, four moles glucogenic C per mole of nitrogen (N; according to the average C/N ratio found in glucogenic amino acids), a proportion of 45 % glucogenic amino acids of total amino acids and a molar weights of 73.1, 89.1, 14.0 and 180.2 g/mol for propionate, L-lactate, N and glucose, respectively, were assumed (Table 5.1, equation 2).

Table 5.1. Equations for the calculation of glucose balance applied in this study.

No	Variable	Equation	Reference
(1)	Glucose balance (GB; mol C)	GS – GD	
(2)	Supply of glucogenic carbon (GS; mol C/d)	(PRO / 1000 × 3) + (GLU / 1000 × 24 × 6) + (LACdiet / 89.1 × 3) + (gAA/ 1000 × 14.01 × 4) + (GLY / 92.1 × 3) + (ALA / 89.1 × 3) + LACendo	
(3)	NPA of propionate (PRO, mmol/d/kgBW)	3.8 + 0.72 × RU_Pro; Sy.x = 1.6	[45]
(4)	Propionate in the rumen (RU_Pro, mmol/d/kgBW)	PROD_VFA (mol/kg DM) × DMI × Pro % / BW × 1000	
(5)	NPA of glucogenic amino acids (gAA, mgN/d/kgBW)	NPA_tAA × 0.45	[47]
(6)	NPA of α-amino-N (NPA_αAA, mgN/d/kgBW)	63 + 0.74 × PDI (mgN/d/kgBW); RMSE = 60	[28]
(7)	NPA of total amino acids (NPA_tAA, mgN/d/kgBW)	NPA_ αAA × 1.3958	[48]
(8)	NPA of L-lactate (LACdiet, g/d/kgBW)	= 0.098 + 0.0098 × RfOM (g/d/kgBW) RMSE = 0.022	[21]
(9)	NPA of glucose (GLU, mmol/h/kgBW)	-0.103 + 0.0913 × StdI (g/d/kgBW); RMSE = 0.035	[21]
(10)	Mobilized glycerol (GLY; mol/d)	ΔLIP × 0.105 × 1000	[24]
(11)	Mobilized alanine (ALA; mol/d)	ΔPROT × 0.044 × 1000	[29]
(12)	Adipose tissue mobilized (ΔLIP; kg/d)	d_L × ΔEBW (kg) × -1	
(13)	Protein tissue mobilized (ΔPROT; kg/d)	d_P × ΔEBW (kg) × -1	
(14)	Empty body weight (EBW; kg)	BW (kg) – TDC	[20]
(15)	Total digestive contents (TDC, kg)	TRC / 0.7	[20]
(16)	Total rumen contents (TRC, kg)	RL × 1.15	[20]
(17)	Rumen liquid (RL; kg)	3.78 × (NDF%BW -1) + 12) / 100) × BW (kg)	[20]
(18)	Reference level of adipose tissue mobilization (ΔLIPref; kg)	$-1{,}315 \times \exp^{(-0.0329 \times DIM)} + 0.124 \times \exp^{(0.0015 \times DIM)}$	[20,49]
(19)	Reference level of protein tissue mobilization (ΔPROTref; kg)	$-0.413 + \exp^{(-0.0488 \times DIM)} + 0.0268 \text{ x } \exp^{(0.00047 \times DIM)}$	[20,49]
(20)	Ratio of adipose tissue mobilized (d_L)	ΔLIPref / (ΔLIPref + ΔPROTref)	
(21)	Ratio of protein tissue mobilized (d_P)	ΔPROTref / (ΔLIPref + ΔPROTref)	
(22)	Endogenous L-lactate (LACendo; mol C/d)	Lac % × GD_{PT}	[19]
(23)	Lac %	$1 – 0.5 \times (ILR / ILR_{ref})$	
(24)	Irreversible loss rate of glucose in peripheral tissue (ILR; $mmol/h/kg^{0{,}75}$)	$-0.35720 \times 10^{-4} \times DIM^3 + 0.00386 \times DIM^2 - 0.08329 \times DIM + 2.42587$	derived from [2]
(25)	Glucose demand (GD; molC/d)	$GD_{MG} + GD_{PT} + GD_{RBC} + GD_{Brain}$	
(26)	Glucose demand of the mammary gland (GD_{MG}; mol C/d)	lactose yield (g/d) / 0.80 / 180.16 × 6	[30,31]
(27)	Glucose demand of protein tissue (GD_{PT}; mol C/d)	PT (kg) × 0.0288 mol/d × 6	[22]
(28)	Protein tissue mass (PT; kg)	(EBW – AT) * 0.215	[20]

(29)	Adipose tissue mass (AT; kg)	0.0377 × ((BCS-0.5) × 8 / 4) × EBW	[50]
(30)	Glucose demand of red blood cells (GD_{RBC}; mol C/d)	4.75 × BW × 10^-4 × 6	[27,34]
(31)	Glucose demand of the brain (GD_{Brain}; mol C/d)	3.58 × BW × 10^-4 × 6	[32,33]
(32)	Energy balance (EB; MJ)	(DMI (kg) × NEL(MJ NEL/kg)) – (ED_M + ED_P)	
(33)	Energy demand for production (ED_P; MJ of NEL)	MY × 1.05 + 0.38 × F % + 0.21 × P %	[51]
(34)	Energy-corrected milk (ECM; kg)	E_P / 3.28 MJ of NEL/kg	[51]
(35)	Energy demand for maintenance (E_M; MJ of NEL)	0.293 MJ/kg × BW0.75 (kg)	[51]

BCS: body condition score; BW: individual body weight; DIM: days in milk; DMI: dry matter in-take (kg dry matter (DM) per day); NDF %BW: level of NDF intake (% of individual body weight); ILRref: reference level of irreversible loss rate of glucose; MY: milk yield; NEL: content of net energy for lactation in the ration (MJ / kg DM); NPA: net portal appearance; PDI: protein truly digestible in the intestines; Pro %: percentage of propionate production of total rumen VFA production; PROD_VFA: total rumen VFA production; StdI: starch digestible in the intestines

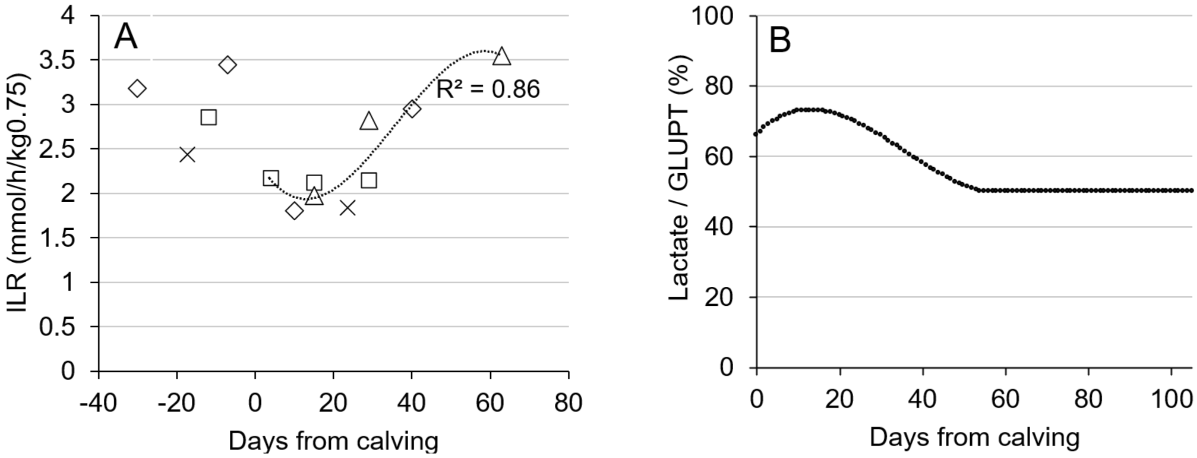
2.1.3. The Mobilization of Glucogenic Precursors from Lipolysis and Proteolysis and L-Lactate Production in Muscle Tissues

The daily rate of the mobilization of glucogenic precursors from lipolysis and proteolysis was estimated from the daily changes in empty body weight (ΔEBW, kg), which can be obtained from individual BW and the level of NDF intake (NDF %BW; kg DM/kg BW) according to INRA (Table 5.1., equations 14 to 17). If ΔEBW was negative (BW loss), the amount of daily lipid (ΔLIP) and protein mobilization (ΔPROT) were obtained by multiplying individual daily ΔEBW with the day-specific share of lipids (d_L) and proteins (d_P) in the daily ΔEBW (Table 5.1., equation 12 and 13). The ratios for d_L and d_P were calculated as the share of daily lipid and protein mobilization (ΔLIPref. and ΔPROTref.; Table 5.1., equation 20 and 21) in the sum of ΔLIPref. and ΔPROTref. The reference values for daily lipid and protein mobilization (Table 5.1., equation 18 and 19) originate from the intra-experiment adjustment of experiments on body lipid and body protein kinetics in dairy cows reported in the INRA feeding system for ruminants [20,49]. It is assumed that all circulating glycerol derives from lipolysis and enters gluconeogenesis. Based on the average molar masses of fatty acids (887 g/mol) and the molar mass of glycerol (92 g/mol), the share of glycerol in the amount of mobilized adipose tissue is 10.5 %. Per mol of glycerol, three moles C were assumed (Table 5.1., equation 10).

Alanine is the only amino acid involved in the transfer of glucogenic C between muscle and liver [2,52]. Its share in the amount of mobilized PT was reported to be 4.4 % [29], with a molar weight of 89.1 g and three moles C per mole of alanine (Table 5.1., equation 11).

Additionally, gluconeogenesis is supported by a substantial inter-organ transfer of L-lactate, emerging from L-lactate production in PT (Cori cycling) [2]. In this study, the individual daily amounts of protein (PT) and adipose tissue (AT) were calculated from BCS and EBW (Table 5.1., equation 28 and 29) [20,50]. Based on the work of Lindsay et al. [53], Rhodes et al. [54] and Martin and Sauvant [19], 50% of glucose being oxidized and 50 % of glucose being recycled through the L-lactate production in PT were assumed as initial values. However, the whole-body irreversible loss rate of glucose excluding the loss of glucose in lactose was found to be reduced during early lactation [35], indicating the reduced oxidation of glucose in peripheral tissues. Based on the data of Bennink et al. [55], Bruckental et al. [56] and Baird et al. [57], as well as unpublished data from Larsen and Kristensen [35], who measured the glucose rate of disappearance during the transition period of dairy cows, a 3rd degree polynomial regression equation was derived to calculate the amount of glucose being oxidized and the amount of glucose being released as L-lactate from muscle according to the day in milk (DIM) (Figure 5.2.A; Table 5.1., equation 22 to 24). The curve obtained showed a drop around parturition followed by an increase up to 3.55 mmol/h/kg0.75 (DIM 55). Thereafter, a constant rate of L-lactate recycling (Lac %) was presumed until DIM 105 (Figure 5.2.B) together with a complete recycling of endogenous L-lactate through gluconeogenesis and three moles C per mole of L-lactate.

Figure 5.2. (A): The irreversible loss rate of glucose in tissue metabolism (ILR) during the transition period in dairy cows; data compiled by Larsen and Kristensen (2013) from Bennink et al. (diamonds), Baird et al. (crosses) and Bruckental et al. (triangles) and unpublished ILR data from Larsen and Kristensen, 2009 (squares). (B): The percentage of glucose carbon being recycled through L-lactate formation in protein tissue based on the regression equation derived from A.



2.1.4 The Sum of Precursors for Gluconeogenesis

Due to uncertainties concerning the hepatic capacity for the conversion of glucogenic C to glucose (see discussion), the sum of fluxes of glucogenic C is chosen as the most consistent and comparable level of aggregation for glucose supply. Based on the estimations of the supplies of propionate, glucogenic amino acids, L-lactate and glucose from the portal-drained viscera and the estimations of glycerol, alanine and L-lactate released by protein and adipose tissue, the sum of glucogenic C from these digestive and endogenous precursors is used as the final value for glucose supply in this study.

2.1.5. Glucose Demand

In dairy cows, Bickerstaffe et al. [58] noted that mammary glucose uptake accounts for almost all of glucose turnover, while only small amounts are left for maintenance functions. As mentioned above, the quantification of the glucose demand imposed by the immune system is far from being clear, mainly due to a lack of robust and practical indicators of immunoactivation. However, the amount of glucose consumed by other major glucose-consuming organs, including the mammary gland, PT, the brain and red blood cells (RBC), does not rely to a similar extent on the effect of environmental stressors. Therefore, the glucose demands of these tissues serve as a proxy for the overall glucose demand of a dairy cow and were subtracted from overall glucose supply to calculate the amount of glucose reserves available for immunoactivation. The demands were estimated from the levels of MY and L-lactate yield (mammary gland), the metabolic BW and assumptions made on the carcass compositions of individual cows (PT, RBC and the brain) according to the equations given in Table 5.1.

Splanchnic Tissues / Portal-Drained Viscera

The glucose demand of the digestive processes in the duodenum as well as the glucose consumed by other processes in the portal-drained viscera is represented with the estimate of NPA of glucose described above. Hence, the glucose demand of these tissues, which accounts for an average of 22 % of the whole-body rate of appearance of glucose [44], is integrated in the input part of the model.

Mammary Gland

According to studies on catheterized animals and radioisotope labelling [30,58], the average of 70 % of glucose taken up by the mammary gland for lactose synthesis is often used for the calculation of the mammary glucose requirements in lactating dairy cows. However, the ratio varies greatly between and within studies measuring both glucose arteriovenous differences across the mammary gland and lactose yield [59–61]. Lemosquet et al. [62] found that the ratio shows a parabolic curve when related to the supply of glucogenic nutrients (rumen propionate + duodenal starch) in the range of 2.12 to 3.95 mmol C/h. In high-yielding dairy cows during early lactation, however, Galindo et al. [31] found up to 88 % of mammary glucose flux being used for lactose synthesis during early lactation in control cows and 92 % in cows being abomasally infused with casein + amino acids. Based on the latter study and assuming that the mammary gland will prefer to use energetic nutrients other than glucose for oxidative processes in the mammary gland during early lactation hypoglycemia, a proportion of 80 % of mammary glucose uptake being used for lactose synthesis was assumed in our study (Table 5.1., equation 26).

Muscle/Protein Tissue

Galindo et al. [22] presented data on the whole-body rate of disappearance of glucose and the fluxes across mammary and splanchnic tissues in cannulated Holstein cows (77 ± 13 DIM; ~ 30kg ECM/d) and estimated that the remaining residuals (12 % of WbRa) would be left for non-splanchnic, non-mammary tissues, mainly the brain (<3 %) and muscle tissues (~10 %), which resulted in an average of 28.8 mmol glucose/d/kg PT. By multiplying this value by individual daily protein tissue mass (Table 5.1., equation 27 and 28), daily glucose demand of PT was calculated.

Red Blood Cells

According to Basarab et al. [27] and Harvey [34], the glucose consumption of red blood cells (RBC) is approximately .014 mmol/d/ml RBC. In the bovine, blood volume averages 10 % of BW with a hematocrit of 33 % [63]. Thus, the glucose demand of RBC was calculated from individual body weight (Table 5.1., equation 30).

Brain

As mentioned above, Galindo et al. [22] estimated that less than 3 % of the whole-body rate of disappearance might be attributed to the brain metabolism. The glucose consumption of the human brain averages 5.6 mg/min per 100g of brain tissue according to Mergenthaler et al. [32]. For the calculations applied in our study, the mean weight of the brain of dairy cows was estimated at .08 % of BW according to Ballarin et al. [33] (Table 5.1., equation 31).

The whole-body glucose demand excluding the glucose demand of the immune cells (GLU-OUT, mol C/d) was calculated as the sum of the demand of the mammary gland, protein tissue, red blood cells and the brain, while daily GB (mol C/d) was calculated as the difference between glucose supply and glucose demand.

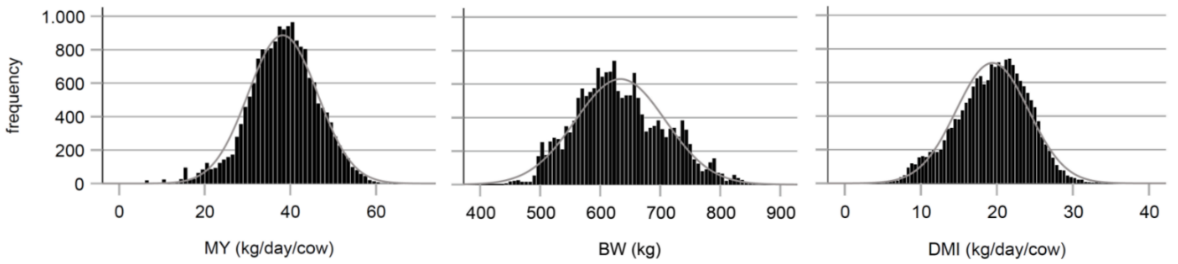
2.2. Data

2.2.1. Animals

The data were recorded at the Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle from January 2015 to November 2016 (23 months) for the project OptiKuh (for details see [64]) and the data were made available by the persons responsible. Animals were kept in a loose pen. The data on 157 Holstein cows (201 lactations) from lactations 1 to 11 were analyzed. The data sampling encompassed the daily recording of MY (kg), daily recording of individual feed intake, daily BW measurement, weekly measurements of milk ingredients and chemical analysis of feedstuffs (Figure 5.3, Table 5.A1). Animal data were analyzed for the first 105 days in milk. Individual feed intake was recorded daily using feeders equipped with a weighing unit and automatic cow identification (Roughage Intake Control, Insentec B.V., Marknesse, The Netherlands). Cows were milked twice daily using a combination of a herringbone and a side-by-side milking parlour manufactured by GEA Farm Technologies (located in Bönen, Germany). BW was measured automatically after every milking via walk-over scale and daily values were derived by averaging morning and evening BW. The data from the milking parlour were recorded via the herd management system Dairy Plan C21 (GEA Farm Technologies, Boenen, Germany). Due to different housing during the first DIM, the collection of the cow's DMI, BW and MY data started between days 1 and 8. Daily MY was recorded at morning and evening milking, and milk was analyzed for fat, protein and lactose via infrared analyzer (MilkoScan FT-6000, Foss Analytical A/S, Hillerod, Denmark; LKV Rheinland-Pfalz-Saar e.V., Bad Kreuznach, Germany). BCS was assessed once every two weeks.

Measurements of milk ingredients and BCS, as well as values for MY and DMI missing due to technical errors were inter-/extrapolated linearly up to three consecutive days. If more values were missing, the lactations were excluded from the analyses. After the removal of values differing by more than 10 kg EBW (see below for the calculation) from the previous or succeeding day (or up to 50 kg within 5 days), BW was smoothed across the 105 DIM – period for each cow using a cow-specific 5th-degree polynomial function.

Figure 5.3. The distributions of the daily recordings of milk yields (MY), dry matter intakes (DMI) and body weights (BW).



For further analyses, daily energy demand for production (ED_P; MJ of NEL/d) and daily energy demand for maintenance (ED_M; MJ of NEL) were calculated according to GfE [51]. Energy intake was calculated from the energy content of the diet (MJ NEL) and individual DMI.

The sum of the energy demands was subtracted from the energy intake to obtain the individual energy balance (EB; MJ of NEL) (Eq. 32-35; Table 5.1).

2.2.2. Feeding Rations

All cows were fed a total mixed ration (TMR), consisting of grass silage, corn silage, pressed sugar beet pulp silage, hay, straw, vegetable oils, extruded rape seed, extruded soybean, corn, barley, soybean hulls, molasses, minerals, urea, salt, vegetable oils and calcium carbonate (Table 2). Over the period of two years, the ration composition was adapted on a monthly basis to ensure equal levels of metabolizable energy intake (11.5 ± 0.2 MJ ME / kg DM). Cows were fed ad libitum. TMR and feedstuffs were characterized monthly and/or if ration composition changed by Weende and van Soest analysis for dry matter, organic matter, crude nutrients (protein, fat, fibre, starch and sugar) as well as ash-free acid detergent fibre and ash-free neutral detergent fibre (Table 5.2). The energy contents of the diets were calculated according to GfE (2001). Subsequently, information on ration composition was assigned to each day and cow.

Table 5.2. The means of the proportions of feedstuffs and the contents of energy and nutrients of the total mixed rations fed during the study period.

Feed	**DM**	**DM**	**ME**	**OM**	**CP**	**CL**	**CF**	**ST**	**S**	**ADFom**	**aNDFom**
	%	g/kg FM	MJ/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM
Grass silage, wilted, with additive	25.4 ±3.5	404.5 ±13.6	10.6 ±0.1	905.1 ±2.2	157.9 ±1.3	40.5 ±1.5	247.6 ±3.7	0.0 ±0.0	14.0 ±1.6	286.7 ±8.7	445.7 ±4.1
Hay, permanent grassland	4.3 ±1.1	916.0 ±0.0	7.3 ±0.0	922.0 ±0.0	100.0 ±0.0	18.0 ±0.0	306.0 ±0.0	0.0 ±0.0	69.0 ±0.0	370.0 ±0.0	233.0 ±27.8
Corn silage, whole crop	20.9 ±3.6	330.9 ±3.5	11.3 ±0.1	958.7 ±1.7	78.8 ±2.1	36.9 ±0.6	163.2 ±6.6	345.3 ±3.5	0.0 ±0.0	196.5 ±8.2	656.0 ±0.0
Pressed sugar beet pulp silage	12.2 ±4.1	295.2 ±10.3	11.7 ±0.2	920.5 ±23.4	84.4 ±5.9	13.5 ±1.7	169.8 ±8.0	0.0 ±0.0	12.9 ±1.0	203.0 ±7.9	337.0 ±5.9
Vegetable oils	0.8 ±0.0	990.0 ±0.0	28.7 ±0.0	875.0 ±0.0	0.0 ±0.0	840.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	419.1 ±12.8
Barley straw	1.6 ±0.7	860.0 ±0.0	5.9 ±0.0	954.0 ±0.0	34.0 ±0.0	12.0 ±0.0	380.0 ±0.0	0.0 ±0.0	8.0 ±0.0	440.0 ±0.0	0.0 ±0.0
Concentrates*	34.8 ±0.7	883.1 ±2.8	13.0 ±0.2	938.8 ±12.3	268.0 ±17.5	38.7 ±3.8	101.0 ±25.2	253.6 ±17.2	69.1 ±6.0	141.5 ±34.5	668.0 ±0.0
Total mixed ration	100.0 ±0.0	420.3 ±18.2	11.5 ±0.2	930.9 ±4.3	160.7 ±1.7	33.5 ±1.1	160.1 ±2.7	222.1 ±6.2	58.9 ±5.9	219.7 ±8.0	334.6 ±4.8

ADFom: ash-free acid detergent fibre; aNDFom: ash-free neutral detergent fibre; CF: crude fibre; CL: crude lipids; CP: crude protein; DM: dry matter; ME: metabolizable energy; OM: organic matter; S: sugar; ST: starch; * extruded rape seed, extruded soybean, corn, barley, soybean hulls, molasses, minerals, urea, salt, vegetable oils, calcium carbonate

Further feed values required for the calculation of ruminal and duodenal flows of nutrients with the Systool web application were taken from the INRA feed tables (INRA, 2018, Table 5.3.). The FA content of forages was predicted from their CP content (INRA, 2018).

Table 5.3. The tabulated values of digestibility and further nutrients required for the analyses in Systool Web according to the INRA Feeding system for ruminants (INRA, 2018).

Feed	OMd	Ed	ED6_N	ED6_S	FA	PF	FLref
	%	%	%	%	g/kg DM	g/kg DM	% BW
Grass silage, wilted, with additive	70	66	77	-	22	85	1.39
Hay, permanent grassland	62	59	68	-	16	0	-
Corn silage, whole crop	73	70	75	70	28	80	1.53
Pressed sugar beet pulp silage	86	82	66	-	5	140	1.28
Vegetable oils	89	100	100	-	840	0	1.63
Barley straw	44	40	68	-	6	0	-
Concentrates*	80	86	52	85	37	0	2.47

Ed: apparent digestibility of gross energy; ED6_N: effective degradability of nitrogen; ED6_S: effective degradability of starch; FA: fatty acid content; FLref: reference feeding level; OMd: organic matter digestibility; PF: fermentation products in silages; *extruded rape seed, extruded soybean, corn, barley, soybean hulls, molasses, minerals, urea, salt, vegetable oils, calcium carbonate

The Systool web application (Version 1.2, 2017; for details see 46), which is based on a series of regression equations described in the INRA feeding system for ruminants [20], was used to calculate nutrient fluxes for a total of 26 rations. The digestive processes modelled in the application are modified by species (bovine), feeding level, proportion of concentrate in the diet and rumen protein balance. Therefore, the digestive fluxes were calculated for all rations at six different levels of DMI and five different levels of BW (Table 5.4). Subsequently, the digestive fluxes were attributed to each cow and day according to the corresponding BW, DMI and the ration fed.

Table 5.4. The categories of body weight (BW) and dry matter intake (DMI) for the calculation of nutritional values via Systool Web for the 26 total mixed rations fed during the study period as well as the numbers and means of the daily measurements in each category.

BW level (kg)	n	Mean BW (kg)
<500	357	485
500 to 600	6,015	561
600 to 700	8,007	644
700 to 800	3,416	738
>800	300	824
DMI level (kg)	n	Mean DMI (kg)
<10	650	8.6
10 to 15	2,621	12.9
15 to 20	6,138	17.7
20 to 25	12,859	20.1
25 to 30	1,880	26.5
>30	136	31.4

The main pathways of the digestion of glucose and glucogenic metabolites that were modelled in the application included: organic matter degradability, the amount of ruminal fermentable organic matter, the amount of neutral detergent fibre (NDF) digestible in the rumen, the whole-tract non-digestible NDF, the production of volatile fatty acids (VFA) in the rumen, the proportion of propionate in the total VFA production, the degradation of starch in the rumen, duodenum and large intestine, the amount of starch truly digestible in the intestine, the degradation of proteins, the synthesis of microbial proteins and the total flow of proteins digestible in the intestines (Table 5.5.). Detailed explanations on the development and validation of the databases and equations involved are described by Nozière et al. (2013) [17] and in the INRA feeding system for ruminants (2018) [20].

