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# Protein nutrition of dairy cows

Some relevant further aspects of protein nutrition of dairy cattle, when reducing protein nutrition in dairy cows in view of societal demands

A. Bannink, A. Van Ruitenbeek, W. Spek, R. Zom, J. Dijkstra

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Some relevant further aspects of protein nutrition of dairy cattle, when reducing protein nutrition in dairy cows in view of societal demands

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## Samenvatting NL

Het doel van de huidige deskstudie is om een overzicht te geven van verschillende aspecten van eiwitmetabolisme in melkkoeien die weinig of geen aandacht krijgt met de eiwitwaardering in de huidige praktijk. Een aantal aspecten kunnen echter wel relevant zijn voor met name voedingsomstandigheden waarbij er sprake is van een verlaagd ruw eiwitgehalte van het rantsoen en aanbod van darm verteerbaar eiwit (metaboliseerbaar eiwit) aan de koe om de stikstofexcretie te beperken. Ten eerste, een wisselend of oscillerend aanbod met tijdelijke deficiëntie voor de duur van enkele dagen lijkt weinig invloed te hebben op de prestatie van de koe. Het is echter onduidelijk of negatieve bijwerkingen uitblijven van ongeacht het eiwitgehalte van het rantsoen, en of dit ook op de langere termijn volgehouden kan worden. Ten tweede, keuze voor aanbod van glucogene in plaats van lipogene nutriënten lijkt vooral interessant om de vastlegging van stikstof in melkeiwit te stimuleren. Maar bij een laag aanbod van darm verteerbaar eiwit kunnen ook vetbronnen een toename geven van de omzetting van aminogene nutriënten naar melkeiwit. De koe blijkt een grote flexibiliteit te hebben in de aanmaak van melkeiwit, -vet en -lactose uit het aanbod van lipogene, glucogene en aminogene nutriënten waar beter gebruik van gemaakt zou kunnen worden om darm verteerbaar eiwit maximaal te benutten bij een laag aanbod aan de koe. Ten derde, de weinige lange-termijn studies naar het effect van een laag eiwitgehalte in het rantsoen geven aan dat dit een zeer effectieve manier is om de efficiëntie van stikstofbenutting te verbeteren en de stikstofexcretie en -emissies te verlagen, zonder grote gevolgen voor de energiebalans en gezondheidsstatus van de koe. Een niveau van 14% tot 16% ruw eiwit in de rantsoen droge stof voor koeien die 10.000 kg melk per jaar produceren lijkt de stikstofexcreties te minimaliseren. Verdere verfijning van het rantsoen naar de verschillende lactatiestadia is echter nodig. Ten vierde, lijkt het raadzaam om rekening te houden met de behoefte aan extra darm verteerbaar eiwit ten behoeve van de groei van viscerale organen tijdens de start van de lactatie. Hoewel slechts een indirecte indicator van deze behoefte, geven onderzoekresultaten vooralsnog verschillende inzichten in de duur van de negatieve stikstofbalans. Ten vijfde, er lijkt slechts een beperkte mogelijkheid voor het verbeteren van de efficiëntie van microbiële eiwitsynthese in de pens en van het transport van ureum vanuit bloed naar het pens milieu, als opties om aan verbetering van de stikstofbenutting te werken. Er is slechts een marginale winst te behalen met het defaunatie van de pens (verlaging van de predatie op bacteriën door protozoën) om op die wijze de efficiëntie van stikstof benutting te verbeteren, evenals met het wisselen tussen niet-eiwit-stikstof en eiwit-stikstofbronnen in het rantsoen. Ten slotte, lijkt een gerichte aanvulling met pens bestendige essentiële aminozuren een belangrijke oplossing kan zijn voor het verbeteren van de stikstofbenutting richting voor een rantsoen met een laag aanbod aan darm verteerbaar eiwit en een ruw eiwitgehalte tussen 12 en 14% (mits het geschikt profiel aan essentiële aminozuren bevat). Een niveau van efficiëntie voor stikstofbenutting van ~35% lijkt haalbaar, vergeleken met een ~25% bij een gehalte van 16% ruw eiwit wat ongeveer de huidige gemiddelde Nederlandse praktijk is. Voor deze ontwikkeling / innovatie in de melkveehouderij is het van belang dat een breed profiel aan pens bestendige essentiële aminozuren beschikbaar komt.

## Summary UK

The aim of the current desk study is to provide an overview of various aspects of protein metabolism in dairy cows that do receive little or no attention with protein evaluation in current practice. However, a number of aspects may be relevant, in particular under feeding conditions with a reduced dietary crude protein content and supply of intestinal digestible protein (i.e. metabolizable protein) to the cow in order to limit nitrogen excretion. Firstly, a fluctuating or oscillating supply and temporary deficiencies for a few days seems to have little impact on the cow's performance. However, it is unclear whether there are no negative side-effects regardless of the protein content of the ration, and whether these temporal deficiencies can be sustained in the longer term. Secondly, the choice of glucogenic instead of lipogenic nutrients seems to be interesting in particular to stimulate the fixation of nitrogen in milk protein. But with a low supply of intestinal digestible protein, fat sources can also increase the conversion of amino nutrients to milk protein. The cow appears to have great flexibility in the production of milk protein, fat and lactose from the supply of lipogenic, glucogenic and aminogenic nutrients that could be better used to make maximum use of intestinal digestible protein when the cow has a low supply. Thirdly, the few long-term studies on the effect of a low crude protein content in the ration indicate that this is a very effective way to improve the efficiency of nitrogen

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utilization and to reduce nitrogen excretion and emissions, without major consequences for the energy balance and health status of the cow. A level of 14% to 16% crude protein in the dry matter ration for cows producing 10,000 kg of milk per year seems to minimize nitrogen excretions. However, refinement of the ration to the different stages of lactation is needed. Fourthly, it seems advisable to take into account the need for extra intestinal digestible protein for the growth of visceral organs during the start of lactation. Although only an indirect indicator of this need, research results so far provide different insights into the duration of the negative nitrogen balance. Fifthly, there seems to be only limited potential for improving the efficiency of rumen microbial protein synthesis and of the transport of urea from blood to the rumen environment, as options to improve nitrogen utilization. Only a marginal gain can be made from rumen defaunation (reduction of predation on bacteria by protozoa) to improve the efficiency of nitrogen utilization, as well as from switching between non-protein-nitrogen and protein-nitrogen sources in the ration. Finally, a targeted supplementation with rumen-bypass essential amino acids seems to be an important solution for improving nitrogen utilization towards a ration with a low supply of intestinal digestible protein and a crude protein content between 12 and 14% (provided it contains an appropriate profile of essential amino acids). A level of nitrogen utilization efficiency of ~35% seems achievable, compared to a ~25% at a content of 16% crude protein, which is roughly average current Dutch practice. For this development / innovation in dairy farming, it is important that a broad profile of rumen-bypass essential amino acids becomes available.