Table 5.5. The means (SD) of nutritional values of a total of 780 calculations (26 rations for six levels of DMI and five levels of BW, see Table 5.4) performed with the Systool Web application Version 1.2 (2017). In addition to the levels of DMI and BW, digestive interactions are based on ration composition, the type of the animal (dairy cow) and the existence of urea in the ration.

Intake level	**OMD**	**RfOM**	**RdNDF**	**VFA Prod**	**Pro %**	**PDI**	**STdI**
(kgDM/ kgBW)	%	g/ kgDM	g/ kgDM	mol/ kgDM	mol/ 100mol	g/ kgDM	g/ kgDM
<2	73.0 ±1.1	506.5 ±11.5	186.1 ±12.8	4.3 ±0.1	19.4 ±0.7	96.2 ±2.8	24.3 ±2.8
2-3	70.2 ±1.1	469.8 ±11.3	167.3 ±12.7	4.0 ±0.1	21.8 ±0.7	99.0 ±3.0	28.4 ±3.2
3-4	67.2 ±1.1	431.6 ±11.3	147.8 ±12.6	3.6 ±0.1	24.5 ±0.7	102.0 ±3.3	32.6 ±3.6
4-5	64.3 ±1.2	395.2 ±12.8	129.4 ±12.5	3.3 ±0.1	27.0 ±0.8	104.8 ±3.5	36.6 ±4.1
>5	60.1 ±1.5	344.9 ±17.0	105.8 ±10.8	2.9 ±0.1	30.7 ±1.2	109.1 ±3.9	42.5 ±4.9

OMD: organic matter digestibility; PDI: protein truly digestible in the intestines; Pro%: the percentage of propionate production of the total VFA production in the rumen; RdNDF: neutral detergent fibre digestible in the rumen; RfOM: organic matter fermentable in the rumen; STdI: starch digestible in the intestines; VFAProd: volatile fatty acid production

2.3. Statistical analyses

Calculation of daily and weekly supplies, demands, GB and EB were performed with Microsoft Excel®. Weekly means of individual lactations were calculated if three or more measurements were available in the respective week. Zero-order, partial and semi-partial correlations between GB and animal data as well as between GB and ration composition were calculated using the procedure “linear regression” in IBM® SPSS®. Separate analysis of these correlations was performed for the first week of lactation (DIM 1 to 7) to evaluate the effect of animal and ration characteristics on GB during the period of highest metabolic stress.

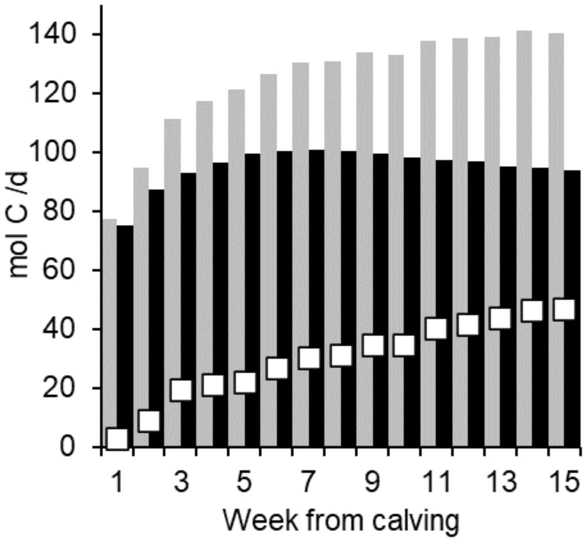
3. Results

The average weekly MY, ECM, percentages of milk fat (F %), milk protein (P %) and milk lactose (L %), DMI, BW, EBW, and mass of AT and PT as well as the BW-loss were within the expected ranges for high-producing dairy cows and the lactation stage investigated (Table 5.A1). Across the 105-day period, MY, ECM yield, F %, P % and L % averaged 38.1 ± 7.2 kg, 36.3 ± 8.1 kg, 3.7 ± 0.6 %, 3.1 ± 0.4 % and 4.8 ± 0.2 %, respectively. DMI, BW, EBW, AT, PT and BW-loss averaged 19.5 ± 3.0 kg, 634 ± 75 kg, 554 ± 65 kg, 105 ± 22 kg, 96 ± 11 kg and -

0.6 ± 0.8 kg/d, respectively. The average EB ranged from -49.8 ± 20.9 MJ NEL/d during the first week of lactation (DIM 1 to 7) to -1.3 ± 18.9 at the end of the 105-day period.

The mean GB reached levels close to zero (3.2 ±13.5 mol C per day) during the first week of lactation, followed by a steep increase during the second and third weeks of lactation, and a moderate but continuous increase until 105 DIM (Figure 5.4, Table 5.A2). Accordingly, the highest weekly averages were observed at the end of the monitoring period (14th week of lactation (DIM 92-99): 46.7±17.4 mol C per day).

Figure 5.4. The weekly means of daily measurements of the supply with (grey bars) and the demand for glucogenic carbon (black bars) and the glucose balance (squares) from calving to 105 days in milk. Supply encompasses the portal appearance of digestive precursors as well as endogenous precursors. Demand encompasses the mammary gland, protein tissue, red blood cells and the brain.



The results for GB varied greatly between cows (Figure 5.5.). In the first week of lactation, average GB varied from -32.6 to 37.2 mol C/d (without outliers </> 1.5 x interquartile range (IQR)) between cows. Across all days and cows, the rolling mean (-3 days) of GB ranged from -63 to 145 mol C per day, respectively.

The contributions of the precursors to the overall glucose supply varied according to the stage of lactation (Figure 5.6, Table 5.A2). The contributions of digestive precursors averaged 54.2 ± 3.7 %, 30.7 ± 2.4 % and 5.2 ± 0.4 % for propionate, amino acids and L-lactate, respectively. Weekly averages of the NPA of glucose were negative throughout the period. The contributions of endogenous precursors ranged from 24.7 ± 7.5 % (during the first week of lactation) to a basic level of 6.8 ± 1.4 % in periods with less (adipose) or no (protein) tissue mobilization (DIM 43 -105; Figure 5.7). Accordingly, the contributions of endogenous L-lactate (glycerol and alanine) ranged from 13.9 ± 1.9 % to 6.2 ± 0.8 % (8.0 ± 5.9 % to 0.8 ± 1.3 % and 1.0 ± 0.7 % to 0.0 ± 0.0 %).

Figure 5.5. Variations in glucose balance from calving to 105 days in milk (box-and-whisker-plots; dots represent outliers (greater or smaller than 1.5 x the interquartile range). The dashed line represents the amount of glucose infusion (17.7 mol C/d) required to maintain euglycemia in cows challenged by lipopolysaccharide (Kvidera et al., 2017).

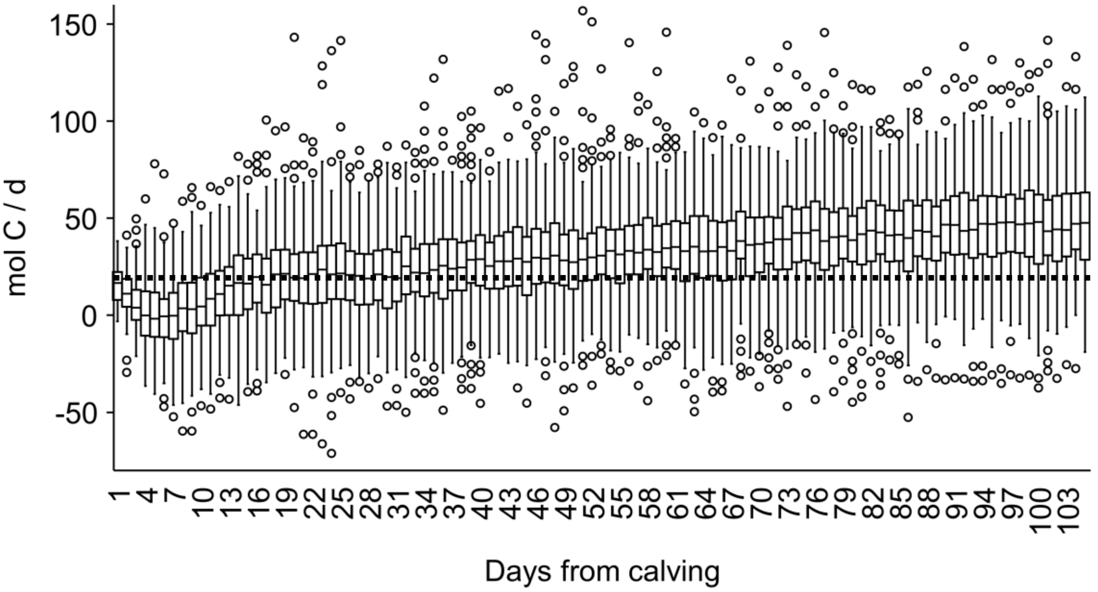


Figure 5.6. The proportional contributions (%) of the endogenous precursors glycerol (GLY), alanine (ALA) and L-lactate (LAC_endo), and the digestive precursors propionate (PRO), glucose (GLU), glucogenic amino acids (gAA) and L-lactate (LAC_diet) to overall glucogenic supply from calving until 105 days in milk. GLU represents the net portal appearance as estimated by equation 9 (Table 5.1) and reflects the difference between duodenal glucose absorption and glucose consumption by portal-drained viscera, which averaged negative values.

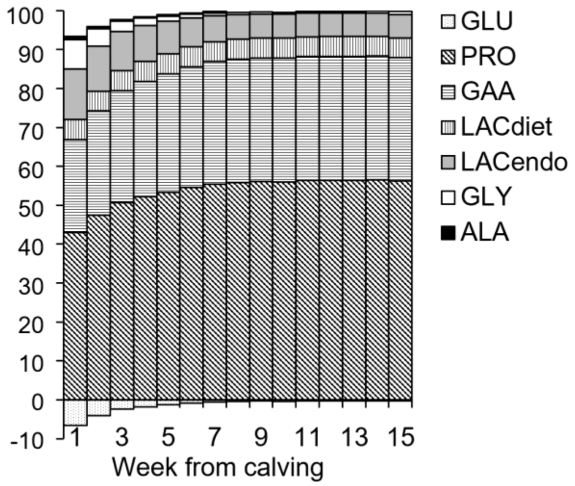
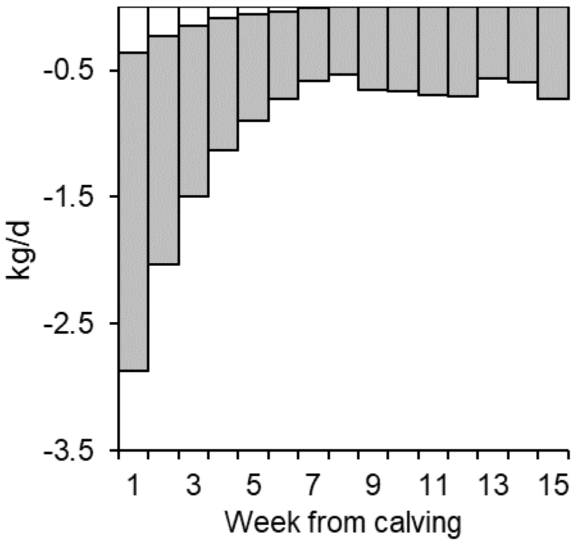
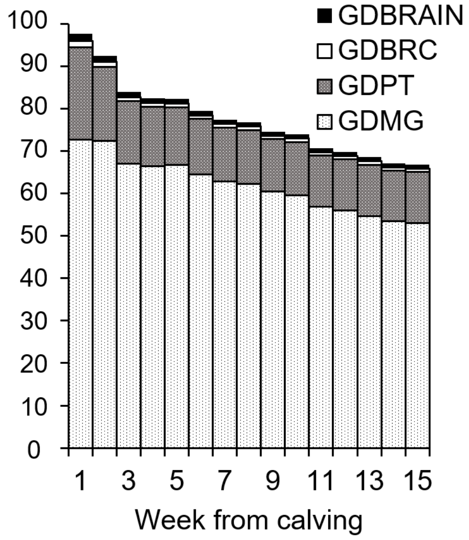


Figure 5.7. The weekly means of daily body weight loss (negative Δ empty body weight (EBW)) and the proportional contributions of adipose (ΔLIP; grey bars) and protein tissue mobilization (ΔPROT, white bars) according to day in milk (DIM).



The mean glucose demand of the mammary gland ranged from 56.4 ±13.1 mol C per day in the 1st week of lactation to 81.8 ±14.4 mol C per day in the 7th week. The means (SD) demand imposed by protein tissue, red blood cells and the brain were constant over the 105-day period at 16.6 ± 0.2, 1.1 ± 0.0 and 1.4 ± 0.0 mol C per day, respectively. With an average of 76.1 ± 15.6 mol C/d, GDMG far exceeded the glucose demand of other tissues (Figure 5.8).

Figure 5.8. The proportions (%) of glucogenic carbon supply (portal appearance of digestive precursors and endogenous precursors) utilized by the mammary gland (GDMG), protein tissue (GDPT), red blood cells (GDRBC) and the brain (GDBRAIN)



Assuming the glucose demand in the case of an immune challenge by lipopolysaccharide that is not covered by reductions in milk production (17.7 mol C/d) according to Kvidera et al. [8], 86.3 % (72.8, 51.8 %) of the weekly mean GB observed in the 1st (2nd and 3rd) week of lactation were below this value. This means that cows would not have been able to meet the challenge without reducing glucose consumption of other tissues. On average, the weekly mean GB reached this value at 20 DIM. However, due to (1) the uncertainty regarding dairy cows ability to reduce glucose consumption in peripheral tissues in case of glucose shortage and due to (2) the statistical variance in the response equations for the NPA of the precursors, maximum GB was calculated assuming no glucose demand in muscle tissue, RBC and the brain on one side and the maximal NPA of the precursors (+standard error) on the other. For maxGB, 9.8 % (7.2 and 3.6 %) of the 1st (2nd and 3rd) weeks of lactation were below this threshold.

Semi-partial correlations between GB and main input variables across the whole dataset revealed that DMI had the strongest effect on GB, followed by MY, BW loss, BW, L % and DIM, while lower correlation coefficients were obtained for the contents of metabolizable energy, crude fibre, sugars, ash-free neutral detergent fibre and the percentage of concentrate in the diet (Table 5.6). The zero-order correlation coefficients between EB and GB varied from r = .53 to r = .99. between lactations. The median day of the lowest GB (Nadir) was 13 with an interquartile range of 24 DIM and was identical with the nadir of EB for 51 % of the lactations investigated.

Table 5.6. The correlations between glucose balance (mol C), animal and ration characteristics.

	p	**Correlation Coefficients DIM 1-105**			***p***	**Correlation Coefficients DIM 1 to 7**		
		Zero Order	**Partial**	**Semi-Partial**		**Zero Order**	**Partial**	**Semi-Partial**
DMI	.000	.819	.987	.849	.000	.332	.956	.716
MY	.000	-.004	-.964	-.491	.000	-.521	-.944	-.628
L %	.000	-.028	-.552	-.090	<.001	-.239	-.473	-.118
BW	.000	.064	-.667	-.121	<.001	-.055	-.448	-.110
BWLOSS	.000	.244	-.698	-.132	<.001	-.276	-.839	-.339
DIM	.000	.467	-.284	-.040	.501	-.285	-.023	-.005
LACT	<.001	.021	-.054	-.007	.691	-.009	-.014	-.003
ME	.001	.039	-.024	-.003	<.001	-.052	-.141	-.031
CP	.396	-.097	-.006	-.001	.358	.115	-.031	-.007
CF	.005	-.045	.021	.003	.827	.042	.007	.002
ST	.973	-.029	.000	.000	.201	-.036	-.044	-.010
CS	<.001	.110	-.048	-.007	.489	-.035	-.024	-.005
aNDFom	<.001	.090	.037	.005	<.001	-.055	.117	.026
CON %	<.001	.129	.120	.016	<.001	.100	.226	.051

aNDFom: amylase-treated, ash-free neutral detergent fibre (g/kg DM); BW: body weight (kg); BWLOSS: body weight loss (kg/d), CF: crude fibre (g/kg DM); CON %: proportion of concentrate in the diet (% of DM); CP: crude protein (g/kg DM); DIM: days in milk; DMI: dry matter intake (kg/d); L %: lactose percentage in milk (%); LACT: lactation number; ME: metabolizable energy content (MJ ME/kg DM); MY: milk yield (kg/d); S: sugar (g/kg DM); ST: starch (g/kg DM).

4. Discussion

The aim of this study was to develop a method for the calculation of the GB of dairy cows from individual lactose yield, BW, DMI and nutrient composition of feedstuffs and to evaluate glucose reserves during early lactation. For this purpose, the amounts of digestive glucose, propionate, glucogenic amino acids and L-lactate as well as the amount of glycerol and alanine from adipose and protein tissues mobilization, the amount of endogenous L-lactate and the demand imposed by major glucose-consuming organs within the cow were estimated. Given that mean GB values of a sample of 201 dairy cows reaching levels close to zero, glucose availability was severely compromised in the immediate postpartum period. However, GB varied considerably across dairy cows within and between lactation stages.

4.1. Estimation of the Supply with Glucogenic C in Cows during Early Lactation

4.1.1. Assessing Digestive and Portal Fluxes

The calculations within the digestive part are based on the estimation of the relevant proportions of nutritional fractions within the rumen and duodenum of the cow from the chemical analyses of feedstuffs, individual DMI and BW via the Systool Web application [46]. Subsequently, the NPA of the digestive precursors were assessed according to Loncke et al. [21] and Martineau et al. [28]. These models exhibited higher R^2 and lower standard deviation of the residuals compared with other published models of VFA production and absorption [65–68]. Nonetheless, regression-induced deviations, which over- or underestimate values in specific ranges of the data have to be considered. In this regard, Loncke et al. [69] pointed out that the models of nutrient NPA applied in this study are based on data encompassing intakes up to 41g DMI per kg BW per day, while the majority of their data are related to intake levels below 35g DMI/kgBW/d. The median (IQR) intake level of the cows investigated in this study was 31.5g (9.4g) DMI/kgBW/d. Values greater than 41g DMI/kgBW/d (6 % of the data) were not excluded from the calculations, assuming the linearity of the relationship between the ruminal and duodenal fluxes of nutrients and their net transfer to the portal vein. However, limitations in the absorptive capacity of PRO, gAA and GLU are related to increased rumen concentration of VFA [70], increased duodenal concentrations of amino acids [71] and the capacity of starch hydrolysis [1], respectively. Thus, the flux of digestive precursors across splanchnic tissues is likely to be compromised in cows with higher levels of DMI, suggesting in these cases an overestimation of GB. In general, deviations between calculated values and the amount of glucose available to an individual cow emerge from individual variation in digestive and metabolic capacities that are not depicted by the applied regression equations.

4.1.2. The Contribution of Digestive Precursors

Across all (digestive and endogenous) precursors, rumen propionate production was the main source of glucogenic C both in terms of absolute and proportional values. Compared to the data of lactating ruminants presented by Loncke et al. [69], NPA of propionate was somewhat higher (1.53 ± 0.41 vs. 1.16 ± 0.29 mmol/kg BW/h), while the share of propionate in the overall supply with glucogenic C (54.4 ± 9.2 %) was slightly lower than maximal contributions to gluconeogenesis (60.9 ± 10.1 %). According to the results of several studies [35,72–78], the proportional contribution of propionate to liver glucose release measured in multicatheterized cows between DIM 1 and 105 averaged 57 %, with a tendency to increase as lactation progressed. Hence, the proportion of propionate to glucogenic C calculated in our study is in accordance with these previous results.

The methodological considerations of the approach presented here assume that alanine, glutamate and glycine are the only amino acids that contribute to hepatic gluconeogenesis. Although other amino acids are considered glucogenic, their availability for gluconeogenesis is thought to be severely restricted in lactating dairy cows, as great amounts are required for milk protein synthesis. In fact, it was proposed that the rate of liver uptake is low for amino acids other than alanine, glycine and serine [2]. Bergman and Heitmann [79] found that the rate of conversion of glucogenic C to glucose is low in amino acids other than alanine and glutamine. Young [80], who compiled evidence on the splanchnic amino acid metabolism from single injection and radioisotope tracer studies identified a substantial conversion of C from glutamine to glucose. However, it was postulated that increased fractional contributions of amino acids other than alanine to glucose synthesis are unlikely during the periparturient period [74]. Therefore, we used the value of 45 % glucogenic AA in overall AA appearing in the portal blood proposed by Loncke et al. [24], which was derived from publications, re-porting the NPA of total AA as well as the NPA of alanine, glycine and glutamate. With average weekly contributions of 25.5 ± 5.1 % (1st week) to 31.9 ± 5.0 % (12th week), the calculated gAA still made up a large share of glucogenic supply. While the amount of amino acids maximally converted to glucose amounted to 37 % in deprived cows in the study by Lomax and Baird [81], other values reviewed by Larsen and Kristensen [2] were found in the range between 7.1 to 21 % for lactating dairy cows, with a tendency to decrease as lactation progresses.

The values calculated for the NPA of LACdiet (0.14 ± 0.02 mmol/kgBW/h) were lower than the averages for lactating ruminants (0.24 ± 0.08 mmol/kgBW/h) calculated by Loncke et al. [21] and lower than the net portal flux of L-lactate in cows during early- or mid-lactation with indwelling catheters in the portal vein [31,82]. In addition to the different utilization rates within the PDV at different stages of lactation, differences between studies and datasets in the content of the predicting variable (ruminal fermentable organic matter) might play a relevant role. Generally, the measurement of NPA of L-lactate does not differentiate between the amounts of digestive L-lactate and endogenous L-lactate derived from glucose metabolism in gut tissues [83]. However, although glucose oxidation rates are reduced in muscle tissue during the periparturient period, Loncke et al. [21] did not observe any effect of the physiological status on the regression equation for the prediction of NPA of digestive L-lactate. This suggests a rather constant conversion rate of glucose to L-lactate in gut tissues, providing a continuous downhill gradient for glucose absorption.

Finally, the NPA of duodenal absorbed glucose, which is a result of the simultaneous processes of absorption from the lumen and utilization within the PDV, varied widely with low and high levels of bypass starch of, respectively, -10.2 to +11.2 mol C /d, which is in the range described by Galindo et al. [22,31]. The authors observed a positive NPA of glucose averaging 11.2 mol C/d in the control cows at 77 ± 13 DIM and a negative NPA of glucose during early lactation averaging at -6.3 mol C/d. Although the glucose rate of appearance was shown to increase with enhanced starch intake and glucose absorption from the gut in lactating dairy cows [25,84], limitations to the hepatic conversion of glucogenic C at high levels of bypass starch must be considered. It was supposed that these may emerge from an associated increase in propionate leading to a lower conversion rate of propionate [69] and/or effects associated with the insulin/glucagon ratio [85].

4.1.3. The Contribution of Endogenous Precursors

The amount of endogenous precursors from tissue mobilization and in particular, cori-cycling of L-lactate, was supposed to contribute considerably to the total afferent flux of glucogenic C to the liver [2]. With 19.3 ± 5.9 mol of glucogenic C, endogenous sources contributed more than 25 % to the overall glucose supply in the first week of lactation in our study. When expressed in grams of glucose, this value is in close agreement with a rough estimate given by Drackley et al. [86], who calculated that glucose supply from feed intake may fall short of glucose demands by 500g/d (16.7 mol C) in the immediate postpartum period in high-yielding dairy cows.

The shift in precursor supply is also reflected by a shift in mRNA-expression and in the activity of the enzymes related to gluconeogenesis. The increase in pyruvate carboxylase after parturition increases the entry of L-lactate and alanine via the Krebs cycle [1,2], while glycerol enters the glucogenic pathway through the action of glycerol kinase [87]. The averages of GLY (0.95 ± 1.65 mmol/kg BW/d) and ALA (0.07 ± 0.17 mmol/kg BW/d) calculated from the weight losses of cows between 1 and 105 DIM (1.07 ± 0.64 and 0.06 ± 0.04 mmol/d/kg BW, respectively) are in the same range with averages calculated from lactating cows in NEB between DIM 11 to 240 by Loncke et al. [69], when expressed in the same unit. The proportional contribution of

GLY and ALA to overall glucogenic supply was low, with the exception of weeks 1, 2 and 3, where GLY contributed 8 ± 6 %, 5 ± 4 % and 3 ± 3 %, respectively. Accordingly, the loss of BW correlated more strongly with GB during the first week of lactation compared with the whole 105-day period. In studies with multicatheterized cows, GLY contributed a maximum of 4.9 % to hepatic glucose release in fed cows during their first week of lactation [2]. Galindo et al. [31] measured similar values (4.6 %) at 5 DIM, while 8.1 % (17 and 22 %) were measured by Lomax and Baird [81] in cows deprived for several days. According to the model of Guo et al. [14], glycerol provided between 12 and 17 % of the glucose demand.

The calculation of the proportion of L-lactate derived from anaerobic glycolysis within muscle tissues contributed between 13.9 ± 1.9 % in the first week and 6.0 ± 0.7 % in week 15 to overall glucogenic C. The dynamics of L-lactate assumed in our study are in accordance with Reynolds et al. [73], Benson et al. [76] and Larsen and Kristensen [2]. Similar to our results, the greatest contributions of alanine and L-lactate to the net hepatic glucose release were reported during the first week of lactation [35]. Physiologically, this is reflected in both a higher net hepatic uptake of L-lactate during this period [74,76] and a reduced irreversible loss rate of glucose (excluding loss in lactose) in the peripheral tissues [2]. However, the metabolic and nutritional influences on the ratio between glucose being oxidized to CO2 and glucose being converted to L-lactate are worth being investigated in more detail to advance metabolic models of the nutrient partitioning in dairy cows.

4.1.4. Hepatic Turnover of Glucogenic Carbon

The calculation of GB presented in this study assumes that the NPAs of PRO, gAA, LACdiet and GLU represent the general gluconeogenic potential of the cows. It does not account for the capacity of the hepatic conversion of precursors to glucose. In fact, the hepatic conversion of digestive precursors to glucose was shown to not follow first-order kinetics but rather to follow a curvilinear course in ruminants [69]. The same authors concluded from the results of a meta-analysis that the total digestive precursors for gluconeogenesis accounted for only 63 % of the net hepatic glucose release in dairy cattle. This estimation is in accordance with those of Larsen and Kristensen [2], who reviewed the maximal contributions of precursors to gluconeogenesis and summarized that in most studies, precursor supply was not sufficient to explain the hepatic glucose release at various stages of lactation. At first, it seems that these results are anything but self-evident, because the net flux and true flux of glucose across the liver were found to be nearly identical and thus, a minimal hepatic glucose consumption from plasma must be assumed [31]. Yet the great flexibility of hepatic metabolism through storage and release or conversion of glucose, the conversion of glucogenic C to glucose in other tissues and metabolites other than those investigated (see 4.1.5.) might partially explain the lack of glucogenic C relative to liver glucose release. Due to the uncertainties associated with the individual hepatic capacity for gluconeogenesis, we assume that the supply with glucogenic C represents the most consistent measure for the calculation of GB. However, because high liver fat contents, as typically observed in dairy cows, particularly in early lactation, were shown to impede hepatic gluconeogenic capacity [88], glucose availability and therewith, glucose balance, as calculated in this study, may be overestimated.

4.1.5. Other Sources of Glucogenic C

In our study, weekly GB across cows averaged positive values throughout the period investigated, but GB was negative for an average of 10.8 ± 8.4 days per cow. If no glucose consumption was assumed in tissues other than the mammary gland and no maximal standard errors of the NPA of precursors were assumed (maxGB), this value decreased to 0.9 ± 1.9 days per lactation. Physiological explanations for cows in negative GB according to the concept presented in this study include the depletion of glycogen stores, the withdrawal of glucose from the plasma pool or other sources of glucogenic C not accounted for. Regarding the plasma pool of glucose, the timepoint of the lowest average GB concurs with the drop in plasma glucose described for the immediate postpartum period in high-yielding dairy cows [89,90]. However, even in the high range of values, the decrease in plasma glucose would hardly surpass 2 mmol/L and thus would provide less than 1 mol C in dairy cows of 600 kg. Moreover, the depletion of glycogen stores in the liver and muscle of dairy cows was not included in our calculations, as it was assumed that these play a minor role in the provision of glucogenic C during early lactation. In contrast with monogastric animals, where the synthesis of glycogen is needed to overcome postprandial hyperglycemia, a constant supply of glucogenic precursors from rumen fermentation compensates for discontinuous feed intake in ruminants. Accordingly, ruminants store only small amounts of glucose, mainly in the liver and in muscle tissue. This may play a role in maintaining the short-term homeostasis of blood glucose (liver glycogen) as well as for a rapid supply of energy for exercise (muscle glycogen). The hepatic glycogen pool comprises maximally 20 mol glucogenic C before calving [91,92]. Similarly, the amount of glycogen stored in muscle tissue (less than 0.4 % of wet weight; [52,93]) would amount to ~20 mol C for maximal weight of protein tissue observed in our data (132 kg). However, both hepatic [12,94,95] and muscle glycogen [52] were shown to be depleted almost completely immediately after calving. Hammon et al. [89] showed significantly lower glycogen stores in the livers of cows with high vs. low fat content. Interestingly, Galvão et al. [96] also found reduced glycogen concentrations in polymorphonuclear neutrophils at calving, which are associated with the occurrence of subclinical endometritis and metritis. At reference levels of blood volume and neutrophil counts, the estimated glycogen content of neutrophils (~30mg/109 cells [97]) would be less than one mol C in cows of 600 kg and would not be sufficient to cover the glucose demand of an activated immune system [8]. Although these have been thought to be of minor quantitative importance, the pyruvate from catabolism of amino acids [75], the formation of D-Lactate in the gastrointestinal tract, the pyrimidine bases of deoxyribose and ribose from the breakdown of ruminal microbes, and the propionyl-CoA from beta-oxidation of C15 and C17 fatty acids [2] must be considered to have a share in providing C for gluconeogenesis. Moreover, glucogenic C provided by the involution of the uterus might not be negligible, particularly in cows during early lactation [69].

4.2. Glucose Demand

4.2.1. Quantitative Glucose Metabolism in Non-Mammary Tissues

The amount of glucose consumed by non-mammary tissues, i.e., the glucose requirement for maintenance functions that was suggested earlier (~200 g/d; [26]) is thought to be a "bare-bone minimum" [25]. Accordingly, Baldwin [98] reported a turnover rate of glucose in ruminants under basal conditions ranging from .03 to .05 mol/d/kg BW0.75, which is equal to 655 g to 1,092 g of glucose for a cow of 600kg. Similar values were obtained for postpartum dairy cows within the model of Guo et al. [14], while the proportion of whole-body glucose flux not being used by the udder is in the range of 20 to 50 % [22,31,84,99], indicating a substantial demand of glucose for tissues other than the mammary gland, even in high-producing dairy cows. After the estimation of the organ-specific glucose demands of individual cows according to their BW, EBW and body composition, the results obtained in our study suggest a substantial glucose demand by muscle tissue, while the glucose demand of red blood cells and the brain were fairly low (1.1 ± 0.2 and 1.4 ± 0.2 mol C/d, respectively). However, a constant supply of glucose to erythrocytes is crucial because glucose oxidation through the pentose-phosphate-pathway and glycolysis usually is the only source of energy for these cells [100]. The small variations in individual values reported in this study, being attributable only to differences in BW, is thus thought to reflect the physiological function of glucose supply to erythrocytes. Accordingly, the enzyme activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in erythrocytes did not vary according to physiological status in high-producing dairy cows [101]. Similarly, the low variation in glucose utilization by the brain, which was estimated from data presented by Ballarin et al. [33] can be assumed to be due to the importance of glucose for neurometabolism and low variations in the weight of the brain of dairy cows (< 1%). The low brain weight of the domestic Bos taurus also leads to a much lower proportional glucose consumption compared with humans, where at least 20 % of the body's energy consumption is attributed to the brain [102].

The calculation of the glucose demands of protein tissue performed in this study is based on the work of Galindo et al. [22]. The authors assumed that the difference between the whole-body rate of appearance and the glucose consumed by the mammary and splanchnic tissues could be largely traced to muscle glucose metabolism. However, the calculated values are lower than the average values reported for the in-vitro muscle tissue of steers [54] but higher than the values measured in the hind-limb muscle tissue of Merino ewes [103]. Glucose utilization by the muscle was shown to be tightly regulated through plasma insulin, insulin sensitivity and receptor expression [52]. Thus, reductions not only in the amount of glucose oxidation but also in the amount of glucose uptake to muscle cells must be assumed and should be investigated in more detail. Guo et al. [14] as well as Martin and Sauvant [18] presented interesting approaches in this respect. In fact, peripheral glucose sparing might be more exhaustive during early lactation. A 40 % depression in glucose transporter (GLUT) 4 expression and protein levels within the first month of lactation was shown [52]. The estimation of glucose demand of protein tissue performed in our study is based on data on high-producing dairy cows at 77 ± 13 DIM [22]. Due to similar metabolic adaptations between lactation and inflammation [11], reductions in the glucose demand of protein tissue may thus also play a role in providing glucose for immunoactivation.

Additional glucogenic C both in case of high mammary demand and for immune processes may be provided by splanchnic tissues, which on average extract 22 % of whole-body utilization [44]. However, the glucose consumed by these tissues is integrated in the digestive part of the GB calculation presented in this study and could not be calculated separately. This is because the net portal appearance of glucose is a result of both glucose absorption and glucose consumption during digestive processes.

While acetate is supposed to be the major source of C for lipogenesis in ruminants [104], glucose was shown to provide 1-10 % of the acetyl units in subcutaneous adipose tissue and 50-75 % in the intramuscular fat depots of Angus steers [105]. Due to these low values in adipose tissues, and because the cows we investigated were in early lactation, no glucose consumption was assumed for adipose tissues.

4.2.2. Quantitative Glucose Metabolism of the Mammary Gland

The glucose demand of the mammary gland of high-producing dairy cows overrides the glucose demand of other tissues by far. Linear correlations between glucose uptake and milk or lactose yield are described in the literature [60,106–109]. In contrast with previous work in the field, however, we assumed a ratio of 80 % glucose uptake to the mammary gland being used for lactose synthesis during the lactation stage we investigated (see method section). This higher value leading to lower overall values for mammary glucose demand was chosen because Galindo et al. [31] found a higher ratio of mammary lactose output to glucose uptake in cows during early lactation. Accordingly, homeorhetic changes occurring during postpartum hypoglycemia are likely to favor the use of energetic nutrients other than glucose for oxidative processes in the mammary gland. In fact, the dynamics of mammary glucose uptake and consumption in dairy cows are tightly interrelated with the uptake and utilization of other energetic metabolites and precursors. A high degree of metabolic flexibility was observed by Amaral-Phillips et al. [26] and confirmed by Lemosquet et al. [110]. The latter showed that variations in the levels of milk solids are not explained by increases in whole-body or mammary glucose availability from plasma but are a result of the mammary partition of acetate, glucose and other glucogenic C between oxidation, lactose, fat and protein synthesis. Thus, the partitioning of mammary nutrients is likely to adapt to the nutritional status of the animal [111]. GLUT1, which is a non-insulin dependent glucose transporter on the apical and basal membrane of bovine mammary epithelial cells [112] is a major factor contributing to a constant rate of glucose uptake to the mammary glands of dairy cows. GLUT1 expression is regulated through local concentrations of growth hormone (GH) releasing factor and local hypoxia in response to the high metabolic activity of the mammary gland [68,113]. Therefore, the rate of glucose uptake to the bovine mammary gland remains fairly constant across a wide range of plasma glucose concentrations [114].

4.3. Glucose Balance as a Measure of the Cow's Ability to Respond to Immune Challenges

In the past, the quantification of the availability of energy and nutrients at the individual animal level to ensure both the health and the productivity of individual cows focused on overall energy balance. Negative energy balance (NEB) has been related to metabolic dysbalances and the occurrence of disease in dairy cows [115]. However, questions remain about the differences between healthy and diseased cows at similar levels of NEB [116,117]. In fact, energy balance (EB) is a highly aggregated measure and information is lacking on the nutrient-specific trade-offs as well as on the compensation through mobilization or through metabolic flexibility in nutrient utilization. This is supported by the heterogeneity in the plasma concentrations of energy metabolites between cows suffering from similar levels of NEB [118,119]. Accordingly, the quantification of separate nutrient fluxes at the whole animal level, i.e., "moving from joules to moles of molecules or groups of molecules" was claimed by Ortigues-Marty et al. [120] to advance nutritional concepts related to high-producing animals.

GB highlights the immunometabolic bottleneck associated with limited glucose availability during the postpartum period, when mammary and immune cells simultaneously impose great demands for milk production as well as uterine reorganization and other stressors associated with e.g., calving and regrouping, while feed intake lags [11,116]. Because immune functionality is not only essential for pathogen elimination but also is part of the coordinated reaction of the organism to all kinds of stressors [121] and because glucose plays a pivotal role for immune functions, GB is supposed to specifically reflect the cow's ability to adapt to immune challenges [11], compared with the estimation of EB only. However, EB and GB correlated well for most cows and days investigated in our study. This might be related to the homogeneity of the rations fed, which were fairly constant regarding the energy, fibre and protein contents and ration composition, resulting in few differences between the intake levels of glucogenic C and energy. Accordingly, the highest semi-partial correlations were observed between DMI and GB, while correlation coefficients for diet characteristics were low. Apart from variation related to the metabolic part of GB calculation, correlation between EB and GB is thus expected to vary between rations differing more than those fed in our study.

Given that the glucose receptors GLUT1 and GLUT3 of immune cells were found to be negatively correlated to lactose yield [122], metabolic conflicts are expected to arise in situations where both mammary and immune functions impose great demands while the supply of glucose is limited. This is in line with Kvidera et al. [8], who calculated a total glucose deficit of 1,553 g during the 12h following LPS-injection, which is equal to 103.5 mol C on a daily basis. The glucose deficit described by the authors is composed of 530 g (17.7 mol C/d) of glucose infusion required to maintain euglycemia, while the remaining glucose is derived from reductions in the cow's milk production. The results of our study suggest a depletion of glucose reserves during the first weeks of lactation and thus indicate a limited availability of glucose for regulatory processes such as immune defense during this period. According to the dominant role of mammary glucose demand for whole-body glucose metabolism, particularly during early lactation, the correlations between MY and GB were found to be greatest in the immediate postpartum period (DIM 1 to 7, Table 5.6) in our study. This is in line with Gross et al. [90], who observed that cows were less able to cut down milk synthesis during negative energy balance in early in lactation, compared with a similar challenge in later lactation phases. Although metabolic prioritization of the mammary gland is part of the physiological adaptation to lactation in all mammals, it was hypothesized that homeorhetic trade-offs between self-sustainment and the survival of the offspring are dysbalanced during early lactation in cows being bred intensively for high milk yields [117]. For instance, changes in the somatotropic axis related to decreased glucose availability for immune cells, such as hypoinsulinemia [123] and hepatic GH-resistance leading to decreased levels of IGF-1 and its stimulating effect on immune cells [124], are more profound in cows with high genetic merit for milk production [125–127].

5. Conclusions

The methodology presented in this article integrates previous research work on quantitative glucose metabolism in dairy cows, providing a consistent physiologic model for the estimation of glucose reserves. Due to the specific role of glucose for both mammary and immune functions, this parameter highlights a central trade-off of quantitative energy metabolism during the challenging postpartum period. GB might help to advance scientific knowledge as well as data-driven management measures associated with cow-specific glucose requirements to ensure sufficient availability for both regulatory and productive functions. The estimation of glucose reserves for 201 lactations in high-producing dairy cows fed energy-dense diets showed that most cows are facing glucose shortage in the immediate postpartum period, while it is uncertain to what degree reductions in glucose demands of other tissues (mammary and muscle) or other sources of glucogenic C provide additional glucogenic C in case of immunoactivation. Further investigations should focus on the relationships between GB and different feeding regimes, management practices and production outcomes, including the risk for the development of production diseases.

Author Contributions:

Conceptualization, J.H. and A.S.; methodology, J.H..; resources/data collection, C.K.; data curation, C.K. and J.H..; writing—original draft preparation, J.H..; writing—review and editing, A.S., C.K., P.C.; visualization, J.H., P.C.; All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was carried out according to the guidelines of the Declaration of Helsinki and in accordance with the German animal protection act and was approved by institutional review. Data collection was approved by the local authority for animal welfare affairs (Landesuntersuchungsamt Rheinland-Pfalz; G 18-20-073) in Koblenz, Germany.

Data Availability Statement:

Restrictions apply to the availability of these data. Data were obtained from the Educational and Research Centre for Animal Husbandry Hofgut Neumuehle and are available with the permission of a third party.

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Conflicts of Interest:

The authors declare no conflicts of interest.

Appendix A

Table 5.A1. Number of lactations enrolled in the study (N) and means of cow data according to week of lactation.

week	N	MY	ECM	F %	P %	L %	DMI	BW	EBW	AT	PT	BWloss	EB
1	187	29.0 ±6.4	27.2 ±9.2	3.8 ±0.6	3.2 ±0.4	4.6 ±0.2	11.3 ±2.0	658 ±79	576 ±70	124 ±27	97 ±12	-2.5 ±1.9	-49.8 ±20.9
2	199	34.5 ±7.4	33.2 ±8.3	3.8 ±0.7	3.2 ±0.4	4.8 ±0.2	14.4 ±2.8	646 ±80	565 ±69	118 ±24	96 ±12	-1.8 ±1.4	-45.9 ±23.7
3	198	37.1 ±7.8	35.7 ±8.8	3.8 ±0.7	3.2 ±0.4	4.8 ±0.2	17.1 ±3.3	637 ±77	556 ±68	112 ±23	95 ±11	-1.2 ±1.2	-35.0 ±24.1
4	194	38.6 ±8.2	37.3 ±8.9	3.8 ±0.7	3.1 ±0.3	4.8 ±0.1	18.0 ±3.2	631 ±76	551 ±66	108 ±22	95 ±11	-0.8 ±0.9	-32.3 ±24.7
5	188	40.1 ±7.6	38.4 ±8.6	3.8 ±0.7	3.1 ±0.4	4.8 ±0.1	18.9 ±3.1	627 ±76	547 ±66	105 ±22	95 ±11	-0.5 ±0.8	-30.5 ±20.8
6	186	40.5 ±7.4	38.8 ±8.2	3.7 ±0.6	3.1 ±0.4	4.8 ±0.2	19.8 ±2.9	626 ±75	547 ±65	103 ±21	95 ±11	-0.4 ±0.6	-24.4 ±20.2
7	182	40.6 ±7.5	38.7 ±8.1	3.7 ±0.6	3.1 ±0.4	4.8 ±0.2	20.5 ±3.0	627 ±74	547 ±65	102 ±21	96 ±11	-0.3 ±0.5	-19.5 ±21.3
8	178	40.5 ±7.2	38.7 ±8.2	3.7 ±0.6	3.1 ±0.4	4.8 ±0.2	20.8 ±3.0	628 ±73	548 ±64	101 ±20	96 ±11	-0.2 ±0.5	-18.6 ±20.2
9	176	40.1 ±6.9	38.3 ±8.0	3.7 ±0.7	3.1 ±0.4	4.8 ±0.2	21.1 ±3.0	629 ±72	549 ±63	100 ±20	96 ±11	-0.2 ±0.7	-14.1 ±19.7
10	170	39.4 ±6.9	37.3 ±7.9	3.7 ±0.7	3.1 ±0.4	4.8 ±0.1	21.0 ±3.3	629 ±72	549 ±63	100 ±20	97 ±11	-0.2 ±0.4	-13.1 ±21.7
11	164	39.0 ±6.8	37.0 ±7.6	3.7 ±0.7	3.1 ±0.3	4.8 ±0.1	21.6 ±3.0	631 ±72	551 ±63	101 ±20	97 ±10	-0.2 ±0.5	-7.9 ±20.2
12	160	38.6 ±7.1	36.4 ±7.6	3.6 ±0.6	3.1 ±0.4	4.8 ±0.1	21.7 ±3.0	632 ±72	552 ±63	101 ±21	97 ±10	-0.2 ±0.5	-5.4 ±17.7
13	157	37.9 ±6.6	35.8 ±7.5	3.7 ±0.6	3.1 ±0.4	4.8 ±0.1	21.8 ±3.1	635 ±72	554 ±63	101 ±21	97 ±11	-0.2 ±0.5	-2.7 ±18.6
14	152	37.9 ±6.6	36.0 ±7.4	3.7 ±0.7	3.2 ±0.4	4.8 ±0.1	22.1 ±3.1	636 ±73	555 ±64	102 ±21	98 ±11	-0.2 ±0.5	-1.4 ±19.4
15	136	37.3 ±7.0	36.0 ±8.0	3.8 ±0.6	3.2 ±0.4	4.8 ±0.2	21.9 ±3.2	640 ±75	559 ±66	103 ±21	98 ±11	-0.3 ±0.6	-1.3 ±18.9

AT: the mass of adipose (kg); BW: body weight (kg); BWloss: daily loss of body weight (kg/d); DMI:, dry matter in-take (kg/d); EB: daily energy balance (MJ/d); EBW: empty body weight (kg); ECM: energy-corrected milk (kg); F %: percentages of milk fat; L %: percentage of milk lactose; MY: milk yield (kg); P %: percentage of milk protein; PT: the mass of protein tissue (kg)

Table 5.A2. Means of daily glucose balance and daily precursor supplies (mol C/d) according to week of lactation.

		GLU_{IN} from digestive precursors					**GLU_{IN} from endogenous precursors**			
week	**GB**	**total**	**PRO**	**gAA**	**LACdiet**	**GLU**	**total**	**LACendo**	**GLY**	**ALA**
1	3.2 ±13.5	59.0 ±12.1	38.8 ±7.0	21.5 ±4.3	4.7 ±0.6	-5.9 ±1.3	19.3 ±5.9	11.7 ±1.6	6.8 ±5.0	0.9 ±0.6
2	8.8 ±15.1	78.8 ±17.6	49.7 ±10.0	28.0 ±6.1	5.4 ±0.8	-4.3 ±1.5	17.3 ±4.5	12.0 ±1.7	5.1 ±3.9	0.6 ±0.4
3	19.4 ±16.3	97.0 ±20.6	59.7 ±11.7	34.0 ±7.1	6.0 ±0.9	-2.8 ±1.6	15.4 ±4.1	11.8 ±1.6	3.4 ±3.3	0.3 ±0.3
4	21.2 ±17.6	103.8 ±20.9	63.6 ±12.0	36.1 ±7.1	6.2 ±0.9	-2.1 ±1.7	13.7 ±3.4	11.3 ±1.4	2.4 ±2.7	0.2 ±0.2
5	22.1 ±15.3	109.3 ±20.1	66.6 ±11.6	37.9 ±6.8	6.4 ±0.9	-1.7 ±1.5	12.2 ±2.8	10.6 ±1.2	1.6 ±2.3	0.1 ±0.2
6	26.7 ±14.8	116.0 ±18.9	70.5 ±11.1	40.1 ±6.3	6.6 ±0.8	-1.1 ±1.5	10.9 ±2.4	9.7 ±1.1	1.2 ±2.0	0.1 ±0.1
7	30.2 ±16.3	121.0 ±20.2	73.4 ±11.9	41.6 ±6.6	6.8 ±0.8	-0.7 ±1.7	9.8 ±2.0	8.9 ±1.2	1.0 ±1.8	0 ±0
8	31.2 ±14.8	122.4 ±19.7	74.1 ±11.7	42.0 ±6.5	6.8 ±0.9	-0.6 ±1.6	9.1 ±1.8	8.3 ±1.1	0.8 ±1.7	0 ±0
9	34.8 ±15.2	125.2 ±20.1	75.8 ±11.8	42.9 ±6.6	6.9 ±0.8	-0.4 ±1.7	9.1 ±2.4	8.2 ±1.2	0.9 ±2.4	0 ±0
10	34.6 ±18.1	123.8 ±22.5	75.0 ±13.1	42.5 ±7.4	6.9 ±0.9	-0.5 ±1.9	8.9 ±1.8	8.3 ±1.1	0.7 ±1.5	0 ±0
11	40.3 ±17.2	128.7 ±21.0	77.9 ±12.4	44.0 ±6.8	7.0 ±0.8	-0.2 ±1.8	9.0 ±1.9	8.3 ±1.1	0.7 ±1.8	0 ±0
12	41.8 ±15.5	129.3 ±20.6	78.3 ±12.2	44.2 ±6.7	7.0 ±0.8	-0.2 ±1.8	9.1 ±1.8	8.4 ±0.9	0.7 ±1.8	0 ±0
13	43.7 ±15.9	129.7 ±21.2	78.5 ±12.5	44.3 ±6.9	7.1 ±0.8	-0.2 ±1.8	9.1 ±1.8	8.4 ±0.9	0.7 ±1.7	0 ±0
14	46.4 ±16.8	131.9 ±21.3	79.8 ±12.6	45.0 ±7.0	7.1 ±0.8	0.0 ±1.9	9.3 ±1.9	8.4 ±0.9	0.9 ±1.8	0 ±0
15	46.7 ±17.4	130.8 ±22.3	79.3 ±13.1	44.7 ±7.3	7.1 ±0.9	-0.3 ±1.9	9.6 ±2.0	8.5 ±1.0	1.2 ±1.9	0 ±0

ALA: alanine; gAA: glucogenic amino acids; GLU: glucose (negative values for GLU indicate a net consumption of duode-nal glucose by portal-drained viscera); GLY: glycerol; LACdiet: L-lactate from feed; LACendo: endogenous L-lactate from muscle catabolism; PRO: propionate

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7. Publication #3 - Dairy Cows Are Limited in Their Ability to Increase Glucose Availability for Immune Function during Disease

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Simple Summary

Both immune defense and milk synthesis require large amounts of energy, particularly from glucose, resulting in competition between these functions if glucose availability is limited. Based on comprehensive data on cow nutrient intake and on cow health records, the magnitudes and dynamics of glucose and energy balances of dairy cows kept on an experimental farm were evaluated in the weeks before, of, and after a production disease was diagnosed. Because dry matter intake (DMI) decreased during the phase of disease and was not adequately compensated by a reduction in milk yield (MY), glucose availability dropped when it was most needed.

Abstract

Shortages of energy and glucose have been hypothesized to play a key role in the development of and responses to production diseases in dairy cows during early lactation. Given the importance of glucose for immune functions, we used a recently established method for the estimation of glucose balance (GB) to evaluate glucose availability during disease phases. A dataset comprising ration analyses as well as individual daily milk yields (MY), dry matter intake (DMI), body weights, and health records of 417 lactations (298 cows) was used to calculate individual daily GB and energy balance (EB). The magnitude and dynamics of MY, DMI, GB, and EB were evaluated in the weeks before, at, and after diagnoses of inflammatory diseases in different stages of early lactation from week in milk 1 to 15. Diagnoses were categorized as mastitis, claw and leg diseases, and other inflammatory diseases. Mixed linear models with a random intercept and slope term for each lactation were used to evaluate the effect of diagnosis on MY, DMI, GB, and EB while accounting for the background effects of week in milk, parity, season, and year. When unaffected by disease, in general, the GB of cows was close to zero in the first weeks of lactation and increased as lactation progressed. Weekly means of EB were negative throughout all lactation stages investigated. Disease decreased both the input of glucose precursors due to a reduced DMI as well as the output of glucose via milk due to a reduced MY. On average, the decrease in DMI was −1.5 (−1.9 to −1.1) kg and was proportionally higher than the decrease in MY, which averaged −1.0 (−1.4 to −0.6) kg. Mastitis reduced yield less than claw and leg disease or other diseases. On average, GB and EB were reduced by −3.8 (−5.6 to −2.1) mol C and −7.5 (−10.2 to −4.9) MJ in the week of diagnosis. This indicates the need to investigate strategies to increase the availability of glucogenic carbon for immune function during disease in dairy cows.

1. Introduction

Reducing the incidence of production diseases (PD) in dairy farming is of pivotal importance. They are of major importance for the economic viability of dairy farms [1,2]. They also raise public concerns about animal welfare [3] and undermine social acceptance of dairy farming. PD are multifactorial diseases. Their occurrence is the result of interactions between cow-specific factors, such as the cow's endocrine and nutritional status, and numerous environmental factors such as climatic and hygiene conditions [4,5,6].