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# Table of contents

<b>Foreword</b>	<b>7</b>
<b>Summary</b>	<b>9</b>
<b>1 Introduction</b>	<b>11</b>
1.1 Aim	11
<b>2 Effect of oscillating dietary protein content in dairy cattle</b>	<b>13</b>
2.1 Literature on oscillating dietary protein	13
2.2 Results and discussion on results dairy cattle	15
2.3 Conclusions and recommendations	18
<b>3 Effect of dietary protein and lipogenic/glucogenic diets on production, energy balance and milk yield persistency in dairy cows</b>	<b>19</b>
3.1 Flattening the curve; effect of protein concentration on production, energy balance and persistency	19
3.1.1 Milk production response to dietary protein content in early lactation	20
3.1.2 Milk production response to altering dietary protein in the second half of lactation	21
3.1.3 Effect of altering protein concentration on persistency	22
3.1.4 Effect of different energy sources on milk production, energy balance and persistency	24
3.1.5 Effects of glucogenic or lipogenic diet on milk yield persistency	25
3.1.6 Discussion and ideas for new studies	25
<b>4 Long term effects of low protein diets on dairy cattle performance, health and fertility</b>	<b>29</b>
4.1 Long term studies available from literature	29
4.2 Overall conclusions from long-term studies on low CP nutrition	34
<b>5 Low protein allowance and capacity of visceral organs</b>	<b>35</b>
<b>6 Reduced dietary CP content and rumen function</b>	<b>36</b>
6.1 Modelling studies	36
6.1.1 Rumen function	36
6.1.2 Excreta composition	38
6.2 Improving efficiency of microbial protein synthesis and of N recycling to the rumen	38
6.2.1 N recycling to the rumen	38
6.2.2 Efficiency of microbial N synthesis	39
<b>7 Targeted supplementation of MP allowance with rumen-bypass essential amino-acids</b>	<b>40</b>
<b>8 Conclusions and research recommendations</b>	<b>43</b>
<b>References</b>	<b>45</b>

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# Foreword

This study was conducted within the research programme “Feed4Foodure”: a public-private partnership between the Dutch Ministry of Agriculture, Nature and Food Quality and a consortium of various organizations within the animal feed industry and the animal production chain. Feed4Foodure aims to contribute to sustainable and healthy livestock farming in the Netherlands, simultaneously strengthening our competitive position on the global market.

The present literature study entitled “Protein nutrition of dairy cows - Possibilities to reduce and alter protein nutrition in dairy cows in view of societal demands” was written to investigate consequences of abandoning the safety ranges of protein nutrition in an attempt to increase N use efficiency and N excreted and associated ammonia emission on several aspects.

André Bannink  
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# Summary

The aim of the current desk study is to provide an overview of various aspects of protein metabolism in dairy cows that do receive little or no attention with protein evaluation in current practice. However, a number of aspects may be relevant, in particular under feeding conditions with a reduced dietary crude protein content and supply of intestinal digestible protein (i.e. metabolizable protein) to the cow in order to limit nitrogen excretion. Firstly, a fluctuating or oscillating supply and temporary deficiencies for a few days seems to have little impact on the cow's performance. However, it is unclear whether there are no negative side-effects regardless of the protein content of the ration, and whether these temporal deficiencies can be sustained in the longer term. Secondly, the choice of glucogenic instead of lipogenic nutrients seems to be interesting in particular to stimulate the fixation of nitrogen in milk protein. But with a low supply of intestinal digestible protein, fat sources can also increase the conversion of amino nutrients to milk protein. The cow appears to have great flexibility in the production of milk protein, fat and lactose from the supply of lipogenic, glucogenic and aminogenic nutrients that could be better used to make maximum use of intestinal digestible protein when the cow has a low supply. Thirdly, the few long-term studies on the effect of a low crude protein content in the ration indicate that this is a very effective way to improve the efficiency of nitrogen utilization and to reduce nitrogen excretion and emissions, without major consequences for the energy balance and health status of the cow. A level of 14% to 16% crude protein in the dry matter ration for cows producing 10,000 kg of milk per year seems to minimize nitrogen excretions. However, refinement of the ration to the different stages of lactation is needed. Fourthly, it seems advisable to take into account the need for extra intestinal digestible protein for the growth of visceral organs during the start of lactation. Although only an indirect indicator of this need, research results so far provide different insights into the duration of the negative nitrogen balance. Fifthly, there seems to be only limited potential for improving the efficiency of rumen microbial protein synthesis and of the transport of urea from blood to the rumen environment, as options to improve nitrogen utilization. Only a marginal gain can be made from rumen defaunation (reduction of predation on bacteria by protozoa) to improve the efficiency of nitrogen utilization, as well as from switching between non-protein-nitrogen and protein-nitrogen sources in the ration. Finally, a targeted supplementation with rumen-bypass essential amino acids seems to be an important solution for improving nitrogen utilization towards a ration with a low supply of intestinal digestible protein and a crude protein content between 12 and 14% (provided it contains an appropriate profile of essential amino acids). A level of nitrogen utilization efficiency of ~35% seems achievable, compared to a ~25% at a content of 16% crude protein, which is roughly average current Dutch practice. For this development / innovation in dairy farming, it is important that a broad profile of rumen-bypass essential amino acids becomes available.



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# 1 Introduction

The Dutch government adopts stringent reduction targets for nitrogen (N) emissions of 26% in 2030 en 50% in 2035. N emissions from the livestock sector are mainly in the form of NH<sub>3</sub>, and in the Netherlands dairy cattle farming is the major source of NH<sub>3</sub>. Considering the open nature of cow houses and the confinement of cows for long periods during the year, housing and flooring systems may have limited effect on reducing N emissions. Other strategies to decrease N emissions are related to improved manure management (i.e., dilution with water), a reduction in cow numbers, or increasing efficiency of nitrogen utilisation for milk protein by cows through nutrition. Nitrogen use efficiency at the cow level can be increased by a reduction of nitrogen intake while preventing a similar reduction in milk protein output, or by improving milk protein output while not changing nitrogen intake. Apart from practical hurdles to achieve this on dairy farms, there are limits to possibilities to reduce the dietary crude protein without seriously compromising cow performance or on-farm roughage production which is likely to stay grass-based in the Netherlands. Current protein evaluation systems applied in practice handle protein requirements in a rather generic manner and estimate protein requirements for a dairy cow. They cover the various aspects of cow protein metabolism that may come into play when feeding below these requirements, or when feeding in non-conventional manners, to a limited extent only. A theoretical handling on the maximum milk N utilisation to be achieved is available already (Dijkstra et al., 2013) (see Textbox 1). However, less information is available on the impacts and argumentation to follow when reducing dietary crude protein content or digestible protein intake on cow protein metabolism and utilization. Furthermore, such information is available in a much more scattered way in literature and needs to be integrated.