The metabolic load resulting from the increase in the milk synthetic capacity over the past decades has been put forward as a major risk factor for the occurrence of PD [7,8]. However, high milk yields do not necessarily imply an increased health risk, as nutritional imbalances and metabolic stress occur only when the performance is not met by an adequate energy and nutrient supply. Generally, the capacities for milk synthesis and feed intake are highly correlated [9]. However, due to increases in body weight and the corresponding maintenance requirements and due to limitations regarding the energy density as well as the time to eat and ruminate, increases in overall energy intake are not sufficient to meet the increased demand during early lactation of cows selected for high milk yields [6,10]. Moreover, selection for milk yield has led to higher feed conversion efficiencies for milk production, i.e., a higher amount of milk produced per unit of dry matter intake. Changes in this ratio are often evaluated as the "residual feed intake" (RFI), with low RFI being associated with higher feed efficiency for milk production [11,12]. Because feed costs are assumed to decline with low RFI [13], this criterion is often used for selection in dairy breeding [12,14]. However, metabolic imbalance and increased lipomobilization arising from strongly negative energy balance (NEB, a negative ratio between energy intake and energy requirement) is a major concern associated with low RFI because it increases the risk for the development of diseases [5,15,16,17]. Excessive lipomobilization causes impaired liver [18,19] and immune functions [20,21] following the hepatic accumulation of triglycerides as well as the increase in plasma levels of non-esterified fatty acids and β-hydroxy-butyrate. Besides metabolic imbalances, a low RFI also implies that the proportion of feed energy intake attributed to maintenance functions decreases. This effect is termed "dilution of maintenance" [22]. Thus, if cows are in NEB, a low RFI also implies that cows have fewer remaining energy sources and essential substrates available for functions other than milk synthesis.

Due to limitations in concentrate supply, rumen fill, and hepatic gluconeogenesis, competition for glucose in particular between the mammary gland and immune cells has been outlined [23,24]. Kvidera et al. (2017) investigated the drop in milk yield and the level of glucose infusion required to maintain euglycemia in mid-lactating dairy cows infused with lipopolysaccharide (LPS) and found that a fully activated immune system may need amounts of glucose similar to those required for the production of ~40 kg of milk [25].

While the amounts of glucose required for a specific inflammatory condition remain unknown under practical conditions, a methodology indicating the quantities of 'residual' glucose available to individual cows (which are expected to reflect a cow's ability to respond to an inflammatory challenge) has been developed previously. The methodology is described in more detail elsewhere [26]. Briefly, it encompasses the estimation of the glucose demands of major glucose-consuming tissues (mammary gland, muscle, red blood cells, and the brain) as well as major sources of glucogenic supply (digestive and endogenous) from daily measurements of milk yield, body weight, and feed intake as well as the chemical composition of feedstuffs ingested. Here, we have applied the methodology to quantify the level as well as weekly changes (Δ) in the energy and glucose availability resulting from changes in DMI, MY, and body weight before, during, and after inflammatory disease events in dairy cows.

2. Materials and Methods

2.1. Animals

During and outside different experimental periods between January 2015 and February 2022, daily milk yields (MY, kg), feed intakes (kg), and body weights (BW, kg) of Holstein cows kept in freestall barns were recorded at the Educational and Research Centre for Animal Husbandry Hofgut Neumuehle. Experiments were part of the OptiKuh project [27], which includes feeding trials on phosphorus and nitrogen reduction, amino acid supplementation, and processing of corn. None of the trials intended to induce (NEB). Milk solids and body condition scores were assessed routinely (biweekly or monthly). The individual feed intake was recorded using feeders equipped with a weighing unit and automatic cow identification (Roughage Intake Control, Insentec B.V., Marknesse, The Netherlands). The cows were milked twice daily using a combination of a herringbone and a side-by-side milking parlor manufactured by GEA Farm Technologies (located in Bönen, Germany). The cows' BWs were measured automatically after each milking via a walk-over scale, and daily values were derived by averaging morning and evening BW. Data from the milking parlor was recorded via the herd management system Dairy Plan C21 (GEA Farm Technologies, Boenen, Germany). The start of recording of feed intake, BW, and MY data of cows varied between days one and eight due to variation in the timing of entry after calving into the main housing system for lactation. The daily MY was recorded at morning and evening milking. The milk was analyzed for fat, protein, and lactose via an infrared analyzer (MilkoScan FT-6000, Foss Analytical A/S, Hillerod, Denmark; LKV Rheinland-Pfalz-Saar e.V., Bad Kreuznach, Germany).

Measurements of milk solids and BCS as well as values for MY and DMI that were missing due to technical errors were inter-/extrapolated linearly up to five consecutive days. The respective part of lactation was excluded from the analyses where more values were missing. After removal of values differing by more than 10 kg of empty body weight (EBW) from the previous or succeeding day (or up to 50 kg within 5 days), BW was smoothed across the first

150 days in milk (DIM) for each cow using a cow-specific polynomial function up to the fifth degree.

In total, data from 5048 weeks (Weeks 1 to 22 in milk corresponding to DIM 1 to 150) of 417 lactations of 298 cows were entered into the final dataset used in our analysis. Due to the variation in the timing of entry to the main herd after calving and due to technical constraints leading to missing records, weeks in milk 1 to 22 were not equally represented in the dataset. Of the 5048 weeks observed, only 98 (1.9%) and 214 (4.2%) were first and second weeks of lactation, respectively. Because some of the experimental recordings stopped at 105 DIM, the proportions of single weeks in milk between week 16 and 22 were also lower (between 2.5% and 4.5%) than those of weeks 3 to 15, which made up between 4.8% and 5.7% of the data. The curves for MY, DMI, GB, EB, and EBW followed the pattern typically observed in dairy cows during early lactation. To investigate differences in the way dairy cows adjust DMI and MY during disease, weeks 1 to 22 were divided into three stages of early lactation (stage 1: week 1 to 7, stage 2: week 8 to 14, and stage 3: week 15 to 22). Averages of MY, DMI, GB, EB, and EBW as well as their weekly change during different stages of early lactation are given in Table 1.

Table 6.1. Means of milk yield (MY, kg/d), dry matter intake (DMI, kg/d), glucose balance (GB, mol C/d), energy balance (EB; MJ of net energy for lactation (NEL)), and empty body weight (EBW, kg) as well as means of the weekly change (Δ) in average daily MY, DMI, GB, EB, and EBW during different stages of early lactation (data of all cows and lactations enrolled in the study).

	Week in Milk		
	1 to 7	**8 to 14**	**15 to 22**
MY (kg)	40.0 ± 0.2	41.1 ± 0.2	39.0 ± 0.2
ΔMY	1.3 ± 0.1	−0.3 ± 0.0	−0.4 ± 0.1
DMI (kg)	18.5 ± 0.0	22.2 ± 0	23.4 ± 0.0
ΔDMI	0.7 ± 0.1	0.3 ± 0.1	0.0 ± 0.1
GB (mol C)	7.2 ± 0.4	16.7 ± 0.4	26.6 ± 0.4
ΔGB	−0.8 ± 0.3	1.9 ± 0.2	1.3 ± 0.2
EB (MJ NEL)	−53.6 ± 0.6	−31.2 ± 0.5	−17.4 ± 0.5
ΔEB	0.8 ± 0.6	3.0 ± 0.3	1.3 ± 0.4
EBW (kg)	564 ± 2	567 ± 1	585 ± 2
ΔEBW	−0.7 ± 0.0	0.1 ± 0.0	0.1 ± 0.0

2.2. Health Records

Diagnoses were made by a veterinarian according to a standardized diagnostic key that is used for the evaluation of health data in Germany [28]. Each diagnosis was classified in one of the following categories: udder disease, metabolic disease, claw and leg disease, genital tract disease, gastrointestinal disease, respiratory disease, and other diseases. Due to few diagnoses in categories other than udder disease and claw and leg disease, all other diagnoses were combined in the category “other diseases”. Given the hypothesis that substantial amounts of glucose are required for immunoactivation, diagnoses that were considered to cause no or minor/local inflammatory reactions, including dermatitis digitalis (DD) stadium M0, M1, M4, mild trichophytia, and ovarian cysts, were removed from the dataset. In the final dataset, all cases of descriptive mastitis diagnosis except those identified as sub-clinical and chronic built up the category “mastitis”. Apart from this, no further differentiation regarding the severity of mastitis cases was made. “Claw and leg disease” included ulcers, white line abscesses, interdigital phlegmons, acute DD stages, DD-associated inflammation as well as high-grade swelling of coronet and bulb, as they are supposed to be associated with the presence of infections [29]. In the category of “other diseases”, retained placenta, endometritis, metritis, vaginitis, and a few other infections, such as pneumonia, were compiled. In total, the number of inflammatory diagnoses made was 60, 34, and 26 for mastitis, claw and leg disease, and other diseases, respectively. This is equivalent to an incidence of 29 cases of inflammatory disease per 100 lactations or a 2.4% risk per cow-week (Table 2). Health records were cleaned stepwise by marking the day of the first diagnosis of each cow in each category as well as the following 10 days (“sick days”) with “1” and all other days with “0”. Second (third, fourth, and fifth) diagnoses of the same cow in the same category were considered only if the timing did not fall within the ”sick days” period of the preceding diagnosis to avoid double recordings of the same case of disease. In total, 83 cows were diagnosed once, 14 cows were diagnosed twice, and 3 cows were diagnosed three times within the first 22 weeks of lactation.

Table 6.2. Number of diagnoses according to disease category and stage of lactation.

	Week in Milk		
	1 to 7	**8 to 14**	**15 to 22**
Mastitis	17	25	18
Claw and Leg Disease	11	10	13
Other Disease	13	12	1

2.3. Rations

All cows were fed a total mixed ration (TMR) with varying compositions. All diets included grass silage, corn silage, and a mix of concentrates and were complemented by one or more of the following feedstuffs: pressed sugar beet pulp silage, hay, straw, vegetable oils, urea, and a mixture of synthetic amino acids (Table 3). Ration composition was adjusted regularly to ensure adequate levels of metabolizable energy intake. Cows were fed ad libitum. TMR and feedstuffs were analyzed monthly and/or if ration composition changed using Weende and van Soest analysis for dry matter, organic matter, crude nutrients (protein, fat, fiber, starch, and sugar) as well as ash-free acid detergent fiber and ash-free neutral detergent fiber. Means and standard deviations (SDs) of crude nutrient composition of the TMRs are given in Table 3.

Table 6.3. Mean, minimal, and maximal contents of dry matter (DM), net energy for lactation (NEL), organic matter (OM), crude protein (CP), ash-free acid detergent fiber (ADFom), and ash-free neutral detergent fiber (aNDFom) in the rations fed (A) as well as mean, minimal, and maximal proportions of feedstuffs in the diets (B).

A

Variable	Mean	Min	Max
DM (g/kg fresh matter)	468	383	566
NEL (MJ/kg DM)	7.0	6.5	7.3
OM (g/kg DM)	929	918	939
CP (g/kgDM)	152	121	165
Starch (g/kgDM)	197	129	235
aNDFom (g/kgDM)	341	252	375
ADFom (g/kgDM)	213	148	232

B

Feedstuffs	Mean	Min	Max
Concentrates	35.1	29.6	40.1
Maize silage	25.5	15.0	45.5
Grass silage	23.0	9.7	34.3
Sugar beet pulp silage	13.8	0.0	19.0
Hay	4.6	3.1	7.5
Barley straw	1.6	0.0	2.4
Urea	0.4	0.3	0.4
Amino acids	0.4	0.4	0.5
Vegetable oils	0.8	0.8	0.9

2.4. Calculations

Daily energy demand for production (EDP; MJ NEL/d) and daily energy demand for maintenance (EDM; MJ NEL) were calculated according to the Society of Nutrition Physiology, which periodically publishes calculation principles for the evaluation of feedstuffs and rations typically used in the German dairy sector [30]. Daily energy intake was calculated from the energy content of the feed (MJ NEL) and the recorded individual dry matter intake (DMI; kg). The sum of EDP and EDM was subtracted from energy intake to obtain daily individual energy balance (EB; MJ NEL).

The main input variables for the calculation of GB were the individual daily milk yield, feed intake and body weight, and data on ration composition and chemical analyses of feedstuffs. Detailed explanations and equations for the calculation of glucose balance are reported elsewhere [26]. In brief, the calculations have a metabolic and a digestive part. In the metabolic part, the daily release of glucogenic C from endogenous sources is estimated from daily changes in empty body weight (ΔEBW, kg). EBW is calculated from the individual daily BW and the level of NDF intake (NDF%BW) according to INRA [31]. Additionally, the proportion of L-lactate production in protein tissue is taken into account according to a regression equation derived from data on the irreversible loss rate of glucose in protein tissue compiled by Larsen and Kristensen [32]. Glucose demands of major glucose-consuming tissues, including the mammary gland, protein tissue, the brain, and red blood cells, are calculated from the lactose yield (mammary gland) and individual body weight (protein tissue, brain, and red blood cells) according to previously published study in the field [33,34,35,36,37,38].

The estimation of the supply with glucogenic carbon (C) from feed is based on data-driven models that predict ruminal production of propionate as well as duodenal flows of L-lactate, glucogenic amino acids, and glucose from the contents of ruminal fermentable organic matter (RfOM), truly digestible starch (StDI), and truly digestible protein in the intestine (PDI) via the Systool Web application [39]. The calculations account for digestive interactions related to the proportion of concentrate in the diet, rumen protein balance, and the individual feeding level (feed intake in relation to body weight). In a second step, portal flows of glucose and glucogenic precursors were calculated from their ruminal and intestinal flows according to Loncke et al. [40] and Martineau et al. [41]. “Mol of glucogenic C per day” (mol C/d) was used as the general unit for the calculation of input and output fluxes as well as GB.

2.5. Statistical Analyses

The analyses were restricted to the first 22 weeks in milk (154 days) to focus on the early part of lactation when metabolic stress is most severe. Due to strong intra-individual variance from day to day, values were aggregated as weekly means. Two statistical approaches were used.

First, two-sided tests for difference of means between the mean value in a week (wk) relative to the week of diagnosis (wk-2, wk-1, wk0, and wk+1) and the mean of all other weeks (“healthy weeks”) were performed. The following variables were tested: GB, EB, MY, and DMI as well as the development of those variables compared to the preceding week (ΔGB, ΔEB, ΔMY, and ΔDMI). The test was performed for different disease categories as well as for different stages of early lactation (see above). Means in wk-2, wk-1, wk0, and wk1 differing significantly from healthy weeks are indicated at a level of $p = 0.1$ (*) and $p = 0.05$ (**). For the second approach, mixed linear models were used to estimate GB, EB, MY, and DMI as well as ΔGB, ΔEB, ΔMY, and DMI in wk-2, wk-1, wk0, and wk1 compared to healthy weeks. The modeling included repeated measures of lactation weeks within lactations following a first-order autoregressive moving average structure. Each of the models included a random intercept and slope term for each lactation with a covariance structure assuming no correlation between lactations. For the fixed effects of week in milk, polynomials of week in milk up to fourth degree representing the shape of the GB, EB, MY, DMI, and EBW curves were chosen by best fit according to the Akaike information criterion. To account for other confounding effects, the fixed effects of parity, season, and year were included. Finally, a derived variable of five levels representing the week relative to the week of diagnosis ("WeekDia"; two levels for the 2 weeks before the disease event, one level for the week of diagnosis, one level for the week after diagnosis, and one level for all other weeks) was built and added to the models. The model equation is:

$$y_{i,p} = \beta_0 + \beta_1 t_i + \beta_2 t^2{}_i (+\beta_3 t^3{}_i) + (\beta_4 t^4{}_i) + season_j + year_k + parity_l + WeekDia_m + \alpha_0 + \alpha_{1,p} t_i + \alpha_{2,p} t^2{}_i + \varepsilon$$

where $y_{i,p}$ is the i^{th} weekly (t) GB (EB, MY, DMI, ΔGB, ΔEB, ΔMY, ΔDMI, and ΔEBW) measured on cow p; β_0 is a regression coefficient for the intercept; β_1, β_2, (β_3), and (β_4) are regression coefficients for the polynomial terms to estimate weekly values as a function of week in milk across all cows in the herd; $season_j$, $year_k$, $parity_l$ and $WeekDia_m$ represent the fixed effects of year, season, parity, and WeekDia on $y_{i,p}$, respectively; α_0, α_1, and α_2 are the random intercept and slope terms to describe the deviation of cow p's relative weekly values from that of the rest of the herd; and ε represents the random residual error. Residuals were checked graphically against predicted values to test the homogeneity of variance of the error terms. The mixed model was fitted using the mixed linear model procedure in IBM® SPSS® Version 28.0.1.0.

3. Results

On average, GB and EB increased from 3.9 ± 16.8 mol C/d and −73.5 ± 25.7 MJ NEL/d in the first week of lactation to 31.1 ± 17.8 mol C/d and −12.7 ± 21.3 MJ NEL/d in the fifteenth week of lactation.

When evaluated across all stages of early lactation, means of GB were lower in the week a disease was diagnosed (11.3 mol C/d; $p = 0.001$) as well as in the week before diagnosis (13.7 mol C/d; $p = 0.070$) compared to the average of healthy weeks (16.7 mol C/d). Among diseased cows, GB was lowest in cows diagnosed with claw and leg disease. Means of MY were higher in the week before, of, and after diagnosis compared to other weeks during the same lactation stage, although the average daily MY declined by an average of 1.00 kg in the week of diagnosis compared to the preceding week (ΔMY). Means of DMI were higher in the week before and after diagnosis but lower in the week of diagnosis compared to healthy weeks during the same lactation stage. The average reduction in daily DMI in the week of diagnosis compared to the preceding week was 1.06 kg (ΔDMI).

When evaluated for different disease categories (Figure 1) as well as for different stages of early lactation (Figure 1 and Figure 2), however, differences in the way MY and DMI (and thus GB) adapted during disease events were observed.

Figure 6.1. Weekly means of daily milk yield (MY), dry matter intake (DMI), and glucose balance (GB) in the week before (−1), the week of (0), and the week after (1) diagnosis in different stages of early lactation (weeks 1 to 7, 8 to 14, and 15 to 22) for different disease categories: claw and leg disease (x), mastitis (▲), and other diseases (□). Significant differences between means in the respective week compared to the means in healthy weeks of the respective stage (grey line) are indicated at p < 0.1 (*) and p < 0.05 (**).

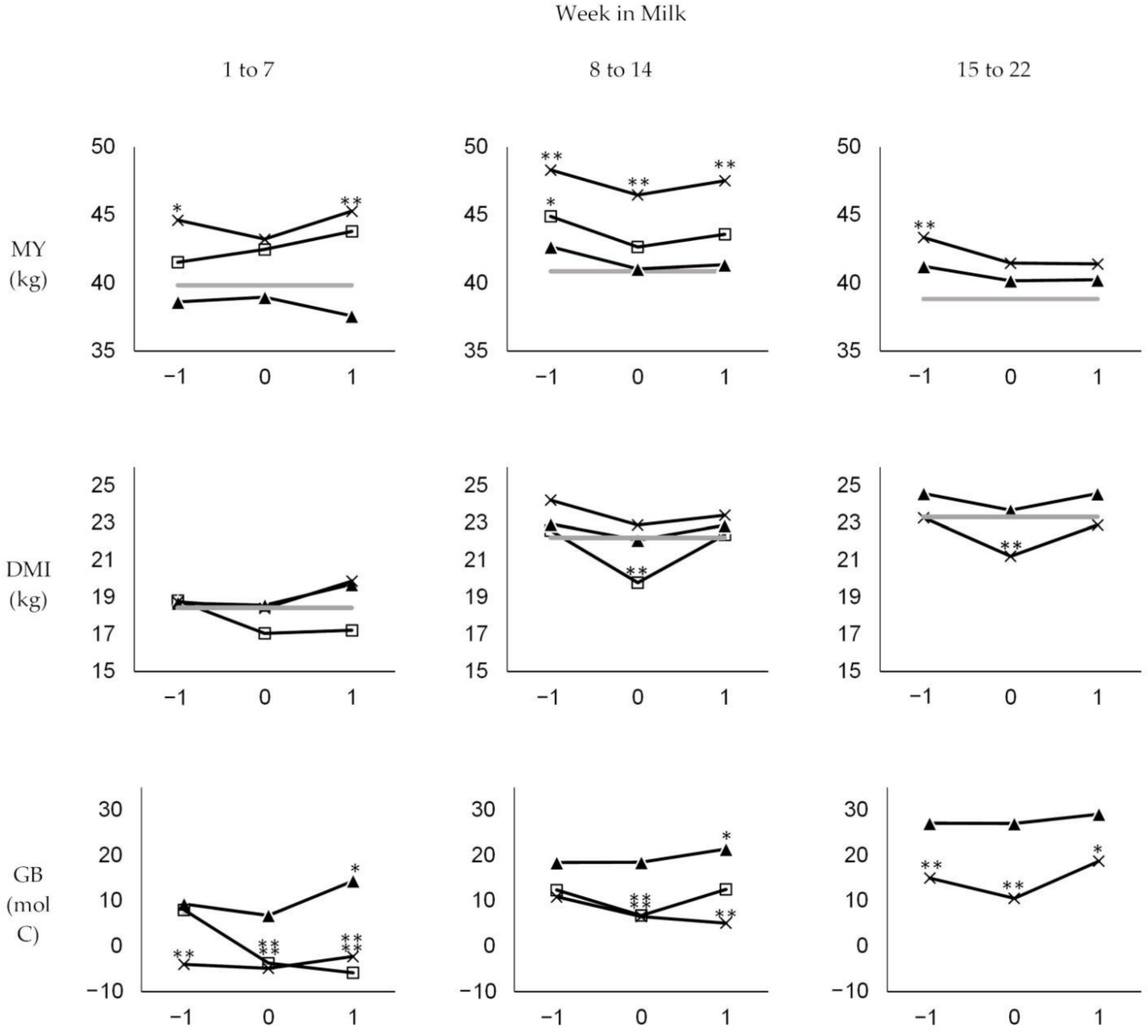
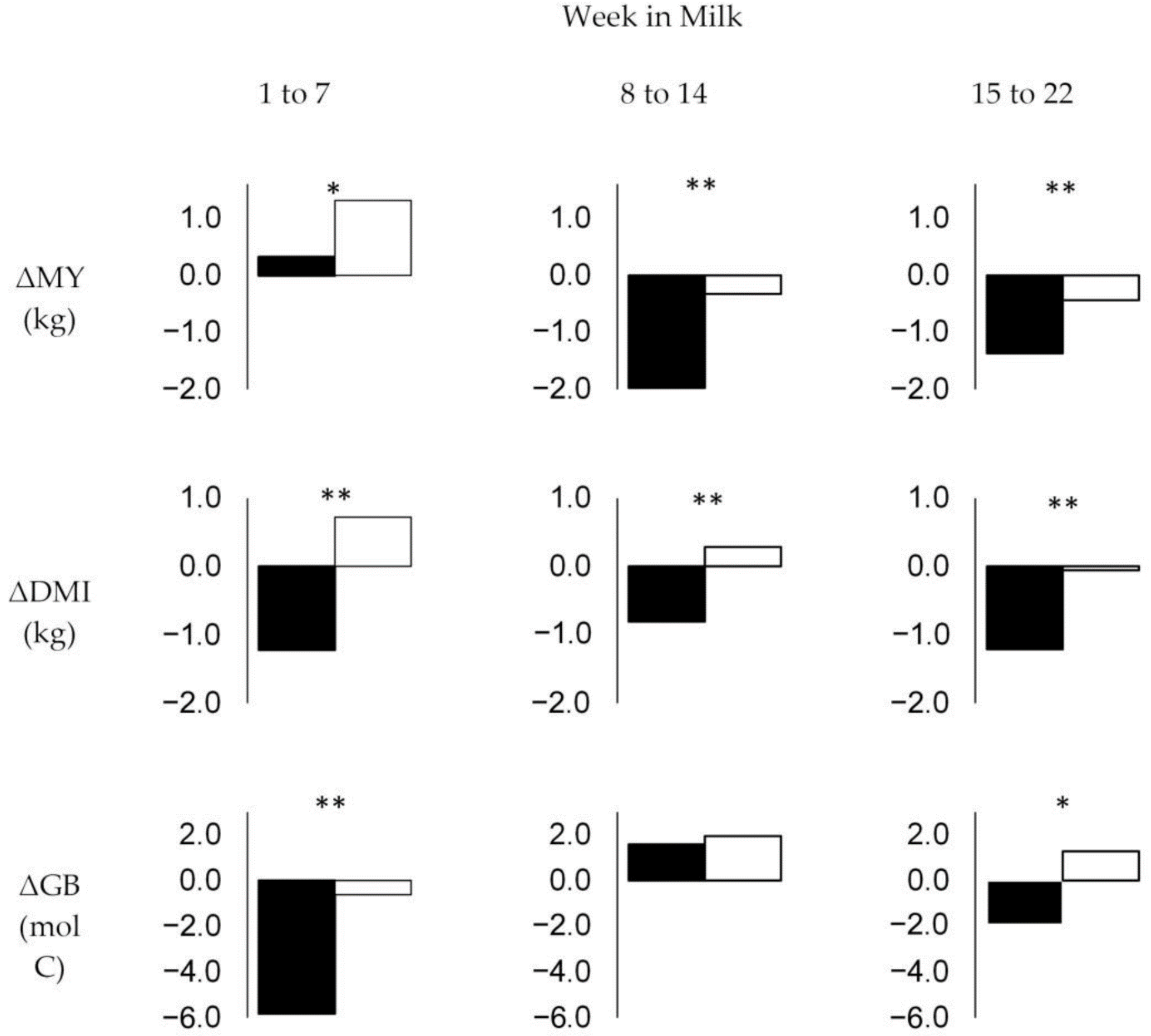


Figure 6.2. Weekly change (Δ) in means of daily milk yield (MY), dry matter intake (DMI), and glucose balance (GB) in the week of diagnosis (black bars) compared to the means of MY, DMI, and GB in other weeks of the respective stage (white bars) for all disease categories in different stages of early lactation (weeks 1 to 7, 8 to 14, and 15 to 22). Significant differences are indicated at p < 0.1 (*) and p < 0.05 (**).



In the first stage (weeks one to seven), average daily MY of diseased cows increased even in the week of diagnosis, although it increased more slowly than in healthy weeks during this stage (ΔMY averaging +0.3 vs. +1.3 kg; p = 0.061). The average daily DMI was reduced by −1.2 kg in the week of diagnosis compared to the preceding week (ΔDMI), while this value was +0.7 kg in healthy weeks during this stage (p = 0.000). This resulted in a more severe decrease in GB in the week of diagnosis compared to healthy weeks (−5.8 vs. −0.6 mol C; p = 0.022), with absolute values of GB averaging 0.9 and 7.4 mol C/d in the week of diagnosis and healthy weeks, respectively (p = 0.007). During the first stage, cows diagnosed with mastitis showed lower MY than cows diagnosed with claw and leg disease or other diseases. However, their average daily MY, DMI, and GB in the week of diagnosis did not change significantly compared to the preceding week and was not significantly different from the average MY, DMI, and GB observed in healthy weeks during this stage. Because their MY dropped in the week after diagnosis, GB in the week after diagnosis was greater than the average GB in healthy weeks during this stage. In contrast, GB was significantly lower in the week of diagnosis as well as in the week after diagnosis compared to healthy weeks in cows diagnosed with claw and leg disease or other diseases.

In the second stage investigated (weeks 8 to 14), MY decreased more significantly in the week of disease compared to healthy weeks, with ΔMY averaging −2.0 vs. −0.3 ($p = 0.000$). Because the decrease in DMI during disease was not as severe as in the first stage, with ΔDMI averaging −0.8 kg in the week of diagnosis compared to +0.3 kg in healthy weeks ($p = 0.002$), no significant differences in ΔGB, which, on average, became positive during this stage, were observed between the week of diagnosis and healthy weeks in this stage (1.6 vs. 1.9 mol C; $p > 0.1$). Accordingly, absolute values of GB in the week of diagnosis and healthy weeks averaged 13.9 and 16.9 mol C/d ($p > 0.1$), respectively. In the second stage, the drop in MY and DMI in the week of diagnosis was observed for all disease categories, but only cows diagnosed with claw and leg disease or other diseases had significantly lower absolute GB values when compared with healthy weeks during the same stage.

In the third stage (weeks 15 to 22), the decrease in MY in the week of diagnosis was not as strong as in the second stage, with ΔMY averaging −1.4 kg compared to −0.4 kg in the healthy weeks of this stage ($p = 0.016$). Because DMI decreased more significantly in the week of diagnosis than in the healthy weeks during this stage (ΔDMI averaging −1.2 vs. −0.1 kg; $p = 0.006$), GB stopped increasing in the week of diagnosis, with ΔGB averaging −1.9 mol C compared to +1.3 mol C in healthy weeks of this stage (p = 0.0.58). GB averaged 20.3 and 26.9 mol C/d in the week of diagnosis and heathy weeks, respectively ($p = 0.018$). GB of cows diagnosed with claw and leg disease in the third stage was significantly lower than the average of healthy weeks. This was due to both higher MY in the week before, of, and after diagnosis and similar (week before and after diagnosis) or lower (in the week of diagnosis) DMI. Although the MY of cows diagnosed with mastitis in the third stage was higher than the average MY in healthy weeks of this period, their GB was similar due to higher DMI.

Besides marginal differences in the absolute values and in the level of significance, EB followed a similar pattern to GB during disease. Pearson correlation coefficients for weekly means of GB, EB, DMI, MY, and ΔEBW across all weeks investigated are given in Table 4.

Table 6.4. Pearson correlation coefficients (upper side) and their p-values (lower side) for weekly means of glucose balance (GB), energy balance (EB), dry matter intake (DMI), milk yield (MY), and the weekly change in empty body weight (ΔEBW) across all lactation weeks investigated.

	GB	**EB**	**MY**	**DMI**	**ΔEBW**
GB	1	0.861	−0.366	0.496	0.127
EB	0.000	1	−0.441	0.520	0.322
MY	0.000	0.000	1	0.526	−0.054
DMI	0.000	0.000	0.000	1	0.247
ΔEBW	0.000	0.000	0.000	0.000	1

Due to the limited number of disease events in each category, the fixed effect of the week of diagnosis included in the mixed linear models (for GB, EB, MY, and DMI) included all diagnoses. Results of mixed linear modeling, which also accounted for the fixed effects of week in milk, year, season, and parity and included a random intercept and slope term for each lactation, showed that average daily GB, EB, MY, and DMI in the week of diagnosis was −3.8 (−5.6 to −2.1) mol C, −7.5 (−10.2 to −4.9) MJ, −1.0 (−1.4 to −0.6) kg, and −1.5 kg (−1.9 to −1.1), respectively, compared to weeks without diagnosis. When testing for the change in GB, EB, MY, and DMI compared to the preceding week while accounting for the same fixed effects, ΔGB, ΔEB, ΔMY, and ΔDMI in the week of diagnosis was −3.1 (−5.0 to −1.1) mol C, −4.7 (−8.0 to −1.5) MJ, −1.2 (−1.7 to −0.8) kg, and −1.4 (−1.9 to −0.9) kg, respectively, when compared to weeks without diagnosis. Although ΔGB, ΔEB, ΔMY, and ΔDMI became positive in the week after diagnosis, overall GB and EB did not recover as coefficients were still negative at −1.7 (−3.3 to 0.0) mol C/d and −3.4 (−5.9 to −0.9) MJ/d, respectively, compared to weeks without diagnosis. Results of the models for GB, EB, MY, and DMI as well as for ΔGB, ΔEB, ΔMY, and ΔDMI are given in Table A1 and Table A2.

4. Discussion

Due to a reduced DMI and the associated limited availability of nutrients in the digestive tract and in the intermediary metabolism during early lactation, metabolic trade-offs exist between productive and other life functions, such as reproductive and immune functions, particularly in high-yielding dairy cows [23,42]. Severe negative energy balance resulting from the mismatch between food energy intake and energy expenditure [5,43] and the severe loss of body tissue mass, i.e., the change in EBW or the change in body condition scores, which is a result of this mismatch, have been brought forward as risk factors for the development of PD in dairy cows [7,44]. It has also been shown that metabolic adaptations to similar levels of NEB differ greatly between individual cows [45]. Because glucose is the central metabolite for both mammary and immune cells, competition for this specific metabolite is at the core of the metabolic conflict [24]. Nevertheless, this conflict has not been addressed intensively in dairy research so far. In this study, a methodology for the quantification of residual amounts of glucose, which includes both the amount of glucose derived from nutrient intake as well as the amount of glucose derived from the intermediary metabolism (from the change in EBW and from the main pathway of glucose recycling via lactate), was applied to evaluate the development of glucose balance of dairy cows during disease.

Although the metabolic burden imposed by the onset of lactation is supposed to be the starting point for subclinical and clinical metabolic disorders and, subsequently, other diseases, it is not predictable, if, at what time, and how animals respond to metabolic stress [6]. Due to the multifactorial character of PD, scientific evaluation of the relationship between individual nutrient availability and the occurrence of PD is difficult. Even if cow-individual data for DMI, MY, BW, and health status are collected in a consistent manner while housing and living conditions are highly standardized, this does not prevent large inter- and intra-individual variations in nutrient supply and other factors, such as the level of exposure to biotic and abiotic noxes or social stress, and the individual capacities and coping strategies [46]. Genomic and metabolomic research investigating, e.g., individual differences in tissue-specific mRNA expression and milk biomarkers, is thought to advance understanding of why animals respond so differently to similar stresses or are able to regenerate differently under identical conditions. [47,48]. However, knowledge of the level of individual reserves is of central importance, as these levels are required for the verification of any (genomic, nutritional, or management) effect. Thus, the cow-specific variation in glucose availability during and outside periods of disease may be linked more directly to individual differences in adaptability.

The incidence of disease recorded in our dataset is lower than what has recently been observed in a large sample of German dairy farms [49]. Generally, comparison of incidences is difficult due to great variation between farms, herds, and the methods and definitions used for disease recording. However, cows enrolled in this study were kept on an experimental farm, and the rather low incidence is likely to be due to the selection criteria applied (inflammatory disease only and exclusion of diagnosis within 10 days after the previous diagnosis) and the proportionally lower number of first and second weeks in milk recorded in our dataset.

In our study, the occurrence of disease was associated with reductions in both milk yield and dry matter intake. Hypophagia during inflammation is a well-known phenomenon and is observed across many species [50,51]. In various studies, dairy cows diagnosed with mastitis, metabolic, or other diseases showed a reduced DMI compared with healthy cows

[17,52,53,54]. Host cytokines such as tumor necrosis factor-α and interleukin−1β, and bacterial endotoxins [55,56] have been found to exhibit appetite-depressing effects.

While dry matter intake decreased significantly in the week of diagnosis throughout all lactation stages investigated, reductions in MY were low during early lactation despite an insufficient nutrient supply in relation to the requirements. In contrast, the ability of dairy cows to reduce milk production during disease was greater in later compared to early lactation stages. This is in accordance with a greater ability to reduce milk yield during nutritional challenges in mid vs. early lactation observed in other studies (e.g., [57]). It has been shown repeatedly that milk yield decreases during disease [58,59]. With regard to the rather low reductions in MY and DMI in cows diagnosed with mastitis observed in our study, interpretation is difficult due to the lack of differentiation of mastitis diagnoses, which is a weakness of this study. However, it can be assumed that the majority of diagnoses were mild mastitis cases and that they were identified and treated at an early stage, with inflammation being limited locally and being of short duration (i.e., drop in milk yield and recovery within a few days instead of weeks). In fact, the ability to reduce milk yield during mastitis or other diseases is not only affected by the stage of lactation but also by the level of inflammation and by the genetic merit for milk production. Endocrine changes such as peripheral insulin resistance and downregulation of hepatic growth-hormone receptors [60,61] favoring the flow of glucose to the mammary gland during the periparturient period [62] are physiologic but are more severe in cows bred for high milk yields [61]. According to our results, it has been shown that reductions in milk synthesis during disease in early lactation are rather low [57,63] even when challenged by intramammary inflammation [64,65].

In all lactation stages investigated, decreases in milk yield did not lead to increased GB and EB, i.e., to greater nutrient availability for self-sustaining life functions. Milk yield reductions were not sufficient to cover the reductions in energy and glucose supply emerging from decreased DMI. Results obtained from the mixed models indicate that the average daily GB was −1.1 to −5.0 mol C (95% CI) in the week of diagnosed diseases. This means that the glucose availability for self-sustaining life functions decreased in periods when glucose was urgently needed. To avoid this decrease in GB following the imbalance between milk yield reductions and reductions in dry matter intake in the week of diagnosis, an infusion of 34 to 151 g of glucose (equivalent to ~700–3000 mL of a 5% glucose solution or ~85–375 mL of a 40% glucose solution), a supplementation of 28–124 g of propionate, or an additional reduction of ~0.5 to 2.1 kg milk yield would have been required on average. Besides therapeutic options to increase the supply with glucose or glucogenic precursors in case of disease, increasing overall glucose availability, and, in particular, glucose availability for functions other than milk synthesis through nutritional interventions, is limited. Overall energy intake is restricted because of the risk of rumen acidosis in case of excessive intake of highly digestible carbohydrates, time to eat, rumen volume, and liver function. Moreover, increases in DMI or in the energy density of the diet during early lactation results in increases in milk energy output at a similar magnitude, with no beneficial effects on energy reserves for functions other than milk synthesis [66]. Regarding the nutrient composition of diets, it has been suggested that feeding glucogenic instead of lipogenic sources of energy favors the allocation of energy towards functions other than milk synthesis, although results are inconsistent [66,67,68,69]. However, avoiding overfeeding in the dry period was shown to alleviate metabolic imbalance related to the carbohydrate metabolism, such as insulin resistance, during early lactation [19].

With regard to the limitations of increasing energy intake and the supply with glucogenic C, and with regard to the inability of cows to sufficiently reduce milk yield during disease, we emphasize that it is possible to reduce milk withdrawal through incomplete milking during phases of disease and severe undernutrition. By supporting the physiological processes of nutrient reallocation in case of disease in this way, the economic loss in revenue from milk sales appears to be of minor importance if, at the same time, the costs of a severe case of disease are avoided. With regard to the risk of mastitis, it can be assumed that an amount of residual milk between 200 and 800 mL per quarter is not related to the incidence of mastitis [70,71,72,73], whereas inconsistent effects of a prolonged milking interval on the incidence of mastitis have been described [74,75,76].

The low reductions in milk yield despite an inflammatory disease, particularly during early lactation, indicate that dairy cows have limited ability to repartition glucose away from the mammary gland. To avoid negative effects on the health following the failure to simultaneously supply productive and self-sustaining life functions, an animal’s ability to metabolically adapt to additional demands (a trait which has been termed ‘plasticity’ [77]) is of major importance. It has been hypothesized that cows with high genetic merit for milk production have a reduced capacity to adapt partitioning of energy and essential substrates in response to additional demands [77]. Although this may explain the low reductions in milk yield and overall reduced availabilities of glucose and energy during disease observed in our study, the severity of disease, the degree of immunoactivation, and thus the demand from immune cells of diseased cows enrolled in this study are not known. Moreover, inflammation induces several metabolic adaptations other than milk synthesis reductions that increase glucose availability during disease which cannot be evaluated by means of GB calculation. Among them, increased glucose removal from the plasma pool [25,78], a shift in glucose transporter expression [79], and the depletion of glycogen stores [43] may increase glucose availability to immune cells to some degree. A reduced energy demand from the digestive tract following hypophagia, as reflected by reduced cell migration and turnover [80,81], may also increase glucose availability for other tissues. In total, however, the contribution of glucogenic C by these adaptations is expected to be low in dairy cows during early lactation, as glycogen depots are generally exhausted after calving [82,83], while homeostasis of plasma glucose is tightly regulated [84]. Hence, the low absolute residual amounts of glucose (on average, less than 20 mol C, which is equivalent to ~600 g of glucose) observed in our study indicate that dairy cows often do not have sufficient glucose available to respond to infections where they do not reduce milk synthesis adequately. Together with low metabolic plasticity, this points to the risks associated with the trend of increasing feed conversion efficiency for milk production. Besides the risk associated with increased levels of metabolic stress following severe NEB [85], it can be assumed that an insufficient supply of self-sustaining life functions with energy and glucose is a major threat to the health and, ultimately, the longevity of high-producing dairy cows [6]. With regard to failure costs of disease events, such as reduced milk yield, discarded milk, medication, labor, and in particular, premature culling [1], monetary gains from high feed efficiency for milk production may thus be offset by monetary losses when the cows' ability to fuel immune functions is compromised. A sound economic evaluation of biological efficiencies should thus include all costs and benefits attributable to the service life of individual cows and herds, including not only feed costs but also the costs of disease and involuntary culling [2].

5. Conclusions

During early lactation, high-yielding dairy cows generally face a shortage in glucose for functions other than milk synthesis, such as the immune system. During phases of diseases, shortage in glucose increases as dry matter intake decreases more than milk yield. Further research on overall glucose balance and the effect of management measures such as the reduction of milk withdrawal, infusion of glucose, or supplementary feeding of propionate to cows challenged by inflammatory diseases should be envisaged.

Author Contributions

Conceptualization, J.H. and A.S.; methodology, J.H. and A.S.; investigation, J.H. and A.S.; data curation, J.H.; writing—original draft preparation, J.H.; writing—review and editing, A.S.; visualization, J.H. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was carried out according to the guidelines of the Declaration of Helsinki and in accordance with German animal protection law and was approved by institutional review. Data collection was approved by the local authority for animal welfare affairs (Landesuntersuchungsamt Rheinland-Pfalz; G 18-20-073) in Koblenz, Germany.

Informed Consent Statement

Not applicable.

Data Availability Statement

Restrictions apply to the availability of these data. Data were obtained from the Educational and Research Centre for Animal Husbandry “Hofgut Neumuehle” and are available with the permission of third party.

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Conflicts of Interest

The authors declare no conflict of interest.

Appendix A

Table 6.A1. Estimated regression coefficients and their standard errors from a mixed linear regression model of factors influencing the weekly change (Δ) in average daily glucose balance (GB, molC/d), energy balance (EB, MJ NEL/d), milk yield (MY, kg/d), and dry matter intake (DMI, kg/d) in dairy cattle.

	ΔGB			**ΔEB**			**ΔMY**			**ΔDMI**		
Variable	**Coeff. (SE)**	***p***	**95% CI**	**Coeff. (SE)**	***p***	**95% CI**	**Coeff. (SE)**	***p***	**95% CI**	**Coeff. (SE)**	***p***	**95% CI**
Intercept	−28.26 (4.1)	<0.001	−36.3 to −2.2	−49.1 (3.2)	<0.001	−55.4 to −42.9	3.42 (0.17)	<0.001	3.08 to 3.76	−1.84 (0.31)	<0.001	−2.5 to −1.2
Week in milk	14.03 (1.47)	<0.001	11.1 to 16.9	27.7 (2.1)	<0.001	23.6 to 31.8	−0.53 (0.02)	<0.001	−0.60 to −0.50	1.15 (0.15)	<0.001	0.9 to 1.4
Week in milk ^{2}	−2.66 (0.34)	<0.001	−3.33 to −1.99	−5.48 (0.48)	<0.001	−6.41 to −4.54	0.02 (0.00)	<0.001	0.02 to 0.02	−0.19 (0.02)	<0.001	−0.2 to −0.1
Week in milk ^{3}	0.24 (0.03)	<0.001	0.17 to 0.31	0.49 (0.05)	<0.001	0.4 to 0.59				0.01 (0.00)	<0.001	−0.01 to 0.01
Week in milk ^{4}	−0.01 (0.00)	<0.001	−0.01 to −0.01	−0.02 (0.00)	<0.001	−0.03 to −0.02						
Season												
March–May	0.03 (0.38)	0.944	−0.72 to 0.77	−0.99 (0.36)	0.007	−1.7 to −0.27	−0.04 (0.08)	0.621	−0.2 to 0.12	−0.09 (0.05)	0.073	−0.2 to 0.0
June–August	0.56 (0.39)	0.153	−0.21 to 1.34	0.07 (0.31)	0.825	−0.55 to 0.68	−0.31 (0.07)	<0.001	−0.5 to −0.2	−0.13 (0.04)	0.002	−0.2 to −0.1
September–November	−0.17 (0.39)	0.663	−0.94 to 0.6	−0.26 (0.35)	0.459	−0.95 to 0.43	−0.08 (0.08)	0.317	−0.2 to 0.1	−0.07 (0.05)	0.171	−0.2 to 0.0
December–February	Reference											
Year												
2015	1.43 (0.82)	0.083	−0.19 to 3.04	3.25 (0.63)	<0.001	2.02 to 4.48	−0.02 (0.14)	0.865	−0.3 to 0.2	.50 (0.09)	<0.001	0.32 to 0.67
2016	2.88 (0.83)	0.001	1.25 to 4.52	3.44 (0.64)	<0.001	2.19 to 4.69	−0.11 (0.14)	0.438	−0.4 to 0.2	.43 (0.09)	<0.001	0.25 to 0.60
2018	3.43 (0.95)	<0.001	1.57 to 5.29	4.35 (0.70)	<0.001	2.96 to 5.73	0.18 (0.16)	0.250	−0.1 to 0.5	0.62 (0.1)	<0.001	0.43 to 0.81
2019	1.71 (0.85)	0.045	0.04 to 3.37	2.97 (0.64)	<0.001	1.72 to 4.21	0.12 (0.14)	0.370	−0.2 to 0.4	0.45 (0.09)	<0.001	0.28 to 0.63
2020	6.00 (1.95)	0.002	2.17 to 9.82	5.31 (1.23)	<0.001	2.88 to 7.74	−0.08 (0.27)	0.770	−0.6 to 0.5	0.46 (0.16)	0.004	0.14 to 0.78
2021	0.75 (0.84)	0.372	−0.90 to 2.41	3.10 (1.03)	0.003	1.08 to 5.13	0.42 (0.21)	0.046	0.01 to 0.83	1.13 (0.16)	<0.001	0.81 to 1.44
2022	Reference											
Parity												
1	−0.68 (0.60)	0.255	−1.86 to 0.49	−1.02 (0.31)	0.001	−1.62 to −0.42	0.24 (0.07)	0.001	0.09 to 0.38	0.09 (0.04)	0.020	0.01 to 0.17
2	−0.80 (0.51)	0.116	−1.80 to 0.20	−0.68 (0.26)	0.010	−1.20 to −0.16	−0.05 (0.06)	0.453	−0.2 to 0.1	−0.03 (0.03)	0.342	−0.1 to 0.03
3	−0.26 (0.55)	0.634	−1.34 to 0.82	−0.48 (0.28)	0.090	−1.03 to 0.08	0.07 (0.07)	0.294	−0.1 to 0.2	0.01 (0.04)	0.689	−0.1 to 0.1
4	Reference											
Week_Dia [1]												
Wk−2	−1.15 (1.15)	0.317	−3.41 to 1.1	−0.99 (1.70)	0.561	−4.33 to 2.35	0.42 (0.28)	0.129	−0.1 to 0.9	0.04 (0.26)	0.885	−0.5 to 0.5
Wk−1	−0.92 (1.03)	0.371	−2.95 to 1.1	−1.69 (1.69)	0.320	−5.01 to 1.64	−0.12 (0.26)	0.644	−0.6 to 0.4	0.12 (0.26)	0.642	−0.4 to 0.6
Wk 0	−3.09 (0.99)	0.002	−5.04 to −1.14	−4.74 (1.64)	0.004	−7.95 to −1.53	−1.23 (0.24)	<0.001	−1.7 to −0.8	−1.41 (0.25)	<0.001	−1.9 to −0.9
Wk+1	2.59 (0.99)	0.009	0.64 to 4.54	6.75 (1.51)	<0.001	3.79 to 9.70	0.66 (0.24)	0.006	0.19 to 1.12	1.37 (0.23)	<0.001	0.93 to 1.81
Other	Reference											
Random effects												
Intercept	96.12 (1.89)		92.5 to 99.9	289.1 (6.8)		276 to 303	6.18 (0.13)		5.9 to 6.5	6.77 (0.16)		6.5 to 7.1

Week in milk	0.68 (-)		-	0.28 (0.03)		0.22 to 0.34	0.50 (0.06)		0.4 to 0.6	00.26 (0.03)		0.2 to 0.3
Week in milk ^{2}	−0.18 (0.02)		−0.21 to −0.15	−0.33 (0.01)		−0.36 to −0.31	−0.18 (0.01)		−0.2 to −0.2	−0.35 (0.01)		−0.4 to −0.3

[1] A derived variable relating weekly values of ΔGB, ΔEB, ΔMY, and ΔDMI to the week of diagnosis

[2, 3, 4] Second, third and fourth polynomial of the fixed effect of week in milk.

Table 6.A2. Estimated regression coefficients and their standard errors from a mixed linear regression model of factors influencing the weekly means of average daily glucose balance (GB, molC/d), energy balance (EB, MJ NEL/d), milk yield (MY, kg/d), and dry matter intake (DMI, kg/d) in dairy cattle.