## 1.1 Aim

The present desk study aims to give an overview of aspects of protein metabolism not receiving much attention in the protein evaluation systems used in dairy nutrition. Nevertheless, these aspects may prove to become more relevant under conditions of reduced metabolizable protein allowance to dairy cows in order to minimize nitrogen losses to the environment and reduce ammonia and nitrous oxide emissions. When abandoning safety ranges in the allowance of digestible protein (DVE) and rumen protein balance (OEB), or when underfeeding cows according to current requirements, cow performance may become more sensitive to various aspects, including: 1) consequences of less constant or oscillating Metabolizable Protein (MP) allowance, 2) effects of long-term feeding low protein diets to dairy cows, 3) effects of MP allowance and glucogenic diets on the persistence of milk yield and udder capacity, 4) effects of a low protein allowance on the protein capacity of visceral organs, 5) effects of low protein diets on feed intake, 6) effects of low protein diets on rumen fermentation capacity and methods to improve efficiency of microbial protein synthesis, and 7) the possibility of targeted supplementation of MP allowance with rumen-bypass essential amino-acids. This review aims to clarify available knowledge on these seven aspects.

### Textbox 1. Maximal theoretical milk N efficiency.

In the Netherlands, currently some 25% of nitrogen (N) consumed by dairy cattle is secreted as milk protein-N. Because N deposition in body (growth; pregnancy) on an annual basis is small, virtually all of the remainder (some 75%) of N is excreted in feces and urine. Dijkstra et al. (2013) identified the maximal theoretical N efficiency at the animal level, and the challenges and opportunities to achieve this maximal milk N efficiency. The theoretical upper limit of N use efficiency in a dairy cow producing 40 kg fat and protein corrected milk/d is 0.43. Lower milk production levels lead to somewhat lower maximal milk N efficiencies, e.g. 0.42 at 25 kg FPCM/d and 0.36 at 10 kg FPCM/d. Inevitable N losses in dairy cattle include losses associated with urinary excretion of urea synthesized from ammonia produced in the rumen; undigested feed or microbial protein excreted in feces; microbial nucleic acids synthesized in the rumen and excreted mainly in urine; fecal and urinary excretion resulting from endogenous secretions; and urinary excretion related to maintenance and milk protein synthesis. Dijkstra et al. (2013) demonstrated there is little or no scope to reduce N losses related to microbial nucleic acid synthesis, recycling of N to the rumen, intestinal digestion of microbial protein, and animal maintenance requirements. To improve milk N efficiency, integration between protein and energy metabolism is essential. A major challenge in dietary strategies to optimize high-fiber diets with human inedible resources for high milk N efficiency will be to avoid increases in enteric methane production associated with dietary strategies.

*Inevitable N losses (g N/day), N output in milk (g N/day) and theoretical maximum milk N efficiency in a dairy cow producing 40 kg fat and protein corrected milk per day and a true protein content of 31.5 g/kg. Adopted from Dijkstra et al. (2013).*

	N in feces	N in urine	N in milk
Inefficiency rumen microbial protein synthesis		35	
Rumen microbial nucleic acids	13	71	
Undigested (microbial) protein	37		
Endogenous secretions	39	19	
Maintenance		13	
Milk		36	198
Total	89	174	198
<i>Maximum milk N efficiency</i>			<i>0.43</i>

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## 2 Effect of oscillating dietary protein content in dairy cattle

An oscillating protein allowance may affect the efficiency at which ruminants can retain nitrogen, by having the protein metabolism adapt to periods of temporal shortage of MP. Furthermore, in case practice seeks to feed close or even slightly below estimated protein requirements in the future to attempt to minimize nitrogenous emissions, there may be many occasions that lead to such oscillations of periods with feeding at requirements intervened by periods with feeding under requirements. For this reason, available evidence on oscillations in protein allowance in dairy cattle was obtained from literature.

### 2.1 Literature on oscillating dietary protein

There have been a number of studies on the effects of feeding oscillating dietary protein content in sheep and beef cattle. The following text from an extensive literature review of Reynolds and Kristensen (2008) is quoted here to precisely indicate their view and interpretation of these protein oscillation studies in sheep and beef cattle. Sentences which convey interesting conclusions and explanations are underlined.

*"In addition to interest in improving rumen synchrony, there also has been considerable research interest in the use of oscillating dietary protein concentration or infrequent protein supplementation as a management tool to reduce labor costs of feeding sheep and cattle but also as a potential dietary strategy to improve N retention and reduce N excretion to the environment (Archibeque et al., 2007b). This dietary strategy represents an example of rumen energy and N asynchrony over periods of days, rather than hours, and emphasizes the importance of N recycling, and perhaps labile N reserves, to the N economy of the ruminant. To our knowledge, the first full report of the use of infrequent protein supplementation for cattle was published by McIlvain and Shoop (1962). Cottonseed meal was fed to steers grazing rangeland in equal weekly amounts on a daily, every third day, or weekly basis, and over the course of 3 winters, no effect on ADG was measured. Similarly, Coleman and Wyatt (1982) reported no effect of supplementation frequency (24-, 48-, or 96-h basis) on N digestion and retention when the same amount of cottonseed meal was provided per 96 h to beef steers. Since these early reports, there have been a number of studies in which dietary protein concentration has been oscillated at intervals ranging from 1 to 2 d or a basal diet has been supplemented with protein at intervals ranging from 1 to 6 d in growing beef cattle (Figure 4; Coleman and Wyatt, 1982; Cole et al., 2003; Farmer et al., 2004; Archibeque et al., 2007c) or lambs (Figure 5; Cole, 1999; Bohnert et al., 2002; Ludden et al., 2002; Currier et al., 2004; Archibeque et al., 2007b). Except when supplementation frequency was extended to more than once every 3 d (Bohnert et al., 2002; Farmer et al., 2004), compared with a daily supplementation control, there were surprisingly few deleterious effects of supplementation frequency, or oscillation, on N utilization and excretion (Figures 5 and 6). Similarly, in a feedlot trial with beef steers fed diets with oscillating protein concentration, there was no effect on ADG or carcass composition, but oscillating protein did increase volatile losses from manure (Archibeque et al., 2007a). It is noteworthy that in 2 cases (e.g., Cole, 1999; Archibeque et al., 2007b), oscillating dietary protein concentration increased N retention compared with feeding a diet providing the same amount of protein on a daily basis as the average protein content of the oscillating diets. In the study by Archibeque et al. (2007b), this increase in N retention was attributable to an increase in the amount of N digested. The lack of a negative effect of oscillating or infrequent protein supplementation on N utilization has been attributed to the recycling of urea to the rumen, which provides N for microbial protein synthesis on days when protein is not supplemented or dietary protein concentration is low (Krehbiel et al., 1998; Archibeque et al., 2007b). This concept is supported by increases in arterial concentration and net PDV removal of urea N in sheep fed oscillating dietary protein (Krehbiel et al., 1998; Archibeque et al., 2007b) and sustained elevations in plasma urea concentrations measured in sheep supplemented with sources of rumen-degradable protein or ruminally undegraded feed CP at 3- or 6-d intervals (Bohnert et al., 2002).*