	GB			**EB**			**MY**			**DMI**		
Variable	**Coeff. (SE)**	***p***	**95% CI**	**Coeff. (SE)**	***p***	**95% CI**	**Coeff. (SE)**	***p***	**95% CI**	**Coeff. (SE)**	***p***	**95% CI**
Intercept	−0.02 (9.53)	0.998	−19.9 to 18.9	−81.95 (1.8)	<0.001	−107 to −57.3	33.7 (2.0)	0.284	−7989 to 8056	12.96 (1.87)	<0.001	9.3 to 16.7
Week in milk	3.67 (1.19)	0.002	1.33 to 6.01	12.47 (1.76)	<0.001	9.0 to 15.9	5.5 (0.3)	<0.001	4.92 to 6.08	4.37 (0.27)	<0.001	3.85 to 4.9
Week in milk ^{2}	−0.99 (0.3)	0.001	−1.57 to −0.4	−2 (0.44)	<0.001	−2.9 to −1.1	−0.79 (0.07)	<0.001	−0.94 to −0.65	−0.67 (0.07)	<0.001	−0.8 to −0.54
Week in milk ^{3}	0.13 (0.03)	<0.001	0.07 to 0.19	0.2 (0.05)	<0.001	0.11 to 0.29	0.05 (0.01)	<0.001	0.04 to 0.07	0.06 (0.01)	<0.001	0.04 to 0.07
Season												
March–May	0.75 (0.75)	0.314	−0.71 to 2.21	−0.83 (1.12)	0.460	−3.03 to 1.37	0 (0.2)	0.984	−0.39 to 0.4	0.36 (0.16)	0.022	0.05 to 0.66
June–August	−1.97 (0.81)	0.015	−3.55 to −0.39	−2.85 (1.21)	0.019	−5.2 to −0.5	−0.41 (0.22)	0.064	−0.85 to 0.02	−0.42 (0.17)	0.012	−0.8 to −0.1
September–November	−1.9 (0.73)	0.010	−3.33 to −0.46	−2.25 (1.1)	0.041	−4.4 to −0.09	−0.66 (0.2)	0.001	−1.05 to −0.28	−0.29 (0.15)	0.056	−0.59 to 0.01
December–February							Reference					
Year												
2015	2.86 (2.16)	0.185	−1.37 to 7.1	−1.03 (3.1)	0.741	−7.12 to 5.07	−3.85 (0.92)	<0.001	−5.66 to −2.05	−3.45 (0.41)	<0.001	−4.3 to −2.6
2016	11.1 (2.16)	<0.001	6.86 to 15.33	0.8 (3.11)	0.796	−5.31 to 6.91	−3.93 (0.92)	<0.001	−5.73 to −2.13	−3.08 (0.41)	<0.001	−3.9 to −2.3
2018	1.27 (2.45)	0.604	−3.54 to 6.09	−9.55 (3.54)	0.007	−16.5 to −2.6	−0.33 (1.03)	0.753	−2.35 to 1.7	−2.1 (0.47)	<0.001	−3.0 to −1.2
2019	−0.48 (2.32)	0.836	−5.04 to 4.08	−14.01 (3.3)	<0.001	−2.6 to −7.5	−0.01 (1.01)	0.994	−1.99 to 1.97	−2.78 (0.44)	<0.001	−3.6 to −1.9
2020	−34.9 (4.4)	<0.001	−43.5 to −26.3	−46.2 (6.3)	<0.001	−59 to −34	1.54 (1.91)	0.422	−2.22 to 5.29	−7.63 (0.83)	<0.001	−9.3 to −6.0
2021	−2.5 (1.24)	0.044	−4.93 to −0.07	−3.2 (1.85)	0.084	−6.8 to 0.4	−0.74 (0.32)	0.021	−1.36 to −0.11	−0.8 (0.27)	0.003	−1.3 to −0.3
2022							Reference					
Parity												
1	−5.42 (1.81)	0.003	−8.98 to −1.85	1.19 (2.56)	<0.001	5.1 to 15.2	−9.42 (0.83)	<0.001	−11.05 to −7.78	−3.98 (0.34)	<0.001	−4.6 to −3.3
2	−2.37 (1.54)	0.124	−5.39 to 0.65	5.58 (2.17)	0.011	1.3 to 9.85	−1.38 (0.71)	0.051	−2.77 to 0.01	−0.23 (0.29)	0.418	−0.79 to 0.33
3	−2.61 (1.67)	0.119	−5.89 to 0.68	1.56 (2.37)	0.509	−3.09 to 6.21	0.59 (0.77)	0.441	−0.92 to 2.1	0.42 (0.31)	0.179	−0.19 to 1.03
4							Reference					
Week_Dia [1]												
Wk −2	−1.1 (0.92)	0.231	−2.9 to 0.7	−2.61 (1.41)	0.064	−5.38 to 0.15	0.28 (0.21)	0.167	−0.12 to 0.69	−0.13 (0.21)	0.536	−0.54 to 0.28
Wk −1	−0.35 (0.9)	0.697	−2.12 to 1.42	−1.64 (1.36)	0.228	−4.3 to 1.03	0.13 (0.22)	0.542	−0.29 to 0.56	−0.1 (0.2)	0.620	−0.5 to 0.3
Wk 0	−3.81 (0.89)	<0.001	−5.55 to −2.07	−7.54 (1.33)	<0.001	−1.2 to −4.9	−0.97 (0.21)	<0.001	−1.39 to −0.56	−1.52 (0.2)	<0.001	−1.9 to −1.1
Wk +1	−1.65 (0.83)	0.047	−3.28 to −0.02	−3.37 (1.28)	0.008	−5.9 to −0.9	−0.17 (0.19)	0.349	−0.54 to 0.19	−0.56 (0.19)	0.003	−0.9 to −0.2
Other							Reference					
Random effects												
Intercept	123.1 (11.5)		102.4 to 147.8	336.1 (56.7)		241 to 468	32.1 (1.7)		16.7 to 61.8	4.8 (0.28)		4.3 to 5.4
Week in milk	0.83 (0.03)		0.76 to 0.88	0.9 (0.03)		0.81 to 0.95	0.96 (0.02)		0.92 to 0.98	0.74 (0.05)		0.6 to 0.8
Week in milk^2	0.63 (0.03)		0.55 to 0.69	0.66 (0.06)		0.54 to 0.76	0.93 (0.02)		0.87 to 0.97	0.48 (0.03)		0.4 to 0.5

[1] A derived variable relating weekly values of ΔGB, ΔEB, ΔMY, and ΔDMI to the week of diagnosis

[2, 3, 4] Second, third and fourth polynomial of the fixed effect of week in milk.

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8. Publication #4 - Income Over Service Life Cost - Estimation of Individual Profitability of Dairy Cows at Time of Death Reveals Farm-Specific Economic Trade-Offs.

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Abstract

Managing a dairy farm requires a farm- and cow-specific assessment of the required resources and the revenues obtained from the production process. Due to scientific and practical reasons, the various types of data from a farm, e.g., economic and biologic data, commonly lack connectivity and compatibility at animal level. This means that the economic impacts associated with differences in rearing, productivity and culling as well as the economic values of cow-individual service lives often remain hidden under average figures. This work is based on a two-fold hypothesis: (1) large differences in cow profitability exist between farms but also between individual cows within one farm, and (2) knowledge about the farm-specific economic impact of service life characteristics provides new options for action. In this article, a farm-specific full-cost calculation that links routine herd management data and key economic figures gained from business sheets of the farm's dairy branch is introduced. The new methodology for the calculation of individual income over service life cost was exemplarily tested on a sample of 4,962 culled cows from 32 German dairy farms. Median income over service life cost of culled cows was negative for 19 farms (59 %) and varied largely between farms (-€3,502 to €3,323). Within a given economic situation, i.e., cows of the same farm, the number of lactations was identified as the major lever to cow profitability for most farms, followed by the individual milk yield level and the number of days in milk at culling during last lactation. This indicates that the economic sustainability of the farm is inherently linked to the ability of cows to cope with their environment. The vastly different monetary value associated with, e.g., productivity and longevity parameters and the heterogeneity in the ranking of their effect sizes between farms also show that the economic impacts of herd characteristics are highly farm-specific. Evaluation of the overall lifetime profitability of culled cows along with identification of economically poor farm-specific herd characteristics can help to pinpoint problems, optimize herd management, and prioritize investment necessities.

1. Introduction

In recent years, many European dairy businesses have been under severe economic pressure. This is associated with global structural changes of the dairy industry and includes intensification of dairy production as well as liberalization of agricultural trade policies, while quota abolishment in 2015 triggered further competition and greater production in Europe (Hemme et al., 2014). For individual dairy farms, these changes manifest in very volatile milk and feed prices, and regulation necessities. To stay in business in such a dynamic dairy market, farmers are forced to develop strategies for cost reduction. In this regard, economies of scale seem to play an important role because the costs of production are thought to decline if milk production expands (Drews et al., 2018; Macdonald et al., 2017). On the other hand, high production costs and/or lower milk yields may be economically offset by the effects of higher milk prices and/or lower total feed costs following increased self-sufficiency in organic farming (McBride and Greene, 2009; Jan et al., 2014; Zekalo, 2015; Naglova and Vlasicova, 2016; Nehring et al. 2021), and may further be encountered by assuring animal health and keeping more older cows in the herd (Slagboom et al., 2016; Roesch et al., 2016). However, there are large differences between dairy farms when it comes to the overall goals and strategies of the dairy business (Poczta et al., 2020) and to the level and allocation of resources such as feed and labor within the production process (Wilson, 2011). Accordingly, differences between organic and conventional farms regarding their individual breeding and feeding strategies, and the emphasis that is placed on animal health and longevity were reported (Slagboom et al., 2016; Jones et al., 2016). Thus, cross-farm comparisons often lack a common ground, and generalizations of economic effects may even be fallacious for the individual farm (Jarvis and Valdes-Donoso, 2018). Accordingly, case studies rather than large-number trials have been suggested to advance understanding of systemic interactions (Teixeira de Melo et al., 2020).

Irrespective of the farm type, assessing the farm-specific ratio between the production output and the sum of resources needed (input) is a basic principle to improve and monitor efficiency and profitability of the business. From a holistic perspective, the input-output ratio also applies to the production output in terms of animal health and welfare and a sustainable production process (Dentler et al., 2020; Grandl et al., 2019). However, in dairy farming and scientific debate, the focus often remains on partial output measures like the milk yield per cow and, in dairy accounting, milk productivity is merely seen in terms of revenues obtained from the milk sold and thus, to the financial liquidity of the business. While this is obviously important for short-time survival, farm-specific economic strategies should be based on the integration of all input variables (costs), which would allow for a more complete evaluation of the profitability of the business (Wilson, 2011), and the identification of weak points for improving economic sustainability. Feed costs are the top driver for cost of milk production in most countries and production systems (Hemme et al., 2014) and account for up to 50% of the total cost of milk production in high producing dairy herds (Evink and Endres, 2017). Therefore, measures like income over feed costs (IOFC; €/kg) or marginal feed costs (per kg dry matter or per kg milk sold) are widely used (Atzori et al., 2013; Shoemaker et al., 2019; Wieck and Heckelei, 2007). Yet, they only reflect the part of profitability that is associated with the average feed-efficiency of the herd, signifying that further costs beyond fodder or feeding are disregarded. Similarly, individual failure costs related to reduced health and longevity are commonly hidden in the aggregated form of business sheets, although they have been shown to play an important role for farm economics (Liang et al., 2017; Mostert et al., 2018; van Soest et al., 2019). In fact, most accounting data from dairy farms lacks cow-specific information, and/or is incompatible with herd data. This is particularly disadvantageous for the identification of economic levers at animal level as it neglects the monetary valuation emerging from the variation in individual cow characteristics such as the ratio between yield and intake (Vandehaar, 1998), the reproductive efficiency (Vries, 2017) and the time and circumstances of culling (Dechow and Goodling, 2008), which are all thought to be main drivers of economic results.

In view of economic pressure, it is an existential challenge for dairy farms to not only analyze partial profitabilities at a purely herd level but to include all farm-specific costs and revenues of the production process and to link these to the biological characteristics of single animals. The full-cost, cow-specific calculation of individual income over service life cost (iIOLC) presented in this article provides this link and contrasts with other approaches in the field of dairy economics in three major aspects: firstly, it regards the individual cow as both the basic biological and the basic economic unit of a dairy farm, which allows for allocation of all costs and revenues of the dairy branch to single cow's service lives; secondly, because only death sets an irrefutable end to return on investments as well as ongoing costs, it focuses on the "dead herd", i.e. culled cows. This allows for a robust estimation of the 'biologic' and the 'economic' success or failure of individual cows. Finally, it assumes that analyzing differences in iIOLC of culled cows which lived and produced within the same farm and thus, within the same economic boundaries, is more suited to identify farm-specific economic optimization potentials than general, industry-wide recommendations and beliefs. In light of the lack of knowledge about the degree and variation of iIOLC at animal level and the farm-specificity of the economic effects associated with key service life characteristics, these assumptions were evaluated for a sample of 32 German dairy farms.

2. Material, Animals & Methods

2.1. Animal and herd data

Our study, which is part of a project known as Tier-Wirt, originally involved 38 farms (TW01 - TW38). Of these, six farms (TW07, TW10, TW16, TW19, TW22, TW23) were excluded either due to missing data transfer or due to termination of the dairy business during the project. The final database for the analysis presented in this article therefore comprises herd data from 32 German dairy farms. However, farms retained their original denomination (TW01 – TW38) to ensure tracking of farms in different publications emerging from the same project (Sundrum et al., 2021; Hoischen-Taubner et al., 2021). The selection of farms covered different regions, structures, breeds, herd sizes and production methods (Table 7.1). Average herd sizes during the monitoring period ranged from 73 to 1,613 cows (median 240; mean 488), Holstein Friesian was the predominant breed (28 farms), others were Simmental and Brown Swiss (2 farms each). Five organic farms (TW02, TW04, TW06, TW08 and TW37) participated in the project. A monitoring period of one year according to the fiscal year of the farm (05/2017 to 04/2018 (13) or 07/2017 to 06/2018 (19)) was chosen to allow for a comprehensive and sound synthesis of economic and animal data as the basis for calculations.

Table 7.1: Structure of the farms studied (n=32)

Herd size (n)		**Feeding system (n)**	
<150 cows	12	Total Mixed Ration	21
150-450 cows	7	other	11
>450 cows	13	**Milk yield (kg/305 days in milk)**	
Regional distribution (n)		Mean	8,568
North Germany	11	Min	5,465
South Germany	8	Max	12,681
East Germany	13	**Culling rate (%)**	
Production method (n)		Mean	28.9
Conventional	27	Min	18.4
Organic	5	Max	42.5
Grazing for lactating cows (n)		**Concentrate feed intake (% of dry matter)**	
yes	9	Mean	38.9
no	23	Min	12.5
		Max	48.9

At animal level, life cycle data of each cow (birth date, first calving, last calving, lactation number, culling date and reasons) as well as monthly or bimonthly milking records of the period monitored (31 and 1 farm/s resp.) and key figures from previous lactations were recorded. For each lactation of the monitoring period, the total and average daily milk yield from calving (or from other entry at start of the year) until the end of the lactation (or until death or the end of the year) was calculated according to the 'test interval method' (ICAR, 2020). Lactations without a test-day record were set at zero. Based on this data, common indices were calculated for each cow: age at first calving, total days of the productive life (days from first calving to death), total and daily lifetime milk yield, total and daily milk yield of the productive life, average daily milk yield during last lactation and days in milk at culling during last lactation. Data of cows leaving the herd alive, i.e., cows sold for breeding or other purposes, were excluded.

Hence, the term ‘culling’, as we use it, refers solely to the death of a cow either on the farm or at slaughterhouse. Overall, a data set of 4,962 culled cows was available.

2.2. Economic data

Because farms use a wide range of cost accounting systems encompassing different economic units, the economic data we received varied both in format and in reliability. Allocation of cost items was thus checked for plausibility by following the widely used approach for farm business budgets (DLG, 2011). This resulted in the development of a standardized economic datasheet for the dairy branch (milk production without rearing heifers) on which we based further analyses. Within this datasheet, the dairy herd is generally considered as one single economic unit, while dairy heifers were "internally purchased" from the economic unit of rearing. Final enterprise budgets encompassed the following main average key figures during the monitoring period: milk price, carcass value, costs of rearing heifers and production costs without rearing costs. Production costs were further subdivided into feed costs and other production costs, which comprised labor, building (maintenance, depreciation), capital costs and proportional general costs. An example of categories included in a full-cost approach of dairy businesses is given by Knierim et al. (2020).

Farms participating in this project presented their data either as a complete dairy branch budget of the monitoring period created by advisors (10) or used the standardized datasheet that was sent to the farm (13). Five farms submitted data from the fiscal year preceding the monitoring period, while no farm-specific economic data was available for four farms. For the latter, we estimated daily rearing and production costs as well as milk price and slaughter values based on average values of other farms in this project. The herd data (number of cows, duration of their productive life, milk kg per cow) was original data from these farms.

2.3. Data adaptation and calculation

By merging accounting and herd data, the following equations were developed for monetary valuation of the lifespan of cows (Table 7.2). Individual revenues from milk were calculated from individual lifetime yield and the average farm-specific milk price during the monitoring period (equation 1). If cows were slaughtered, the farm-specific average revenue from the carcass value was taken into account, whereas no revenues or costs (e.g., for veterinary or rendering services) were considered if cows died on farm (equations 2 and 3). Average daily costs of rearing a heifer were calculated from average total costs of rearing a heifer and median age (in days) at first calving of the herd. Individual costs of rearing a heifer were then calculated from individual age at first calving and farm-specific average daily rearing costs (equation 4).

To allow for a cow-specific attribution of daily production costs, and for consistency between economic datasets, we started with a calculation of cow-years – defined as the sum of all days of all cows present in the herd during the monitoring period (cow-days) divided by the number of days in this period (365). Days present were based on test-day milk record information and dates of calving and culling. Costs were then divided by 365 and by the number of cow-years on the respective farm to obtain the accounting unit ‘per day, per cow’, which then served as a starting point for further adaptations. To reflect the strong association between yield and intake while accounting for feed-efficiency, a linear regression equation was used to estimate average individual dry matter intake per day of the productive life from individual milk yield of the productive life (Britt et al., 2003). Average farm-specific feed costs per kg dry mater intake were calculated from total feed costs and median dry matter intake during the productive life of the herd. Finally, individual feed costs were calculated from individual dry matter intake and average farm-specific feed cost per kg dry matter intake (equations 5, 6, 7 and 8). All other farm-specific production costs such as costs for labor, housing, etc. were equally attributed to cows of the same farm (equation 9). Individual daily production costs were multiplied by the number of days of the productive life and added with individual rearing costs to obtain total individual costs. Individual income over service life cost (iIOLC; €) was then calculated as the difference between individual milk and carcass revenues and individual costs.

Table 7.2: Equations for calculation of individual Income over service life cost (^{i}IOLC).

Parameter	***Equation***	***Eq. Nr.***
Revenues		
Revenue from milk ($^{i}R_{milk}$; €)	= $^{i}MY_{Life}$ x ^{f}MP	(1)
Revenue from carcass ($^{i}R_{C}$; €)	= $^{f}R_{C}$ (if slaughtered); 0 (if died on farm)	(2); (3)
Costs		
Rearing costs ($^{i}C_{H}$; €)	= $^{f}C_{H}$ / medianAFC x ^{i}AFC	(4)
Production costs ($^{i}C_{P}$; €)	= ($^{i}C_{F}$ + $^{f}C_{O}$) x $^{i}d_{PL}$	(5)
Feed costs ($^{i}C_{F}$; €/d)	= $^{f}C_{F}$ /medianDMI x ^{i}DMI	(6)
Dry matter intake ($^{i}DMI_{PL}$; kg/d)	= ^{i}FE x $^{i}MY_{PL}$ / $^{i}d_{PL}$	(7)
Feed efficiency (^{i}FE)[1]	= 0,0304 x $^{i}MY_{PL}/^{i}d_{PL}$ + 0,463	(8)
Other farm-specific production costs ($^{f}C_{O}$; €/d))	= $^{f}C_{P}$ - $^{i}C_{F}$	(9)
Income over service life cost (^{i}IOLC; € per cow)	= $^{i}R_{milk}$ + $^{i}R_{C}$ - $^{i}C_{P}$ - $^{i}C_{H}$	(10)

^{f}: farm-specific; ^{i}: individual/cow-specific; MY_{Life}: lifetime milk yield; MP: milk price (€): R_{C}: average revenue from carcass value per cow (€); AFC: age at first calving (months); d_{PL}: days since first calving/of the productive life; FE: feed efficiency (kg milk/kg DM); MY_{PL}: daily milk yield since first calving (kg/d); [1]Britt et al. (2003)

2.4. Statistical analysis

Descriptive statistics of iIOLC were calculated across farms and for each farm separately. Data sets of nine cows (from 5 farms) were excluded due to missing values. To reveal the economic impact of different service-life characteristics on iIOLC, five variables reflecting key factors of rearing, productivity and longevity were analyzed: lactation number (iLact), days in milk at culling during last lactation (iDIMLL), average daily milk yield during last lactation (iMYLL), age at first calving (iAFC) and the binary variable defining type of death, i.e., whether a cow realized revenue from slaughter or not. To allow for cross-farm comparisons while considering both the heterogeneous, farm-specific economic situation as well as herd characteristics, two statistical approaches were performed. Firstly, cows were grouped as either high profit (HP) or low profit cows (LP). Grouping for positive or negative iIOLC led to the exclusion of a number of farms as few or no cows were in either group. To ensure equal group sizes while accounting for farm heterogeneity, cows were grouped using the farm-specific median iIOLC as threshold. Two-sided tests for equality (Bonferroni, alpha = 0.05) were applied to test for differences of means for iLact, iMYLL, iDIMLL, iAFC and type of death. Bonferroni correction was applied to all pair-wise comparisons. The occurrence of significant differences ($p<0.05$) between HP and LP cows in farm-specific comparisons was summed up across farms.

For evaluation of general trends in the effect sizes of main biological and economic variables of iIOLC calculation (see Table 7.3), a univariate linear regression model (UNIANOVA, Type III sum-of-squares method with alpha = 0.05) including all culls of all farms was performed. For further analysis, 32 farm-specific linear regression models (UNIANOVA, Type III sum-of-squares method with alpha = 0.05) including biological variables only (costs and prices are equal for all cows of a farm) were used to analyze the influence of specific service-life characteristics (iLact, iMYLL, iDIMLL, iAFC and type of death) for all farms separately. In this set of variables, iLact and iDIMLL were used instead of the overall length of the productive life (idPL) to avoid multicollinearity, which was observed for those variables in the cross-farm model. Moreover, using iLact and iDIMLL instead of idPL accounts for differences in the ratio of productive and unproductive days during the productive life. Accordingly, iMYLL was chosen as a variable reflecting the individual yield potential because the milk yield during the productive life (iMYPL) largely depends on the number and length of lactations and the duration of the dry periods, while other key performance indicators were not available for primiparous cows or cows that were culled early in lactation (e.g., 305-/100-days -yield). Within each farm-specific model, effects were considered significant if p was lower than 0.05. Due to the variance in the number of cows per farm and systematic bias in common effect-size values, partial omega square (ωp^2) was chosen as an alternative value for effect-size with lower bias (Okada, 2013). Significant effects were ranked according to ωp^2 and were examined for each farm separately as well as across all farms. The linear regression was chosen as it is the standard procedure for estimation of effect sizes of this type of variables. There were no reasons to assume that other type of regressions would fit better to the data. Estimation of effect sizes, and in particular, estimation of partial omega square, integrates the divergence as well as the magnitude of difference of the data analyzed. Together with the p-value of a variable, these two values reflect the most relevant information of the statistical approach applied. Statistical analyses were performed using IBM® SPSS®.

Table 7.3. Median and interquartile range of herd characteristics, economic variables and the proportion of cows making profit at time of death (^{i}IOLC >0) across farms.

	Variable	***Median***	***IQR***	***Min***	***Max***
Biological	${}^{i}MY_{Life}$ (kg)	29,283	8,290	13,835	41,707
	${}^{i}d_{PL}$ (days)	1,119	343	601	1,890
	^{i}AFC (months)	26.3	1.8	23.4	31.1
	Death on farm (%)	5.7	3.8	1.8	12.2
Economic	^{f}MP (€/kg)	0.37	0.03	0.34	0.53
	${}^{f}R_{C}$ (€/cow)	705	109	377	994
	${}^{f}C_{O}$ (€/cow/day)	4.91	0.88	3.17	6.78
	${}^{i}C_{F}$ (€/cow/day)	4.24	0.79	2.64	6.40
	${}^{i}C_{H}$ (€/cow)	1,570	433	1,200	2,745
	^{i}IOLC >0 € (%)	44.4	32.5	0.0	74.1

^{i} – individual; ^{f} – farm-specific; IOLC – income over service life cost, MY_{Life} – Lifetime milk yield, d_{PL} – days since first calving, AFC – age at first calving, MP – Milk Price, R_C – Revenue from carcass, C_O – other (production) costs, C_F – feed costs, C_H – costs of rearing a heifer

3. Results

The median farm-specific culling rate during the monitoring period averaged at 28.9 % (Interquartile Range; IQR: 11.1 %) and the mortality rate at 5.7 % (3.8 %). On average, 35.4 % (18.2 %, 44.3 %) of the cows were culled during the first 100 DIM (100-200, >200), while 28.7 % of the cows culled during the first 100 DIM died on farm. Across the five organic farms participating in this study, mean culling rate (26.5 %) and mortality rate (3.1 %) were lower, compared to conventional farms assessed (30.1 % resp. 6.2 %). Across farms, approximately three-quarters of the cows were culled for disease or fertility reasons (75.3%, ranging from 61.0 % to 94.1 % for conventional farms and 84.9 %, ranging from 57.9 % to 96.3% for organic farms). Across farms, median (IQR) iMYLife was 29,283 kg (8,290 kg), median daily iMYPL was 25.9 kg (4.6 kg), median iAFC was 26.3 months (1.8 months) and cows left the herd at a median of 199 (61) iDIMLL. Organic farms showed lower average daily iMYPL (22.6 kg vs. 26.3 kg) but on average, had similar total iMYLL (29,994kg vs. 29,478kg) as well as higher average iAFC (28.2 months vs 26.0 months) and iDIMLL (218 vs. 199), compared to the conventional farms.

Herd medians of iMYLife ranged from 13,835 to 41,707 kg and median age of cows at culling ranged from 601 to 1,890 days from first calving. Production costs excluding feed ranged from €3.17 to €6.78 per day and feed costs ranged from €2.64 to €6.40 per cow and day, with similar means for organic and conventional farms (Table 7.4). Milk prices ranged from €0.34 to €0.53 per kg. Milk price was the only economic variable where values of conventional and organic farms did not overlap (€0.34 to €0.45 and €0.48 to €0.53, respectively). Biologic and economic characteristics of the 32 herds participating in the project are summarized in Table 7.3. Across all culled cows of conventional (organic) farms recorded during the monitoring period, iIOLC varied largely from -€11,818 to €12,952 (-€5,672 to €13,680) with average iIOLC being negative for 19 conventional farms (70 %), while all organic farms participating in this study had a positive average iIOLC of culled cows. At cow level, iIOLC was negative for 63 ± 21 % and 43 ± 9 % of the cows in conventional and organic production, respectively (Figure 7.1 and Table 7.4). Assuming iIOLC as an economic rationale for dairy profitability, this means that the majority of cows on conventional farms were not able to return costs. At farm level, median iIOLC of culled cows ranged from -€3,502 to €1,374 (conventional farms) and from -€281 to €3,323 (organic farms).

Table 7.4. Mean of herd characteristics, economic variables and the proportion of cows making profit at time of death (^{i}IOLC >0) according to production method and herd size (small: <150 cows; medium: 150-450 cows; large: >450 cows).

	Variable	***production method***		***herd size***		
		conventional	***organic***	***small***	***medium***	***large***
Biological	$^{i}MY_{Life}$ (kg)	29,478	29,994	30,828	27,660	29,410
	$^{i}d_{PL}$ (days)	1,131	1,342	1,304	1,104	1,067
	^{i}AFC (months)	26.0	28.2	27.1	26.0	25.8
	Death on farm (%)	6.2	3.1	4.4	4.9	7.3
Economic	^{f}MP (€/kg)	0.37	0.50	0.42	0.39	0.36
	$^{f}R_{C}$ (€/cow)	692	761	747	733	645
	$^{f}C_{O}$ (€/cow/day)	5.01	4.71	4.85	4.87	5.11
	$^{i}C_{F}$ (€/cow/day)	4.15	4.40	4.10	4.62	4.04
	$^{i}C_{H}$ (€/cow)	1,538	2,062	1,765	1,608	1,493
	^{i}IOLC >0 € (%)	36.9	57.3	45.3	34.4	38.3

^{i} – individual; ^{f} – farm-specific; IOLC – income over service life cost, MY_{Life} – lifetime milk yield, d_{PL} – days since first calving, AFC – age at first calving, MP – Milk Price, R_{C} – Revenue from carcass, C_{O} – other (production) costs, C_{F} – feed costs, C_{H} – costs of rearing a heifer

Across farms, a median of 37.9 % of the culls occurred during the first or second lactation and only 42.9 % of the cows outlived their 3rd lactation. However, median iIOLC was negative for cows culled during their first, second or third lactation (-€833; -€443 and -€180, respectively) and became positive in lactation 4 or higher (€1,094; Figure 7.2).

Farm-specific maximum iIOLC was not reached before 5th lactation (5th: 5 farms, 6th: 3, 7th: 6, 8th: 3, 9th: 11). For four farms, iIOLC trended towards more negative values in higher lactations, i.e., farm-specific median iIOLC was least negative in 1st (3 farms) or 2nd (1 farm) lactation. This means that culled cows of those farms were on average unable to cover production costs, irrespective of the duration of their productive life (e.g., farm TW27). Figure 7.3 shows an exemplary (4 farms) illustration of farm-specific relationships between longevity and yield potential on one hand and the individual economic result on the other.

Figure 7.1. Variation of individual Income over service life cost (iIOLC) and number of culled cows of 32 German dairy farms participating in the project 'TierWirt' (TW) sorted from highest to lowest farm mean. TW02, TW04, TW06, TW08 and TW37 (grey boxes) are organic farms. Small circles = outliers (1,5 x interquartile range (IQR)); asterisks = extreme values (3 x IQR).

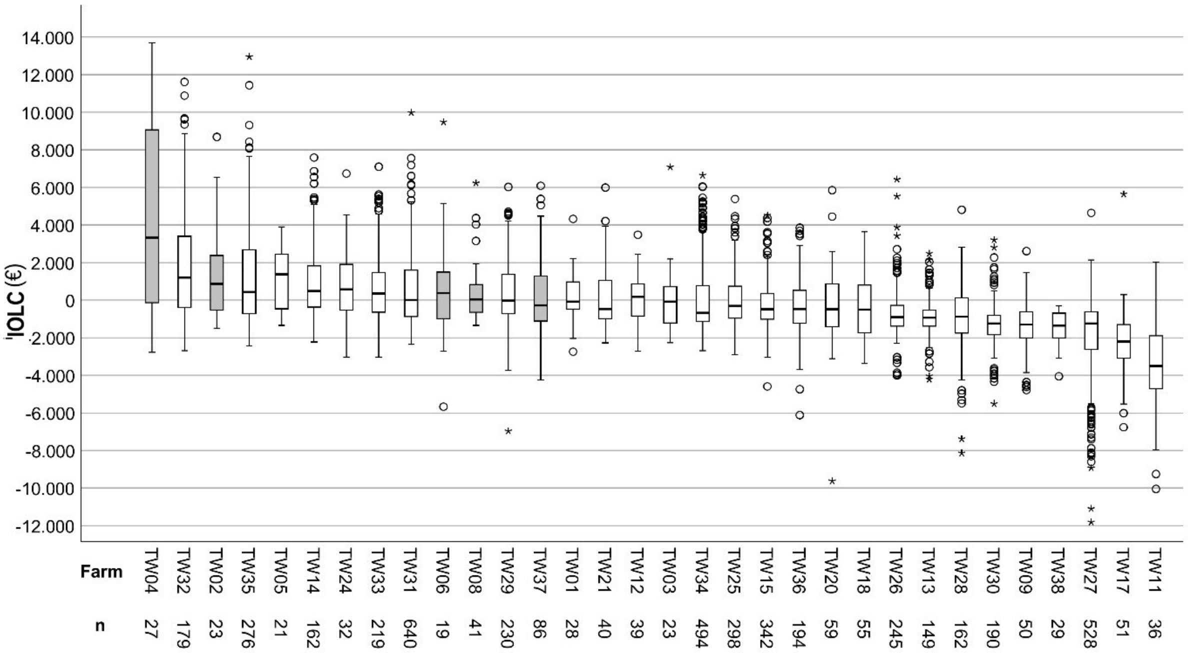


Figure 7.2. Income over service life cost (iIOLC) of cows according to lactation (0 to 9+) and stage of lactation (Lact._Stage; _ 1 = < 100 DIM, _2 = 100 to 200 DIM and _3 > 200 DIM) at death. * iIOLC (lactation 0) = individual rearing costs; ** iIOLC (lactation 9+) = all cows culled in lactation 9 or higher. Small circles = outliers (1,5 x interquartile range (IQR)); asterisks = extreme values (3 x IQR).

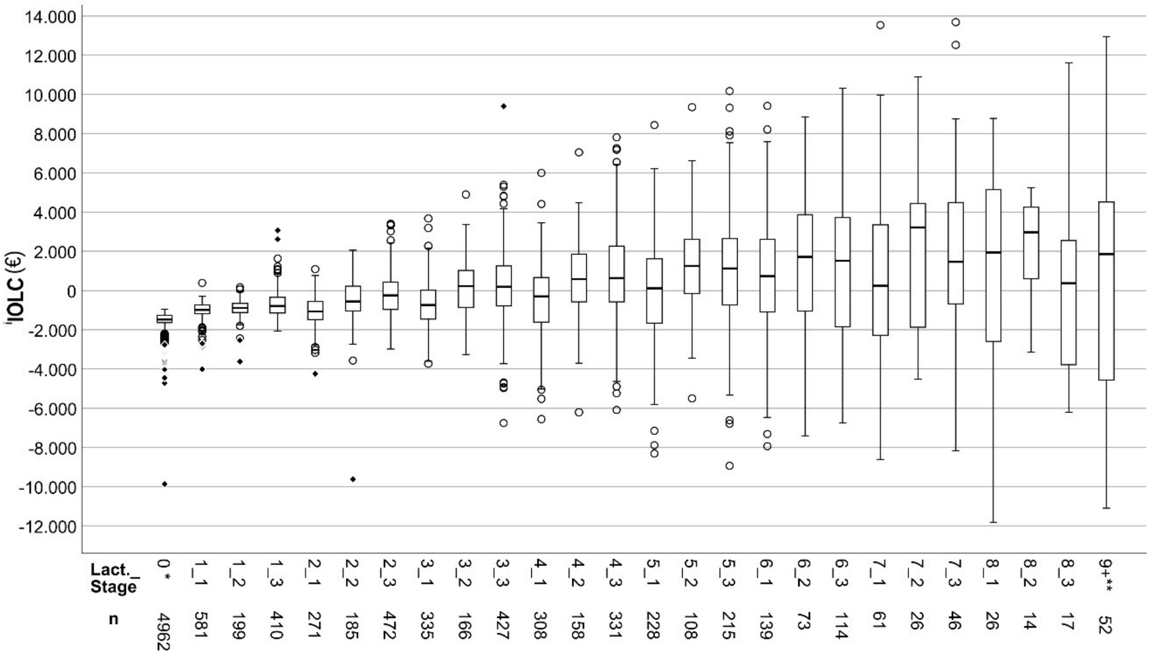
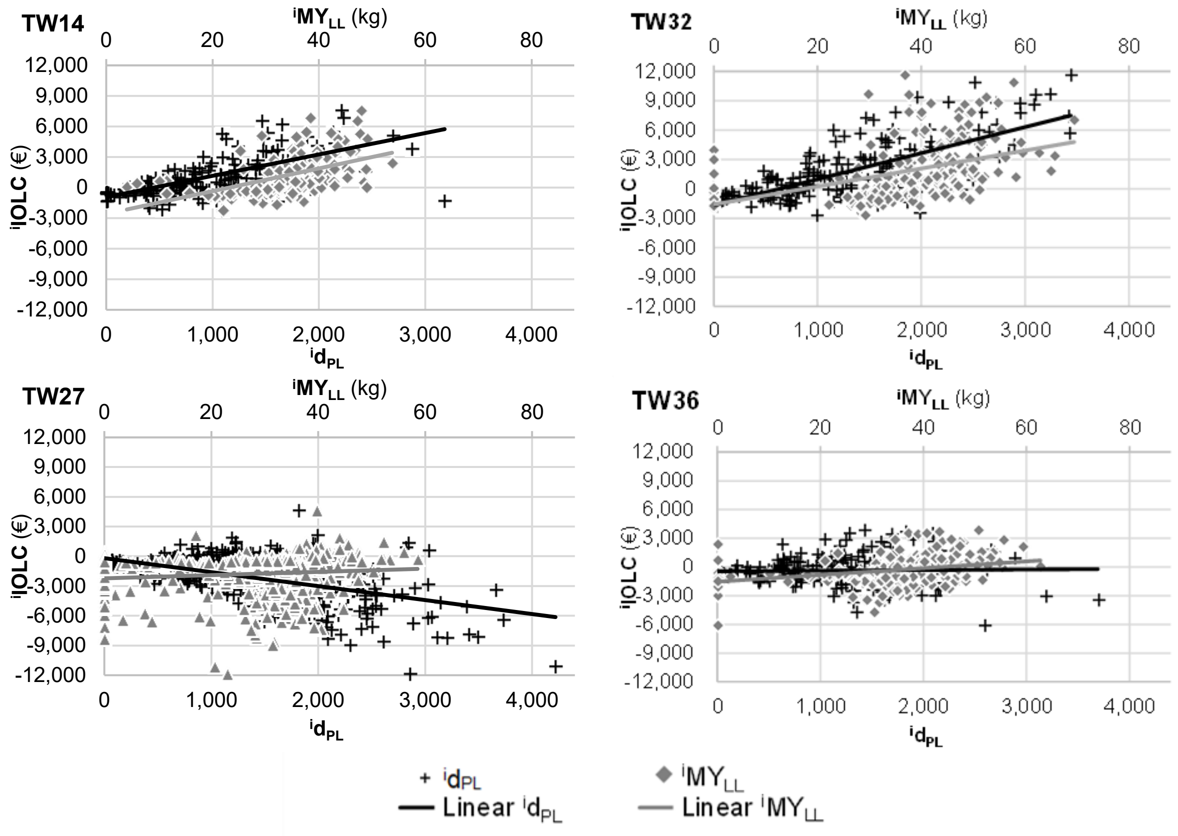
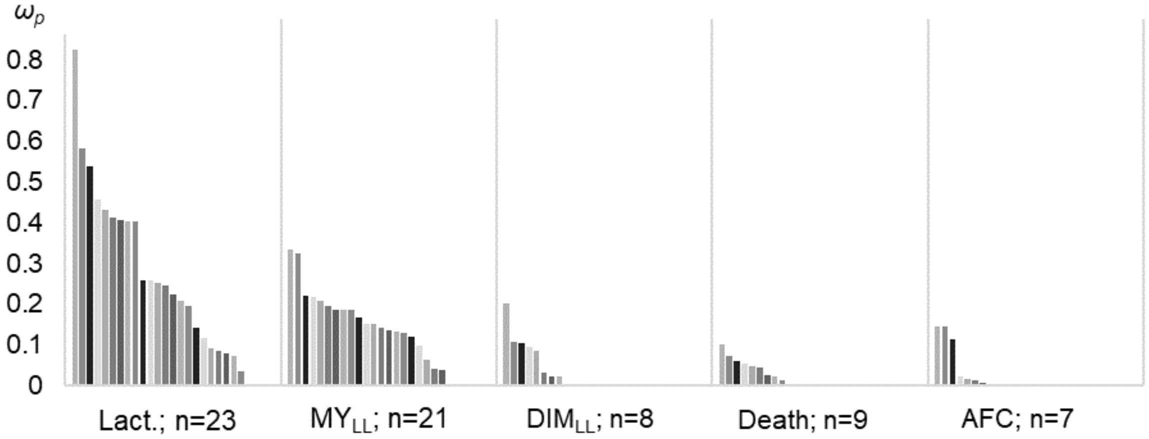


Figure 7.3. Individual income over service life cost (iIOLC) in relation to days of the productive life (idPL) and average daily milk yield during last (culling) lactation (iMYLL) for four farms (TW14, 27, 32 & 36).



Regression analysis involving all culls of all farms (adj. $R^2 = .76$) revealed that the biological variables lifetime milk yield and days of the productive life were the main levers of economic success across farms ($\omega p^2 = .69$ and .62), whereas effect sizes of economic variables milk price ($\omega p^2 = .30$), other production costs ($\omega p^2 = .23$) and feed costs ($\omega p^2 = .12$) were lower. However, to evaluate the effect of service life characteristics on iIOLC within a given economic situation, farm-specific regression models were used for further analysis. Adjusted R^2 of these models ranged from .16 to .95. The number and combination of variables that had a significant effect on both iIOLC and effect sizes of these variables differed strongly between farms (Figure 7.4). Overall, the effect of iLact, iMYLL, iDIMLL, death on farm, and iAFC on iIOLC was significant for 23, 21, 8, 9, and 7 farms, respectively (Figure 7.4). Average effect sizes (ωp^2) of significant effects for iLact, iMYLL and iAFC were .25, .16, and .06 for conventional farms and .48, .20, and .07 for organic farms, respectively. No significant effects of iDIMLL and death on farm were observed for organic farms, while these variables had a small effect (.02 to .20 resp. .01 to .10) for 8 resp. 9 farms. Ranking of effect sizes of significant effects within each farm showed that iLact had the main effect on iIOLC for 16 farms, followed by iMYLL, which was the main effect for 9 farms.

Figure 7.4. Effect sizes (partial omega square; ωp) of significant animal-related variables ($p < 0.05$) on iIOLC on 27 German dairy farms (5 farms without significant effects, not displayed). Lact: lactation number at death; MY_{LL}: average daily milk yield during last lactation; DIM_{LL}: days in milk at death (last lactation); Death: death on farm, i.e., no revenue from slaughter; AFC: age at first calving



Cross-farm testing for equality of means (Bonferroni) between HP and LP cows further confirmed that besides lower milk yield (iMYLL; 28.0 kg in LP cows / 34.3 kg in HP cows, $p<.01$), early death, as reflected by lower lactation number (iLact; 2.5/3.5, $p<.01$), fewer days in milk (iDIMLL; 158/214, $p<.01$) and a high mortality rate on farm (24.9 %/15.3 %, $p<.01$) are major causes of economic failure (Table 7.5).

Table 7.5. 1) Cross-farm comparison of herd characteristics between high-profit (HP) and low-profit (LP) cows, grouped by using the farm-specific median as threshold. 2) Number of farms where herd characteristics differed significantly between HP and LP cows and effect direction (positive/negative = mean value is higher/lower in HP group) on individual income over service life cost (iIOLC).

Variables	Mean values across farms			No. of farms: difference of mean ($p<0.05$) in HP vs. LP cows; effect direction on ^{i}IOLC; N=32	
	HP	LP	p	Pos.	Neg.
^{i}Lact.[1]	3.5	2.5	<.01	19	4
$^{i}MY_{LL}$[2] (kg)	34.3	28.0	<.01	19	0
$^{i}DIM_{LL}$[3]	214	158	<.01	14	0
Death on farm[4]	15.3%	24.9%	<.01	0	11
^{i}AFC[5] (months)	26.1	26.3	<.05	1	3

^{i}= individual; [1] lactation number at death; [2] average daily milk yield during last lactation; [3] days in milk at death (last lactation); [4]death on farm, i.e., no revenue from slaughter; [5] age at first calving; IOLC = Income over service life cost

Although the effect of iAFC, which determines individual rearing costs in a farm-specific range, was significant on some farms, mean differences between HP and LP cows across farms were low (26.3 months for LP cows, 26.1 months for HP cows). In farm-specific Bonferroni tests, iMYLL, iLact and iDIMLL were significantly greater in HP cows than in LP cows for 19, 19 and 14 farms, respectively. A significantly lower average iLact of HP cows was found for four farms and here iLact was negatively related to iIOLC. While average iLact of the culled cows of these farms was similar to mean iLact across farms (3.3 to 3.2), these farms were characterized by high total production costs/day (fCP= €10.3 compared to €9.2 across farms). Mortality rate was significantly lower for HP cows on 11 farms (Table 7.5).

4. Discussion

To our knowledge, the method presented in this article is the first full-cost approach at animal level in which individual total costs and revenues were integrated over the whole service life period of culled dairy cows. It provides a framework for full-cost calculation that is applicable for a broad range of dairy farms and accounting systems. According to the scheme of cost aggregation and cost attribution described in this article, different levels of data availability and accuracy can be integrated. Accordingly, the estimated economic values of the four farms in this study in which farm-specific costs and prices were lacking were included in the study because farm-specific optimization potentials may still be derived from the proportion of the economic effects of service-life characteristics.

The study was based on the dual hypothesis that lifetime profitability of individual cows varies greatly between farms as well as between cows of the same farm and that farm-specific optimization potentials can be revealed by analyzing the effect of service life characteristics on iIOLC of culled dairy cows. While the animal-related variables affecting cow profitability found in this study are well known (e.g., Horn et al., 2012; Vries and Marcondes, 2020; Schuster et al., 2020), the heterogeneity in the economic results and in the ranking of effect sizes between farms support this hypothesis.

Across farms, median iIOLC of culled cows was negative for 19 farms (59 %). Because decades of breeding and managing for higher yields has resulted in an increase in milk performance and a reduction in production costs per kg of milk produced, the low profitability of dairy herds observed in this study and elsewhere (Evink and Endres, 2017; Hemme et al., 2014) raises some general questions about the mutual influences between biological and economic efficiency at both farm and animal level in modern dairy farming. At animal level, higher efficiencies for milk synthesis associated with a dilution of maintenance requirements have been given as reasons for the strong increase in productivity (Baumgard et al., 2017). Yet, it has also been shown that feed intake (Hristov et al., 2005) as well as digestive (Ledinek et al., 2019) and hepatic efficiencies (Loncke et al., 2020) do not increase proportionally to increases in milk production of high-yielding animals and this indicates limitations to the breeding and management of more efficient animals. Studies also indicate that increases in milk yield require progressively greater marginal increases in nutrient supply, which leads to decreasing marginal feed efficiencies in high yielding herds (Bach et al., 2020; Moallem, 2016). In our study, feed efficiency (FE) was estimated from average daily iMYPL to reflect differences in feed costs associated with higher yields. Although FE varies between different stages of lactation and gestation according to the homeorhetic changes, feed costs were estimated from iMYPL because iMYPL is a result of both the yield potential of a cow and the ratio between productive and unproductive days she spent on the farm (i.e., also includes effects of the length of the dry period).

Regarding cow-specific rearing costs, calculations in this study assumed equal daily costs from birth to first calving for all cows of a farm. In fact, true costs per day are likely to differ considerably according to the age of the offspring. However, increases in mean costs of rearing for each extra day of AFC reported in Great Britain (Boulton et al., 2017) were similar to the costs observed in our study. Although iAFC was found to significantly affect iIOLC on some farms, average effect sizes were rather low. This suggests that monetary differences associated with each extra day until first calving seem to play a minor role for overall cow profitability for most farms. However, a lower age at first calving was found to positively affect udder health, lifetime daily milk yield, reproductive performance, and the likelihood of calving for a second time (Eastham et al., 2018) and thus might contribute indirectly to greater iIOLC.

Also due to methodological considerations, iMYLL was selected as a variable based on the assumption that it reflects the milk yield potential of a cow more accurately than does iMYPL, which largely depends on the length of the dry period(s) and the number of lactations, and because other yield measures are not available for primiparous cows or cows that die early during lactation (e.g., 305-days yield/100-days yield). Average daily milk yield during the last lactation, however, is influenced by the exact point in time of culling during lactation.

iMYLL had a significant positive effect on iIOLC of culled cows on 21 farms. Although it was the main biological effect on iIOLC for 9 farms, these results also imply that the individual milk yield level is not a main driver of cow profitability for most farms. In contrast, Drews et al. (2018) identified milk yield performance as the key variable for both economic and biological efficiencies of dairy herds but also concluded that the fluctuations in milk price often mask the mutual influences between the biological efficiency (as reflected by feed efficiency, life efficiency and milk yield from roughage) and the economic efficiency (as reflected by net return, milk price and income over feed cost). This was also confirmed by Wolf (2010), who found that the volatility of milk price is a major confusing factor when evaluating dairy profitability (milk to feed price ratio or IOFC) across farms and financial years. Due to this volatility, which is also observed in the case of feed prices (Merener and Steglich, 2018), the estimations of iIOLC based on one financial year performed in this study would improve through inclusion of longitudinal data of milk and feed prices and attribution of these cost items to the actual lifespan of individual dairy cows.

However, Walsh et al. (2020) identified a higher milk price as a main determinant of profitability for organic farms. In fact, with average total costs per day per cow and average lifetime milk yield being at similar levels but milk price being 35 % higher, culled cows of the organic farms participating in this study showed higher iIOLC values than cows of conventional farms. These results at animal level are in accordance with studies reporting similar or higher profitability (Jan et al., 2014; Zekalo, 2015; Naglova and Vlasicova, 2016; Flaten et al., 2019; Nehring et al. 2021), and reduced economic vulnerability (Bouttes et al., 2019) for organic dairy systems, compared to conventional systems, despite lower milk yield levels in organic farming. Although lower levels of purchased feedstuffs in organic dairy production systems are supposed to decrease total feed costs (Naglova and Vlasicova, 2016; Zekalo, 2015; Bouttes et al., 2019), feed cost per day per cow were at similar levels between organic and conventional farms in our study. Nonetheless, our results support the hypotheses that the higher milk price in organic farming outweighs the reduced feed efficiency in terms of feed cost per kg milk produced at herd level, which is commonly observed due to lower milk yields in organic vs. conventional farming (iMYLL: 25.7 kg vs 31.7 kg in our study). Both due to the farm-specific approach and the limited number of organic farms investigated, differences between conventional and organic farms reported in our study must be interpreted cautiously.

Irrespective of the production method but due to industrial and political demands, however, it is very difficult for individual farmers to act on milk prices, while focusing on a reduction in production costs, such as costs for high-quality feedstuffs, skilled farm labor or disease prevention, can have adverse effects on the productivity and the efficiency of the dairy herd (Barkema et al., 2015; Derks et al., 2014; Põldaru and Luik-Lindsaar, 2020). In contrast, improving disease and fertility management have been shown to also increase feed efficiency and lifetime productivity of individual cows and dairy herds (Knapp et al., 2014). However, costs associated with management practices that aim for increases in productivity, feed efficiency or longevity may not be offset by their positive effects if their economic impact is compared across farms. Hansson and Öhlmér (2008), for example, did not find any effect of animal health practices on long-run allocative or short-run economic efficiency in a set of 169 Swedish dairy farms, while Lawson et al. (2004), who found a positive relationship between the technical efficiency of milk production and higher incidence of lameness, ketosis and digestive disorders, concluded that the level of productivity of the farms outweighs the negative impact of health disorders on efficiency. This might also be true for another cross-farm study enrolling a large set of Dutch dairy farms in which it was recently shown that the gross margins of dairy farms, i.e., the total revenues minus total costs, were not significantly correlated to the longevity of the herds (Vredenberg et al., 2021).

In contrast, the farm-specific full-cost calculation of cow profitability presented in this article, which accounts for differences between profitable and non-profitable cows within a given economic situation, showed that low longevity is the major barrier to individual economic success. In fact, individual cows can only contribute to farmers income, if their individual revenues from milk and slaughter override their individual costs for rearing, feeding and their share of the fix costs of the farm. In this study, only 41.5 % (56.3 %) of the dairy cows in conventional (organic) farms reached lactation 4 or higher, which is in accordance with the average productive life of 3 to 4 years reported for other countries with intensified milk production (Vries and Marcondes, 2020). However, farm-specific maximum iIOLC was not reached before 5th lactation in our study. As a result, median iIOLC of cows at time of death was negative for 70 % of the conventional farms, while all organic farms had an average iIOLC > 0 €. Similar to our data, Horn et al. (2012) calculated that annual profit of Austrian organic cows reached its peak in the 6th lactation. In a situation without involuntary culling, Missfeldt et al. (2015) modelled that maximum profit would be reached after 7 productive years. Somehow unexpectedly, iIOLC trended towards more negative values in higher lactations for four farms in our study. The reasons behind this were ambiguous for two of those farms, but exceptionally high average production costs per cow (€12.95/d; TW17) resulting from a recently made major investment and an enduring rather low milk price (€0.34/kg; TW27) likely influenced the effects of iLact for the other two farms. However, given that culling is not economically desirable before cows reach their productive zenith or can be replaced with more productive cows, considerations about decision-making processes and the circumstances leading to high culling rates evolve. In our study, the median culling rate of 28.9 % is slightly lower and the median mortality rate of 5.7 % is slightly higher than what has recently been reported for a larger sample of German dairy herds (Hoedemaker et al., 2020). Organic farms participating in this study showed a lower overall culling rate (26.5 % vs. 30.1 %) and a lower mortality rate (3.1 % vs. 6.2 %), compared to conventional dairy farms. Lower culling rates together with higher average effect sizes of iLact in organic farms compared to conventional farms may point at the specific potential associated with longevity in an organic cost- and price-structure. Both for conventional and organic farms, culling was primarily due to disease or infertility, which is in accordance with culling reasons reported in other countries with intensified dairy farming (Ahlman et al., 2011; Bascom and Young, 1998; Compton et al., 2017; Rilanto et al., 2020). On one hand, farmers still tend to underestimate costs of disease and replacement (Huijps et al., 2008; Jones et al., 2016). On the other, the aim for genetic progress might contribute to high culling rates although genetic opportunity costs, defined as the loss of keeping the current cow in the herd instead of replacing with genetically superior cows have been shown to not warrant a high culling rate, even if genetic improvement accelerates (Vries, 2017).

While retrospective analyses like the one presented in this article do not include assumptions made on practical and economic feasibility or opportunity costs (economic significance) and exclude the possibility to react in real-time for individual cows, the monetary valuation associated with service life characteristics can help to prioritize when choosing between investments in, e.g., animal health, longevity, or productivity. Our results showed that measuring efficiency at (purely) farm level may hide optimization potentials at cow level. The large variation in iIOLC between cows living and producing within a given cost and price structure, i.e., between cows of the same farm, which was observed in our study, points at the extent of these potentials. Hence, farm-specific iIOLC -calculation can be regarded as a tool for a more strategic and long-term orientation support in, for example, general breeding, culling, and feeding decisions. Given the great proportion of negative iIOLC observed in our data illustrating the economic urgency of many dairy systems, orientation support in practical dairy farming would greatly benefit from further scientific evaluation of efficiency measures that have the potential to mutually improve the biological and economic viability of dairy cows and farms.