Increases in plasma urea concentration and urea transfer to the gut after protein supplementation would be enhanced if kidney urea clearance is downregulated during the period without protein supplementation, and there is a lag in the increase in urea excretion in the urine after protein is consumed (Marini et al., 2006). The lack of a negative effect of oscillating or infrequent dietary protein on N balance in ruminants may be due to more than just a sustained elevation of urea and NH<sub>3</sub> pool size and recycling. Deposition of N in nitrogenous compounds absorbed in excess of requirements on days when protein is provided into pools other than urea and NH<sub>3</sub>, which are subsequently catabolized to generate N for urea synthesis, may also buffer the effects of infrequent protein supply. In this regard, Nolan and Leng (1972) reported that in sheep, a large proportion (62%) of the endogenous urea N degraded in the digestive tract and absorbed as NH<sub>3</sub> does not reappear in urea but is incorporated into pools of nitrogenous compounds that turn over more slowly, providing a more continuous supply of N for synthetic processes. These pools have often been referred to as labile proteins or protein reserves (Paquay et al., 1972; Waterlow, 1999). The identification of these labile nitrogenous compounds is not certain (Waterlow, 1999), but it has long been known that in humans, and other nonruminants, a large short-term increase in body N retention is typically measured after an abrupt increase in dietary protein concentration. Over time, measured N balance then decreases as urinary N increases, until a new steady state is achieved (Oddoye and Margen, 1979). Similarly, there is a dramatic decrease in measured body N balance (N loss) when N intake is abruptly reduced in humans (Oddoye and Margen, 1979), until an adaptation of urinary N excretion occurs that, depending on the magnitude of the change in protein intake, can take weeks to occur. The deposition of N in labile pools on days when protein is fed, which are then degraded to produce urea that can be recycled to the gut on days when protein is not fed, would provide a convenient explanation for the lack of a negative effect of oscillating dietary protein supplementation in ruminants. Previous studies have described a pool of N in cattle that is more rapidly degraded after abrupt reductions in dietary protein (Paquay et al., 1972; Biddle et al., 1975). Biddle et al. (1975) reported that plasma proteins and urea were components of the labile N reserves in growing cattle, and their total labile N pool was equal to 5.6% of body N. Paquay et al. (1972) suggested that in addition to plasma proteins, the liver and other viscera, as well as newly synthesized muscle protein, contributed to the more labile pool of N in the dairy cow. In this regard, Ludden et al. (2002) reported that mass of the small intestine and total gastrointestinal tract plus liver was increased in sheep fed oscillating dietary protein. Finally, the microbial population of the rumen and hindgut may also serve as a reservoir for N, especially if urea transfer to the gut lumen is upregulated when protein supply is reduced. The large increases in N balance measured in nonruminants abruptly changed from low- to high-protein diets has been attributed to a lag in the adaptation of Orn cycle enzymes observed in the classic study of Schimke (1962). Although the total capacity of the Orn cycle probably responds much more quickly than the activity of the component enzymes (Waterlow, 1999), this lag in urea cycle activity, and resulting deposition of absorbed AA N in labile reserves, would reduce the amount of excess N consumed subsequently excreted in the urine, providing a source of N for urea recycled to the gut on days when dietary protein is insufficient. In addition, if the capacity of the urea cycle is reduced by days of not feeding protein, absorbed AA may be spared for anabolic uses on days when the protein is fed. Depending on the diet fed and the resulting dietary and MP requirements of the animals, this may explain the positive effects of protein oscillation on N balance reported (Cole, 1999). However, Waterlow (1999) suggested that factors other than labile proteins and the capacity of the Orn cycle must contribute to the constancy of N balance in humans, who often have sporadic dietary protein intake."

Irrespective of the value of this thorough review by Kristensen & Reynolds (2008) it remains questionable whether findings based on sheep and beef cattle can be translated directly to high producing dairy cattle. Lactating cows have a much higher protein requirement and feeding level compared to sheep and beef cattle which poses different challenges to protein metabolism and includes the metabolically highly active mammary gland. More recently, the effect of feeding oscillating dietary protein to lactating dairy cattle has been studied and reported in a few MSc theses (Brown, 2014; Köhler, 2016) and in peer-reviewed papers of Tebbe and Weiss (2020) and Rauch et al. (2021). In the next section the results of these studies will be discussed in more detail and results are listed in Table 2.1



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## 2.2 Results and discussion on results dairy cattle

Table 2.1 summarizes the results of the four protein oscillation studies with lactating dairy cattle (Brown, 2014; Köhler, 2016; Tebbe & Weiss, 2020; Rauch et al., 2021). These studies were carried out with high producing cows (32 – 38 kg milk/d) demonstrating a high DM intake (22 – 27 kg/d).

### Effect of CP oscillation diets (OSC) on DMI and milk yield

Across the 4 studies the effect of OSC on DMI was slightly negative, being on average 1.1% lower than for the static (or control) diet (effects varying from 0.4 to -4.3%). The effect of OSC on milk yield was slightly negative as well, being on average 2.4% lower than the static (or control) diet (effects varying from 0.6 to -5.0%).

### Effect of CP oscillation (OSC) on MUN and ruminal NH<sub>3</sub> concentration

If oscillating dietary protein would result in a “down regulation” of renal urea excretion in urine or an increase in “renal urea recycling”, as an adaptation mechanism to “conserve” nitrogen, this would likely result in increased concentrations of plasma urea-N (PUN), milk urea-N (MUN), and possibly also ruminal NH<sub>3</sub> compared to “static” CP diets. With respect to the study of Tebbe and Weiss (2020) the MUN level of the OSC diet tended ( $P < 0.10$ ) to be higher (with 0.7 mg/dL) than the static 14.1% CP diet, and in Rauch et al. (2021) a significantly greater MUN level (0.4 mg/dL greater) of the OSC diet was reported. However, in the study of Brown (2014) no significant effect of OSC was observed, and likewise no effect was observed in the study of Köhler (2016) although numerically the OSC-48 h diet had a 1.0 mg/dL higher MUN than the static CP diet. This difference was not observed in PUN concentrations however (PUN in static diet and OSC-48 diets were 20.0 and 20.2 mg/dL, resp.). Furthermore, apparent total tract digestibility of CP (TTD-CP; %) of the OSC diets in the study of Rauch et al. (2021), Tebbe and Weiss (2020) and the OSC-48 diet in the study of Köhler (2016) were higher than for the respective static diets, and this may underlie the differences in MUN.

In the study of Köhler (2016) also ruminal NH<sub>3</sub> concentrations were measured, and these were significantly higher for the OSC diets compared to the static diet. The ruminal NH<sub>3</sub>-N concentrations of the static diet was 11.6 mg/dL compared to 12.6 mg/dL for the OSC-24 diet, 13.9 mg/dL for the OSC-48 diet, and 11.5 mg/dL for the OSC-72 diet. The results of Köhler suggest that dietary oscillation of protein increases urea recycling to the rumen. Overall, however, results of all 3 experiments suggest that oscillation of dietary protein does not substantially affect renal recycling of urea as judged from MUN and PUN results.

### Effect of OSC on N-efficiency

The N efficiency, calculated as milk nitrogen output divided by N intake or directly adopted from the paper, was numerically higher for the OSC diet compared to the static diet in the study of Tebbe and Weiss (2020). However, in the studies of Rauch et al. (2021), Brown (2014) and Köhler (2016) the N-efficiencies were similar or, at least numerically, lower than the static diets. Overall, it is therefore concluded from these 4 studies that OSC diets do not increase N-efficiency in high producing dairy cows in the range of a dietary CP content of 13.4 – 16.5 % in DM.

### Effect of OSC at various dietary CP levels and OSC time schedules

The 4 studies varied with respect to the average CP concentration adopted for the OSC treatments, with 14.9% (alternating from 13.4 to 16.5 % CP), 14.1% (alternating from 11.9 to 16.2 % CP), 13.4% (alternating from 10.3 to 16.4 % CP) and 16.5% (alternating from 13.5 to 19.5 % CP) in the studies of Rauch et al. (2021), Tebbe and Weiss (2020), Brown (2014) and Köhler (2016), respectively. Studies varied as well with respect to OSC time periods, varying from 48 h for the studies of Rauch et al. (2021), Tebbe and Weiss (2014) and Brown (2014), to 24 – 72 h in the study of Köhler (2016).

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In the study of Köhler (2016) the high average dietary CP content of 16.5% and the low dietary CP content of 13.5% with the OSC treatment may have resulted in a MP supply during the low protein feeding period that still did not limit milk production. Therefore, care should be taken to conclude from the study of Köhler (2016) that oscillation periods of 72 h can also be applied to OSC schemes with a lower average dietary CP levels. Nevertheless, it appears that the differences observed for the effect of OSC on performance, N-efficiency, TTD-CP and MUN cannot be attributed to differences in average dietary CP level adopted for the OSC treatments or the OSC time period in these studies. Overall, the results suggest that oscillation of dietary protein in alternating cycles of 48 h low CP and 48 h high CP can be applied with no or only a small negative response in DMI and milk yield compared to cows fed a static CP diet with the same average CP level. These findings suggest that dairy cows, in comparison with sheep and steers, respond differently to oscillating dietary CP regimes.

Despite limited overall effects of dietary CP oscillations, transient effects of changes in dietary CP concentration on milk yield and composition were observed in cows fed the OSC diet in the study of Rauch et al. (2021). These transient effects indicate that dietary CP changes were large enough to obtain transient differences, but that compensatory mechanisms were able to mitigate potential negative consequences over 4-d periods (2 d of low CP followed by 2 d of high CP feeding). Based on transient effects observed in our experiment and on other research findings, Rauch et al. (2021) speculated that the use of primarily RUP sources to oscillate dietary CP may hold potential for future research to increase milk N efficiency in lactating dairy cows.

The duration of the trials listed here can be considered as relatively short, varying from 28 – 50 days. It would be worthwhile to study the effect of oscillating protein allowance on performance for a much longer periods, such as a full lactation or even multiple lactations. Also, application of the OSC concept could also be studied while distinguishing between the different stages of lactation, for example between early, mid and late lactation, in order to accommodate for the change in protein requirements throughout lactation while adopting the OSC concept. It would be particularly interesting to study the OSC concept and its consequences for dietary CP contents which approach a limitation in protein allowance according to current protein evaluation systems (e.g. at average CP levels of 13-14% for example), or for the different stages of lactation.

**Table 2.1** Summary of protein oscillation studies carried out in dairy cattle.

	Adapt. period (d)	Measurement Period (d)	DIM (at the start)	N	Feeding system	CP in DM)	(% DMI (kg/d)	Milk yield (kg/d)	Milk protein Yield (kg/d)	MUN (mg/dL)	TTD-CP (%) <sup>1</sup>	N- efficiency (%) <sup>2</sup>
Rauch et al. (2021)	8	20	150	25	Cont.	14.9	25.5	31.5	1.16	5.3	59.4	31.0
				25	Osc. (48 h)	13.4 – 16.5	25.5	31.5	1.16	5.7	62.4	30.8
Tebbe and Weiss (2020)	0	50	119	10	Cont.	16.2	22.9	36.6	1.10	12.8	65.2	29.0
				10	Cont.	14.1	23.2	35.1	1.06	10.2	61.7	31.7
				10	Osc. (24 h)	16.2 – 11.9	22.2	35.3	1.05	10.9	65.3	32.9
Brown (2014)	0	28	117	21	Cont.	16.4	25.3	35.4	1.00	14.5	67.8	23.6
				21	Cont.	13.4	25.1	34.7	0.99	9.1	63.7	28.8
				21	Osc. (48 h)	16.4 – 10.3	24.7	33.8	0.96	9.0	63.2	28.4
Köhler (2016)	18	12	114	4	Cont.	16.5	26.7	37.6	1.22	15.4	73.2	27.1
				4	Osc. (24 h)		26.8	35.7	1.12	15.2	72.7	24.8
				4	Osc. (48 h)	13.5 – 19.5	26.5	36.4	1.15	16.4	74.9	25.8
				4	Osc. (72 h)		26.6	36.1	1.15	16.1	72.7	25.7

<sup>1</sup>TTD-CP = total tract digestibility of CP. For Brown (2014) this was calculated as a N-intake minus N excreted in milk and urine and where N excreted in urine was determined based on spot samples. In the study of Köhler (2016) N excreted in feces was determined by quantitative collection of feces. However, large N-balances were recorded of more than 150 g N per day (varying from 21 – 29% of total N-intake).

<sup>2</sup>N-efficiency is calculated as: milk protein yield (kg/d) / 6.38 / N-intake (g N/d) × 100.