5. Conclusion

A major barrier for economic optimization of dairy herds is the limited compatibility of economic and biological data at cow level even though the viability of the individual cow and the economic survival of the dairy farm are inherently linked. By breaking down farm-specific costs to the level “per day, per cow” and attribution of costs and revenues to lifetime and performance data of culled cows, however, lifetime profit of individual animals can be calculated and influencing factors can be analyzed. Based on the individual economic results of culled cows from 32 German dairy farms, increasing longevity through measures that increase animal health and thus, reduce involuntary culling due to disease, would appear to be a reasonable aim for a broad range of economic situations. This might be particularly true when taking into account the risks of high-intensity production under conditions of uncertain pay-out and the increasing emphasis that is placed on animal health and welfare issues by consumers and politics. However, the large variation in the economic impact of service-life characteristics between farms indicates the suitability of the method for farm-specific optimization of cow profitability. For on-farm application of the iIOLC -method, continuous recording of the relevant data and up-to-date calculation of iIOLC, including current costs and prices, should be envisaged. Besides on-farm purposes, the method presented should also serve as a starting point for empirical analysis of the effect of management practices on iIOLC.

Declaration of interest

None.

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9. General Discussion

Due to different levels of productivity and different levels of exposure to stresses, not only the nutritional status but also the economic potential of longevity needs to be addressed for each cow individually. Therefore, the present thesis developed novel methods for the quantification of both metabolic and economic trade-offs, which focus expressly on the individual cow.

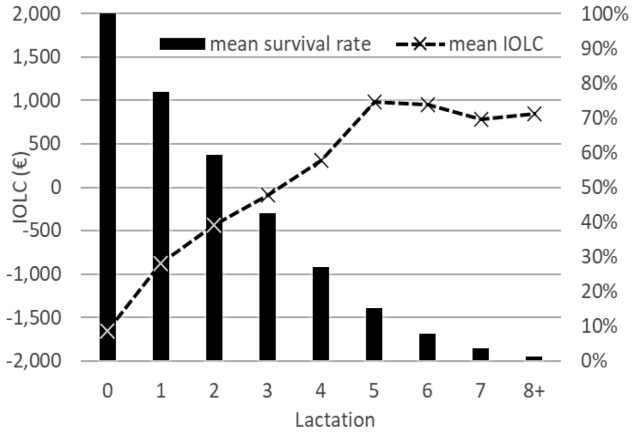
While detailed discussions of the methodologies as well as of the physiological and economic backgrounds are part of the discussion sections of publications #1, #2, #3 and #4, the general discussion of this thesis is intended to cover two additional topics in more detail: (1) the socio-economic relevance of increased longevity (ch. 8.1.) and (2) possibilities and challenges to use the knowledge about animal-individual metabolic trade-offs in the field (ch. 8.2.).

9.1 The Value of Increased Longevity in the Face of Future Challenges to the Dairy Sector

Although cows can live up to 20 years and more, the productive lifespan of dairy cows averages 2 to 4 years in most countries (Compton et al. 2017; Vries and Marcondes 2020). This is supposed to be driven by considerations about efficiency and profitability and in fact, a long lifespan per se does not guarantee increases in profitability, because feed efficiency for milk production during lactation, the length of dry periods and the genetic improvement associated with replacing an old cow by a heifer must be considered when evaluating the economics of longevity (Essl 1998; Horn et al. 2012; Vries 2017; Schuster et al. 2020). Moreover, culling decisions are not only influenced by social and psychological factors (Fetrow et al. 2006; Haine et al. 2017; Rilanto et al. 2020), but also by national policies and the production method. For instance, the culling strategy of farmers producing in supply management systems might be affected by their ability to meet quota while avoiding to overproduce (Schuster et al. 2020). Without accounting for losses related to culling and disease, the economic evaluation of longevity might also be driven by the aim to maximize production per unit of the most limiting factor, which might not be the animal but, milking stalls or hectares (Laven and Holmes 2008; Vries 2017).

The majority of cows in Germany and other countries with industrialized dairy industries, however, is not slaughtered primarily due to economic considerations but is culled due to infertility, mastitis, lameness or other disease (Compton et al. 2017; Hoedemaker et al. 2020). While the annual incidence risk of culling due to low production decreased, the share of mortality and involuntary culling decisions increased (Compton et al. 2017). It is thus not particularly surprising that the majority of culled dairy cows did not produce enough milk to pay-back individualized costs of rearing together with ongoing cost of production in our study (publication #4).

Figure 8.1. Mean Income over service life cost (IOLC, left ordinate) and mean survival rate (right ordinate) of culled dairy cows of 32 German dairy farms.



In the full-cost approach applied, higher average milk yields of farms did not automatically result in increased numbers of culled cows making profit. Instead, the duration of the productive life was the main determinant of cow profitability in all economic scenarios except for those where cows were not able to gain profit at any time during their life due to very high fixed production costs.

It has been shown that failure costs emerging from disease events are commonly not known precisely and thus often underrated by dairy farmers (Huijps et al. 2008). This might be due to the fact that monetary losses associated with PD as well as costs and benefits associated with disease prevention are usually hidden in the aggregated form of business sheets (Demartini et al. 2015). These losses may be further veiled because profits of an agricultural business originate only partly from their economic viability, but also form subsidies. In the European Union, the producer support estimate, i.e. the share of policy transfers in gross farm receipts account for ~ 20 % of (OECD 2022) and may impair improvements in technical efficiencies of dairy farms in Europe (Zhu et al. 2012; Latruffe et al. 2017). However, another common argument from producers is that longevity negatively affects profitability with regard to opportunity costs emerging from slower genetic improvement in milk performance. By calculating costs and benefits of different culling scenarios, however, Vries (2017) showed that genetic opportunity costs do no not warrant low longevity. Moreover, the increase in feed efficiency associated with a „dilution“ of maintenance requirements (Baumgard et al. 2017) in cows with higher genetic merit leading to lower marginal feed costs is limited as increasing milk production in high-yielding herds requires progressively greater marginal increases in nutrient supply (Vandehaar and St-Pierre 2006; Moallem 2016; Bach et al. 2020).

Given the antagonistic relationship between genetic merit for milk yield and health and fitness traits (Veerkamp 2009), profits emerging from higher feed efficiency for milk production may further be outweighed by the costs associated with reduced feed efficiency for self-sustainment. As opposed to the ‚productivist paradigm‘ predominating in the dairy industry (Marechal et al. 2008), the claim for a balanced instead of maximal intensity of production has been brought forward by several authors in recent years, as this may positively affect both animal health and the sustainability of the dairy sector (Ma et al. 2018; Clay et al. 2020).

With regard to the alterations caused by climate change putting a high pressure on many kinds of agricultural resources, agricultural production faces big challenges in the near future. In dairy production, a reduction in the usage of arable land for livestock farming (feed-no-food) and thus, the promotion of (silvo-) pastoral- and/or forage-based systems is required (Broom et al. 2013). This implies, however, that feeding high-producing dairy cows according to their demands to avoid overstressing of self- sustaining life functions and therewith, premature culling, may become more difficult. This is particularly true when accounting for the expected increase in demands of regulatory functions due to climate instability, such as floods, hurricanes and droughts, which have been supposed to make animals more susceptible to disease (Filipe et al. 2020). Besides positive effects on cow resilience and longevity, balanced intensity of production may enable increased self-sufficiency, contributing to farm survival in the long-term. Although both the economic benefits as well as the externalities associated with different intensity of production are inadequately measured for most dairy farms and production systems, lower yield system have the potential to increase farm economics while reducing emissions and nutrient losses (Balmford et al. 2018). With regard to greenhouse gas emissions, increasing longevity may have two major positive effects: Firstly, more methane and more phosphorus are produced per unit of milk in cows with larger proportion of heifers, as heifers account for up to 33% of the enteric methane emissions of the herd (Hristov et al. 2013; Knapp et al. 2014). In the study of Grandl et al. (2019), increased longevity was associated with both reduced greenhouse gas emission and improved profitability of dairy cattle.

Moreover, the societal acceptance of livestock farming, which demands improvements not only to ecological aspects of animal husbandry but also to animal health and welfare should be considered. Besides high incidences of production disease and high rates of culling due to disease, the fact that 10 to 20% of cows die on farm (Pinedo et al. 2010; Compton et al. 2017; Hoedemaker et al. 2020) indicates severe impairments of animal welfare in dairy farming. Although public concerns as well as policies are mainly driven by housing conditions, the importance of animal health as a prerequisite for animal welfare has been outlined (Broom 2006; Sundrum 2020).

Organic agriculture aims at both high levels of animal health and sustainable land use. However, similar prevalence of production disease exists in both production methods across Europe (Krieger et al. 2017a). This might be associated not only to the fact that the occurrence of production disease highly depends on management factors not related to the production method, such as knowledge of the persons responsible, hygiene, monitoring intervals and the time spend for individual animal care, but also to restrictions in the use of antibiotics and to the fact that organic dairy farms often milk cows with genetic merits similar to those in conventional production (Rodríguez-Bermúdez et al. 2019) despite limitations to, e.g., concentrate supply. Moreover, higher milk prices and subsidies in organic compared to conventional farming may lower the farmers efforts to reduce monetary losses associated with, e.g., production disease and culling. In fact, culled cows of the organic farms participating in project Tier-Wirt had on average higher individual profits. Because culling reasons as well as mortality and culling rates differ only slightly in organic dairy farming (Sato et al. 2005; Ahlman et al. 2011; Krieger et al. 2017a), increasing longevity is supposed to be the major lever to increase dairy cow profitability, irrespective of the production method.

9.2 Enabling Dairy Cow Survival by Considering the Individual Ability to Adapt to Immune Challenges

The survival of an organism depends on the ability to cope with its environment, which in turn is related to the function of regulatory systems within the organism (Broom and Johnson 1993). The immune system can be regarded as the most efficacious regulatory system in mammals, as immune action is triggered any time the host system is exposed to excessive environmental stimulation that manifests as stress (Colditz 2009). However, immune cells are part of the nutrient economy of the organism and thus, integrated into systemic physiological processes. To avoid nutrient shortages for immune cells of farm animals in case of additional, and - together with other energy-demanding processes - too large demands, farmers are sought to take management actions that reduce the animal’s exposure to nutritional, environmental, social and infectious stresses. This includes optimal climatic conditions, high hygiene standards within the barn and on pastures, vaccinations, measures to reduce the exposure to helminthic infections and ectoparasites, etc. The load on the immune system may further be kept to a minimum through frequent observation of individual animals and frequent monitoring of all available data related to the health status. In dairy farming, this includes, e.g., somatic cell counts and milk solids, heat intervals and intensity of heat, rumination time, repeated check of body temperature in the first week after calving, etc. to detect diseases before immune actions become excessive and overstressed.

Considering the various, and partially unpredictable stresses imposing a challenge to the immune system of dairy cows, however, farmers should not only strive for a reduction in stresses that are sensitive to management measures, but also enable cows to cope with hazardous challenges. Therefore, management factors related to nutrition and breeding that are likely to affect the cow ‘s ability to adapt are discussed in the following chapters.

9.2.1 The Ability to Adapt to Immune Challenges in Cows with High Genetic Merit for Milk Production

Maybe because selection for milk production has been highly successful in dairy cows, the strive for genetic solutions to the problem of production disease and low longevity is strong. Given the multifactorial character of PD’s as well as the farm-specificity of culling strategies, however, it is not surprising that the heritability of longevity is low (Nejad et al. 2021). Because longevity is only a weak proxy for health and fertility, the implementation of longevity as a breeding goal in many national indices, which developed due to the broad availability of culling data (while health data is not ubiquitous), has thus been criticized (Schmidtmann et al. 2021). Instead, functional traits such as, e.g. udder and claw constitution (Schmidtmann et al. 2021), or low variance and autocorrelation of daily deviations from a predicted milk yield curve have been proposed as a possible breeding goal (Berghof et al. 2019), as they were shown to be genetically correlated with longevity.

On the other hand, the metabolic plasticity, i.e. the ability of cows to reallocate nutrients towards maintenance functions in response to limited resource availabilities has been brought forward as a breeding goal in future dairy farming (Friggens and Newbold 2007; Vandehaar et al. 2016; Gross and Bruckmaier 2019). In evolutionary biology, it has been proposed that the process of adaptation to any kind of external disturbance is costly in terms of energy and nutrients (Sterling 2004; McEwen and Wingfield 2010). This means that cow ‚resilience ‘, ‘plasticity‘ or ‚fitness‘ could be considered as the ability to fuel regulatory functions such as the immune system and that these functions enter a trade-off for resources with other life functions. Moreover, animals that have been selected for high milk production are thought to reallocate resources away from life functions not defined in the breeding goal (Essl 1998; Veerkamp 2009; Oltenacu and Broom 2010). Among them, ‚buffer capacities‘ were hypothesized to be affected in the first place (Rauw et al. 1998; Rauw 2009). Although buffer capacities such as glycogen or fat depots, i.e. the amount of energy and nutrients not assigned to a specific function are considered sub-optimal with regard to the efficiency of nutrient utilization, the suboptimal design is a key characteristic of the evolutionary drive for increased fitness (Garland 1998; Rauw 2009). In fact, natural selection creates intermediate optima for many traits, including milk yield, body weight and immune response, because this allows for the most dynamic adaptation of metabolic priorities over a wide range of environmental stresses (Dunnington 1990; Rauw 2009). Besides overall availability of energy and nutrients, the allocation of resources and the ability to dynamically adjust allocation patterns according to the demands of self-sustaining life functions is thus supposed to be a major factor contributing to the risk of disease

(Glazier 2009; Sundrum 2020). Results of publications #2 and #3 support these hypotheses, as it has been shown that, on average, the cows enrolled in the studies (1) did not have any buffer capacities in terms of glucose during early lactation and (2) did not or only slightly adapt metabolically in terms of overall glucose or energy balance in case of disease. Hence, while direct genetic correlations between milk yield and longevity are inconclusive (Tsuruta et al. 2005; Tokuhisa et al. 2014; Sasaki et al. 2017; Steri et al. 2019), breeding for increased milk yield is thought to indirectly affect longevity by reducing the amount of nutrients partitioned to self-maintenance in case of restricted nutrient availability. In other words, the reconcilableness of both a higher efficiency for milk production and maintaining plasticity is highly questionable (Rauw et al. 1998), because genetic changes associated with intensive breeding for milk productivity are thought to be too fast and too radical, which impedes metabolic trade-offs to gradually adapt (Friggens and Newbold 2007; Rauw 2009).

Regarding glucose availabilities of different life functions, adaptations favoring the glucose supply to the mammary gland were found to be more pronounced in cows selected for high milk yields. For instance, circulating concentrations of growth hormone (GH), which has a pivotal role in regulating periparturient energy metabolism, are higher in early lactation and remain elevated during lactation in cows with high genetic merit for milk production, insulin resistance is increased (Hart et al. 1978; Chagas et al. 2009) and the levels of plasma glucose, insulin-like growth-factor 1 as well as the expression of GH-receptor 1 is decreased (Snijders et al. 2001; Veerkamp et al. 2003; Jiang et al. 2005; Okamura et al. 2009). As the „uncoupling of the somatotropic axis“ (Lucy et al. 2001) is stronger and more sustained in high-genetic merit cows, it can be assumed that the prioritization of mammary glucose supply is less affected by the metabolic trade-offs at whole-body level emerging in case of nutrient shortage or additional stressors in those cows. Accordingly, a strong, linear, negative relationship between the number of glucose transporters expressed on the membrane of monocytes and macrophages and the amount of lactose produced by the mammary gland was observed in cows during early lactation (Eger et al. 2016). Taken together, it must be assumed that glucose shortage for immune functions is a central part of the negative relationship between yield and longevity. In light of the enhanced support of lactation in dairy breeds, it is not surprising that Holstein-Frisian were repeatedly shown to have higher risks of metabolic derailments and immune dysfunction compared to breeds that did not undergo the same degree of unidirectional selection (Urdl et al. 2015; Curone et al. 2018; Lopreiato et al. 2020).

To stay healthy, however, dairy cows with high genetic merit for milk production require optimal management conditions, as these cows were supposed to be both healthy and high producing in ideal environments only, while being increasingly sensitive to environmental disturbances (Beilharz and Nitter 1998). With G x E interactions being investigated in more detail in recent years, this hypothesis is often confirmed (Raffrenato et al. 2003; Kearney et al. 2004;

Petersson et al. 2005; Calus et al. 2006; Roche et al. 2018). The results presented in publication #2 and #3 indicate that even in herds in which the management has a high level (research facility), glucose reserves are exhausted at the start of lactation. If the supply with energy-rich components is disturbed by, e.g., crop losses and/or high market prices for concentrates, high-producing dairy cows are likely to be more susceptible to metabolic and immune dysfunction because their ability to reduce milk synthesis in case of shortage is supposed to be reduced compared to lower genetic merit cows.

9.2.2 Management Measures to Address the Reduced Adaptability to Metabolic and Other Stressors in High-Yielding Dairy Cows

Considering the high demands imposed by immune cells, a sufficient supply with nutrients according to individual needs is required for immune functionality. Yet, diets typically offered to dairy herds or feeding groups can barely meet the specific requirements of individual cows within a herd or feeding group due to variations in milk yield, pregnancy, stage of lactation, age, bodyweight, and their exposure to stresses (Rumphorst et al. 2022). Farmers have confirmed that the individual variation imposes a great barrier to the goal of reducing production diseases (Hoischen-Taubner et al. 2018). Besides reducing heterogeneity in the herd related to different genetic merits for milk production, variation in nutritional supply may be reduced through establishment of as much feeding groups as possible while ensuring sufficiently large feed fences (St-Pierre and Thraen 1999; Huzzey et al. 2007). Additionally, lower culling rates may lead to a greater homogeneity in the social structure of a herd, leading to less competition for feed and less expenditures for aggressive actions as well as for stress regulation in the organism (Gutmann et al. 2015).

Although increasing homogeneity may alleviate the discrepancies between average and individual nutrient balances, accounting for hazards through ensuring the allocation of an adequate level of resources to each cow requires primarily the assessment of the individual nutritional status of each cow. In dairy farming practice, the contents of fat, protein and urea in milk obtained from monthly milking records or automatic milking systems are the most common measures to assess individual nutritional statuses. Compared to the fat content or milk protein content, which were found to not be suitable for the assessment of the metabolic status, the fat-to-protein ratio (FPR) is less affected by the quantity of milk. This ratio is thus often used by farmers, extension and researchers (Heuer et al. 1999; Tremblay et al. 2018; Glatz-Hoppe et al. 2020). In fact, increased FPR is associated with an increased risk for disease and culling (Grieve et al. 1986; Heuer et al. 1999; Kessel et al. 2008; Jenkins et al. 2015). Yet, the definition of thresholds for the fat-to-protein ratio is difficult across breeds and farms (Glatz-Hoppe et al. 2020). In science and veterinary practice, metabolites related to excessive lipomobilization (mainly NEFA and beta-hydroxybutyrate) are used to evaluate metabolic statuses. Interestingly, the catabolic state that is characterized by a strong mobilization of adipose and protein tissue and the concurrent increase in the concentration of these metabolites was shown to decrease immediately when glucose is infused (Lucy et al. 2013; Grossen-Rösti et al. 2018), indicating the central role of glucose for the metabolic balance of transition dairy cows. However, plasma concentrations of glucose lack diagnostic value due to their tight homeostatic control. In general, analyzing individual levels of plasma metabolites routinely and frequently in dairy farming is barely possible as this would require plasma sampling of all cows, which is costly and causes additional stress to the animals. Along with the aim to analyze nutritional imbalances beyond the level of overall energy availability, the limited diagnostic value of plasma glucose concentrations and the low feasibility of routine plasma sampling were additional rationales for the development of the methodology presented in publications #2 and #3.

Based on the explanations presented in publication #1 of this thesis, evaluation of glucose balance might be more suited than evaluation of individual energy balances to monitor the adaptability of dairy cows. While interpretation of individual energy balances is challenged by the fact that cows adapt their metabolism differently at similar levels of NEB (Klein et al. 2012; Tremblay et al. 2018), the lack of data, in particular data on the individual feed intake, is a major barrier to a more widespread use of both animal-individual energy and nutrient balances in practical dairy farms until now. Contrasting feeding throughs equipped with weighing units, which are costly not only in terms of money but also in terms of the labour required to install, clean, etc., camera technologies estimating the height of fodder on the feeding alley might be a feasible option for commercial dairy farms in the future (Shelley et al. 2016; Bezen et al. 2020; Saar et al. 2022). Similarly, the assessment of individual body weights as well as body condition scores is not yet common practice, although automated scales (Dickinson et al. 2013) and scoring systems (Mullins et al. 2019; Zin et al. 2020) are available. In fact, both the change in body weight as well as the change in body condition score were found to have high correlations to the concentration of plasma non-esterified fatty acids ($r=-0.51$ at $p<0.0001$), when compared to the contents of milk solids (Mäntysaari et al. 2019). Thus, at present, the implementation of such measuring systems is the most suitable next step for dairy farmers to advance the assessment of individual metabolic statuses.

If information on feed intake, milk yield and body weight are available in real-time, individual energy and nutrient balance could readily be calculated and several specific management measures could be undertaken to lower the metabolic burden experienced by the individual cow. For instance, the attribution of concentrates via automatic milking systems or feed stations could be adjusted not only quantitatively but also qualitatively to individual requirements, e.g., through different composition of feedstuffs or supplementation of feed additives. In fact, the attribution of concentrates is automated in most dairy systems and is solely based upon lactation stage and/or milk yield. In this regard, it must be considered that automatically reducing the amount of energy-rich concentrates following a drop in milk yield might be contraindicated in situations where milk yield decreases in response to an immune challenge, as this is likely to be associated with a high demand imposed by immune functions and thus, might even lead to increased overall demands. This highlights one of the problems associated with using and relying on big data. In such a case, the feed station data would have to be checked against health data such as rumination time, activity and milk yield. If these data are not matched, the automated attribution of concentrates could worsen a cow's metabolic status.

However, increasing energy and glucose balance in high-yielding dairy cows fed intensively is limited by careful use of concentrates to avoid ruminal dysbiosis. Moreover, time to eat, rumen volume and liver health are to be considered. Feeding glucogenic diets were supposed as another option to increase glucose availability but are unlikely to significantly reverse lactational energy partitioning between milk and body tissue (Kokkonen et al. 2005; van Knegsel et al. 2007; Mesgaran 2010). Taken together, it is doubtable if intensive dairy farming can further optimize precursor supply or gluconeogenic potential and thus, increase total glucose availability in high-yielding dairy cows via nutritional interventions, particularly during the most critical phase of early lactation. On the other hand, improved dry off feeding and heifer management are of outmost importance to alleviate metabolic problems of periparturient cows (Beever 2006). For instance, overfeeding of cows by 50% of predicted requirements during the dry period decreased postpartum plasma glucose and insulin and increased glucagon, BHB, and NEFA concentrations after calving compared with cows fed a controlled energy diet (Mann et al. 2016).

As another option to face severe shortages in individual nutrient availability, short-time modification of the amount of milk withdrawal should be considered. This can be achieved through, e.g., incomplete milking, prolonged milking intervals (Patton et al. 2006; Loiselle et al. 2009; Moallem 2016) or even the use of prolactin antagonists (Lacasse et al. 2019). In fact, decreasing milking frequency or incomplete milking for up to 5 days was shown to not affect milk production in the long-term (Carbonneau et al. 2012; Morin et al. 2018), while longer periods caused sustained reductions in milk yield (Soberon et al. 2011; Phyn et al. 2014). Research investigating the effect of different milking intervals on the incidence of clinical mastitis led to heterogenous results. While Fogsgaard et al. (2015) and Penry et al. (2017) indicated a slightly higher risk for clinical mastitis in cows with increased milking intervals, no increase in the rate of clinical mastitis (Stelwagen et al. 2013) or the prevalence of mastitiscausing pathogens (Holmes et al. 1992) was reported in studies comparing 2 x vs. 1 x daily milking. In contrast, reduced milking frequency can improve reproduction and reduce lameness (Stelwagen et al. 2013). However, reductions in milk withdrawal should only be considered if controlling the possible side effects can be assured. Moreover, the amount of reduction should not be determined according to a simple scheme. Due to a fairly constant amount of glucose utilized by mammary tissue to produce 1 kg of milk (~72 g), the level of glucose balance (if available for individual cows in real-time) would be suited to indicate the amount of reduction that is necessary to alleviate the metabolic burden. However, further research investigating the amount of glucose needed for different immune challenges and for different degrees of immunoactivation (e.g., for a specific level of proinflammatory metabolites) and, preferably, during different physiological stages, is needed to better evaluate the quantities required.

10. General Conclusions

A high risk of production disease, infertility and a short lifespan exists if whole-body demands for glucose are not met by glucogenic supply, e.g., in case of additional demands imposed by the immune system. Low glucose reserves and a limited ability to increase glucose availability for immune cells during disease indicate that high performance and avoidance of production disease (and thus, a long productive lifespan) are difficult to achieve for individual cows. The lack of glucogenic carbon, together with a dysfunctional allostatic regulation in high producing animals that prioritizes lactocyte supply to the detriment of leukocytes is an important part of the multifactorial character of production diseases. Because the metabolic burden of individual cows within a herd varies greatly due to differences in lactation stage, genetic merit and the degree of exposure to stressors, nutrient supply must be optimized to account for the individual demands, a maximum reduction of biotic and abiotic stressors must be envisaged, and, if this is insufficient, milk withdrawal should be reduced.

The inability of cows to cope with their environment resulting in a high prevalence of production disease and high rates of involuntary culling also has major consequences on the profitability of a dairy business. Calculations of the economic result of individual cows presented for a sample of 32 German dairy farms indicate that most cows were culled due to disease and infertility. More than 50 % of culled cows were not able to pay-back their individual share in the costs associated with rearing, feeding and other production costs via milk sales before being culled.

Quantitative knowledge about metabolic trade-offs between productive and maintenance functions, and quantitative knowledge about the monetary valuation of service life characteristics associated with these life functions (productivity, longevity) are essential to improve the cow’s odds to stay healthy and alive. Besides farmers and extension services, these results may be used to prioritize education necessities. Reducing PD and increasing longevity through less involuntary culling is particularly worthwhile when considering not only animal welfare and economic benefits but also public acceptance of livestock farming and the sustainability issue.

Bibliography of the General Introduction and Discussion

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