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## 2.3 Conclusions and recommendations

It is concluded that, in contrast to sheep and beef cattle, a 48 h oscillation period with an alternating high and low dietary CP content in high-producing producing cows does not improve N efficiency and may result in small negative responses in DMI and milk yield although TTD\_CP may be improved. These results indicate that cows are capable to cope with 2 day periods of N-deficiency. Lowering dietary CP in dairy cow rations reduces N-losses to the environment but it also reduces the margin of error allowed with respect to preventing negative consequences of suboptimal levels of dietary CP and MP allowance on milk production. The results of the CP oscillation studies show that imbalances in formulated diets resulting in suboptimal dietary CP levels (too high or too low) can be corrected for within a window of opportunity period of 48 h. Nevertheless, it remains to be established whether allowing temporal shortages in CP allowance (increasing N-efficiency and reducing N emissions compared to the situation in which these are repaired by supplemental feeding) can be allowed irrespective of the average CP level of the diet and for how long such intermittent shortages can be allowed without having negative side-effects.

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### 3 Effect of dietary protein and lipogenic/glucogenic diets on production, energy balance and milk yield persistency in dairy cows

*'Looking forward to an era of feeding low CP diets to high-yielding dairy cows it will be necessary to develop nutritional strategies that optimise N capture in the rumen, enhance N digestion and absorption in the lower gut, and improve post-absorption N utilisation and partition towards the mammary gland.'* (Sinclair et al., 2013)

Around parturition and the first weeks of the lactation are a challenging period for lactating dairy cattle. During the transition from the dry period to lactation the changed metabolism of the dairy cow leads to a higher risk for disease and culling. The negative energy balance (and more temporarily negative protein balance) and strong mobilisation of body reserves is considered to be an important factor contributing to the increased risk for disease. With the onset and rapid increase of milk production the demand of energy, and more specifically demand of digestible protein and glucose, rapid increases, whereas the increase in energy intake with feed does not keep pace with these increased metabolic demands. To compensate for this negative energy balance, the cow starts by mobilizing body fat reserves (and although more temporarily protein reserves as well). Extensive body fat mobilization can result in metabolic diseases such as a fatty liver and ketosis. A negative energy balance is considered to be the most important factor in increasing the risk on metabolic disease, which in turn results in a higher susceptibility for other diseases and which is often accompanied with reduced fertility of the cow.

To reduce the risk of development of diseases associated with a strong negative energy balance, two main strategies can be formulated; (1) reducing the magnitude of the negative energy balance in early lactation, or (2) reducing the amount of transition periods.

(1) Two strategies to reduce the depth of the negative energy balance is by reducing the milk production increase in early lactation or increasing the dietary energy uptake. Means of decreasing the depth of the negative energy balance with dietary strategies is evaluated in section 3.1.

(2) Reducing the amount of transition periods can be achieved by prolonging the length of the lactation. One problem with prolonging the lactation length is the accompanied reduction of milk yield on farm level, due to the reduction in milk production during late lactation. Improving milk yield persistency is important to make a prolonged lactation attractive. Dietary influencing the persistency of cows is reviewed in section 3.2.

#### 3.1 Flattening the curve; effect of protein concentration on production, energy balance and persistency

Citing Whelan et al. (2014), there appears not to be much information on the effect of reduced dietary CP on energy balance and persistency of milk output:

*'Restricting milk production through reducing dietary CP supply may be an effective method of improving EB in early-lactation dairy cows. However, these effects are inconsistent in the literature, with some reports showing improved EB (Ørskov et al., 1987) and others showing no effect (Chapa et al., 2001; Law et al., 2009).'*

In this section an overview is given of available literature and suggestions are made for further research to fill the gap in knowledge.

### 3.1.1 Milk production response to dietary protein content in early lactation

The CP concentration in the diet has an effect on milk production during early lactation. Studies examining the effect of different concentrations of CP during early lactation report fairly consistently that a lower level of CP leads to lower milk production. In a study with 58 multiparous Holstein cows, Wu and Satter (2000) looked at the effect of different CP concentrations with the aim of examining the production response of these cows. From day 1 to 112 in lactation they compared cows which were fed three different concentrations of CP (154, 174 and 193 g/kg of DM) in their diet (Table 3.1). Mean daily milk production during this period was significant lower for cows fed the 154 CP diet compared to cows fed the 174 CP diet. Mean daily milk production for cows fed 174 CP diets was 1.3 kg/d lower than for cows fed 193 CP diets, but this difference was not significant ( $P > 0.05$ ). According to Wu and Satter (2000), it is conceivable that around week 7 the body's reserves of protein were depleted. As a result, cows on the low protein diet could no longer increase their milk production, while cows on the high protein diet could therefore achieve a higher peak production.

**Table 3.1** *Effect of dietary protein concentration on milk production from 1 to 112 DIM (Wu and Satter, 2000).*

Item	Dietary CP concentration (g/kg of DM)		
	154	174	193
DMI (kg/d)	21.2	22.3	21.8
Milk yield (kg/d)	36.9 <sup>b</sup>	39.5 <sup>a</sup>	40.8 <sup>a</sup>
3.5% FCM (kg/d)	39.8 <sup>b</sup>	43.2 <sup>a</sup>	44.3 <sup>a</sup>

In another study of Law et al. (2009a) the effect of different protein levels on milk production during early lactation was tested using 45 primiparous and 45 multiparous Holstein-Friesian dairy cows. The study is interesting in that the effects on calculated energy balance and nitrogen efficiency were evaluated. The effect of three dietary CP concentrations (114, 144 and 173 g/kg DM) was tested during the first 150 days of lactation, with the aim to examine the effects on animal production, energy metabolism, blood parameters, and nitrogen efficiency during early lactation (Table 3.2).

**Table 3.2** *Effect of dietary protein concentration from 1 to 150 DIM (Law et al., 2009a).*

Item	Dietary CP concentration (g/kg of DM)		
	114	144	173
DMI (kg/d)	16.5 <sup>b</sup>	18.0 <sup>a</sup>	18.6 <sup>a</sup>
Milk yield (kg/d)	25.4 <sup>c</sup>	31.8 <sup>b</sup>	35.4 <sup>a</sup>
Milk energy output (MJ/d)	80.4 <sup>c</sup>	96.2 <sup>b</sup>	107.2 <sup>a</sup>
ME requirement (MJ/d)	191.3 <sup>c</sup>	222.3 <sup>b</sup>	242.3 <sup>a</sup>
ME intake (MJ/d)	204.2 <sup>b</sup>	222.8 <sup>a</sup>	231.0 <sup>a</sup>
Cumulative energy status (MJ)	414 <sup>a</sup>	-537 <sup>a</sup>	-1,801 <sup>b</sup>
Efficiency on N use	0.423 <sup>a</sup>	0.391 <sup>b</sup>	0.350 <sup>c</sup>

Decreased dietary protein concentration resulted in a significant decrease ( $P < 0.001$ ) of milk yield, milk fat content, total milk fat yield, total milk protein yield, and milk energy output. Cows fed the CP 114 diet had significantly lower DMI than cows fed the CP 144 and 173 diet. Cows fed the CP 114 diet had significantly lower ME intake than cows fed the CP 144 and 173 diet. Combined with the milk energy output, cows fed the CP 114 diet had significantly greater cumulative energy balance than cows fed the CP 173 diet. It was concluded by Law et al. (2009a) that the higher dietary CP content significantly increased average daily ME requirement due to greater milk production. This conclusion simultaneously supports the concept that reducing milk production by lowering the CP concentration in the diet may be an effective method to improve the energy balance during early lactation (albeit at the cost of milk yield).

Furthermore, Law et al. (2009a) evaluated the effects on milk N-efficiency (i.e. milk N : N intake). They established that a reduced CP content significantly ( $P < 0.001$ ) improved N-efficiency for the first 150 DIM, which is in general agreement with studies on the effect of CP and N excretion and ammonia emissions (e.g. experimentally, Van Duinkerken et al, 2005; in theory, Dijkstra et al., 2013) and model simulations performed with the Dutch Tier 3 for enteric methane emission to quantify the effect of N mitigating feeding measures on N excretion (Dijkstra et al., 2018).

### 3.1.2 Milk production response to altering dietary protein in the second half of lactation

Next to early lactation, the effects of increasing CP concentration in the diet during mid lactation on milk production need to be considered. Both the studies of Law et al. (2009a,b) and Wu and Satter (2000) also investigated the effect of altering protein concentration during mid lactation. Law et al. (2009) changed the CP concentration for half of each CP group at 151 DIM to one of the other CP concentrations. This led to the following six experimental groups: 114/114; 114/144; 144/144; 144/173; 173/173 and 173/144 of which the first number is the original CP concentration and the second number is the altered CP concentration in the diet. Table 3.3 summarizes the main results of the cows from 151 to 305 DIM (Law et al., 2009a).

**Table 3.3** Effect of dietary protein concentration from 151 to 305 DIM (Law et al., 2009a).

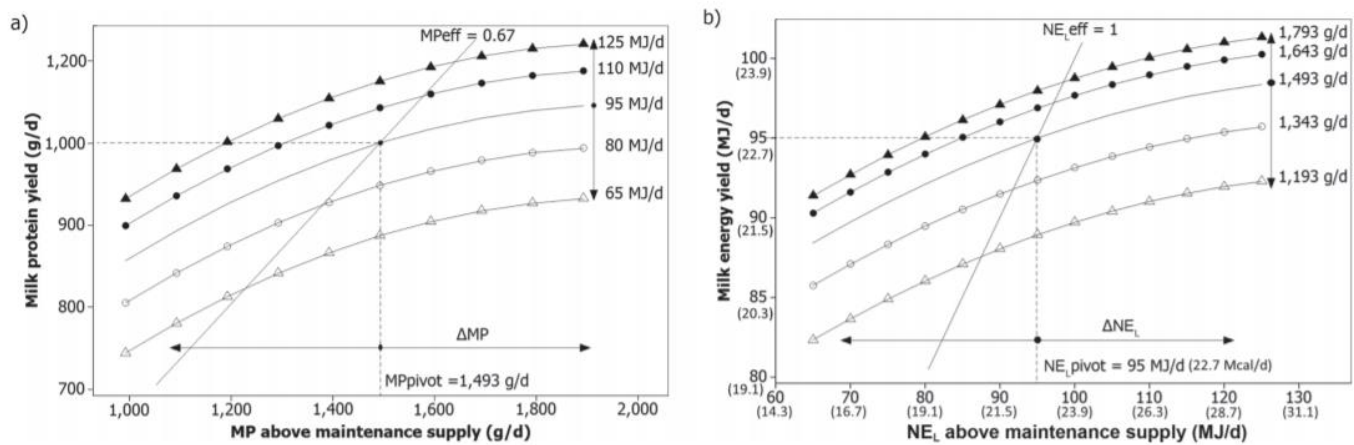
Item	Dietary CP concentration (g/kg of DM)					
	114/ <b>114</b>	114/ <b>144</b>	144/ <b>144</b>	144/ <b>173</b>	173/ <b>144</b>	173/ <b>173</b>
DMI (kg/d)	16.8 <sup>d</sup>	18.0 <sup>bc</sup>	17.8 <sup>c</sup>	19.7 <sup>a</sup>	18.7 <sup>b</sup>	19.3 <sup>ab</sup>
Milk yield (kg/d)	23.0 <sup>c</sup>	26.3 <sup>b</sup>	28.8 <sup>a</sup>	30.7 <sup>a</sup>	29.8 <sup>a</sup>	29.8 <sup>a</sup>
Cumulative energy status (MJ/d)	4,218 <sup>a</sup>	3,202 <sup>b</sup>	987 <sup>cd</sup>	1,790 <sup>c</sup>	27 <sup>d</sup>	716 <sup>d</sup>
Efficiency of N use	0.390 <sup>a</sup>	0.326 <sup>c</sup>	0.368 <sup>b</sup>	0.299 <sup>d</sup>	0.360 <sup>b</sup>	0.300 <sup>d</sup>

Increasing the protein concentration from 114 to 144 g/kg of DM at 151 DIM significantly increased the DMI and the milk yield. Increasing the protein concentration from 144 to 173 g/kg of DM significantly increased the DMI. Milk yield increased 1.9 kg/d if the protein concentration was altered from 144 to 173 g/kg of DM but the difference was not significant ( $P > 0.05$ ).

On the same subject of the effect of dietary CP concentration on cow performance Colnero and Broderick (2006) stated the following:

'... Cunningham et al. (1996) and Leonardi et al. (2003) observed no effect of dietary CP content on DMI and milk yield of dairy cows when dietary CP was increased from 16.5 to 18.5% and from 16.1 to 18.9%, respectively. On the other hand, Broderick (2003) reported a linear increase in DMI when dietary CP was increased from 15.1 to 16.7 and 18.3%; however, milk yield increased from 33.0 to 34.1 kg/d only with the first CP increment, with no further change at 18.3% CP, resulting in lower feed efficiency (milk/DMI) at the highest CP.'

These results and statements appear in general agreement with the findings of Law et al. (2009a) indicated in Table 3.3 and consistent with the conclusions outlined in the review of Ipharraguerre and Clark (2005). More recently, Daniel et al. (2017) performed a meta-analysis on published data and established a similar decline in feed intake and milk yield with decline of MP intake (Figure 3.1). They concluded that milk energy yield and milk component yield responses to dietary change were predicted with good accuracy for various diets and across all stages of lactation. Their results clearly indicate a decline in marginal efficiency of milk protein synthesis, i.e. an increasingly stronger decline in milk yield the more the MP supply above maintenance requirement is reduced.



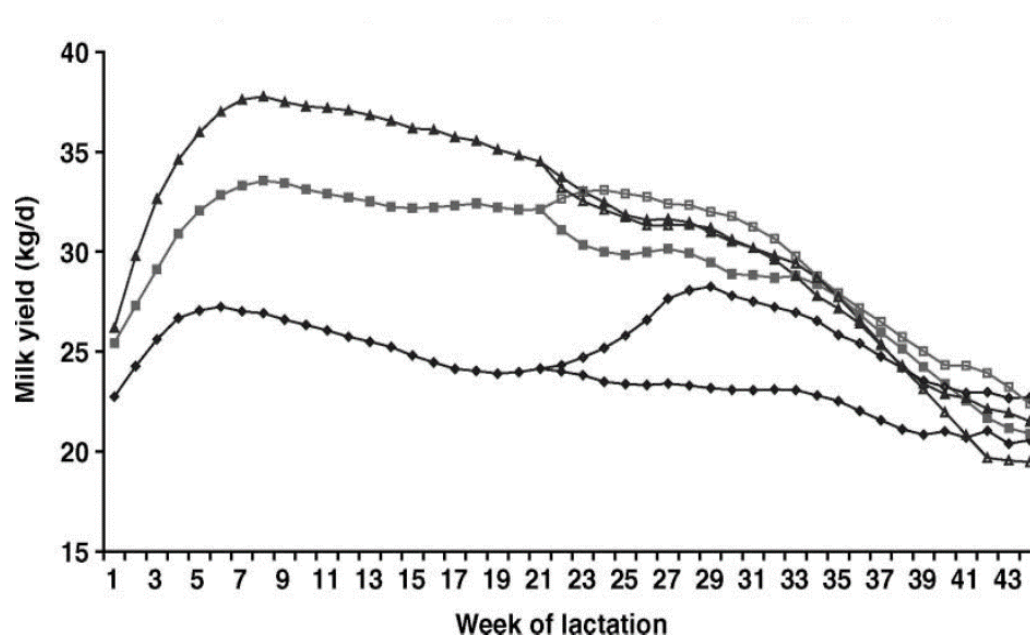
**Figure 3.1** Graphs and legend derived from Daniel et al. (2017). Average milk protein yield (a) and milk energy yield (b) responses to change in MP and NEL supply for a group of animals producing 1,000 g/d of protein and 95 MJ/d (22.71 Mcal/d) of energy at pivots.  $MP_{pivot}$  = level of MP above maintenance supply for which MP efficiency ( $MP_{eff}$ ) is 0.67 and NEL efficiency is 1;  $NEL_{pivot}$  = level of NEL above maintenance supply for which NEL efficiency ( $NEL_{eff}$ ) is 1 and MP efficiency is 0.67. In parentheses, energy values are reported in Mcal/d.

### 3.1.3 Effect of altering protein concentration on persistency

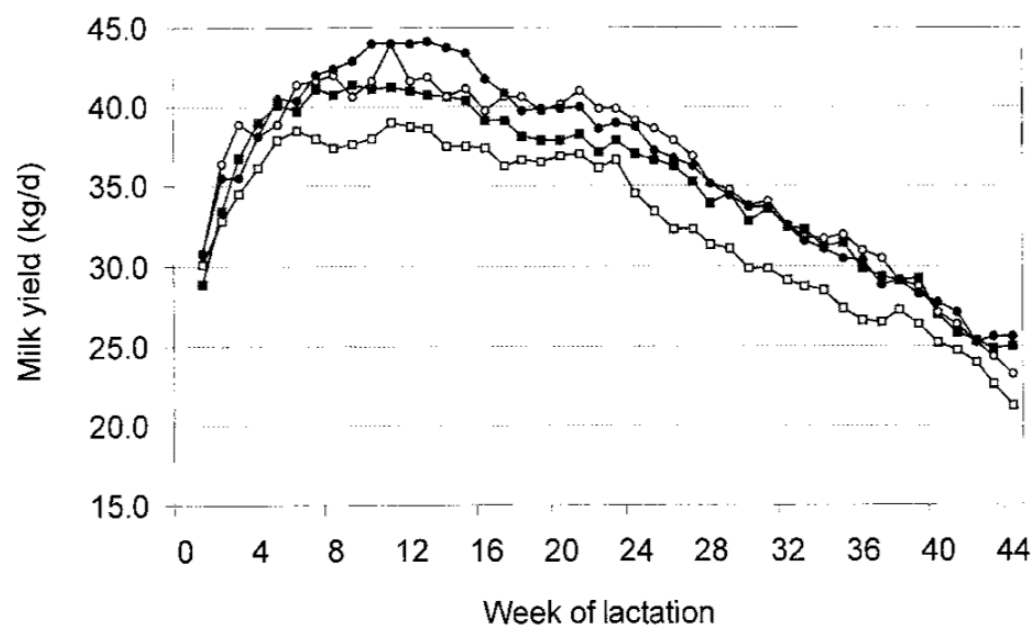
Although both the studies of Law et al. (2009a,b) and Wu and Satter (2000) do not elaborate on milk yield persistency, the presented lactation curves are interesting to compare (Figures 3.2 & 3.3, resp.). Increasing the protein concentration from 144 to 173 g/kg of DM did not significantly increase the milk yield for the period 151 to 305 DIM in the experiment of Law et al. (2009a) as depicted in Figure 3.2. However, the lactation curve of group 144/173 demonstrates to exceed milk yield of the 173/144 and the 173/173 treatment groups after week 23. Because the peak production is lower for 114 compared to 144, and for 144 compared to 173, and the production at 305 d is relatively comparable between all groups, it is conceivable that the persistency of milk production was greater at a lower peak production and lower CP concentration in the diet. On the other hand, it appears that the missed milk yield due to the lower peak production is not compensated by the higher milk yield caused by increasing the CP concentration during the second part of the lactation (from 114 to 144, and from 144 to 173).

Similar patterns in lactation response can be seen when comparing the results of Law et al. (2009a) with the lactation curves for the different protein groups in the experiment of Wu and Satter (2000). Keeping a similar CP concentration of 174 to 179 g CP/kg DM seems to result in a lactation curve that slightly exceeds the curve of the 193 to 179 CP group (Figure 3.2). Which makes it conceivable that cows with a lower peak production may demonstrate a better persistency.





**Figure 3.2** Effect of dietary protein concentration on milk production (from 1 to 150, and from 151 to 305 DIM). After 150 DIM the CP content was either maintained or switched to a new level (figure derived from Law et al., 2009a).



**Figure 3.3** Lactation curves of cows fed diets containing different concentrations of CP: 154/160 (open square), 174/160 (closed square), 174/179 (open circle), 193/179 (closed circle) (legend: Wk 1 to 16/wk 17 to 44). After 17 weeks in lactation a switch towards the same or a new mid-lactation diet was made (figure derived from Wu and Satter, 2000).

### 3.1.4 Effect of different energy sources on milk production, energy balance and persistency

A nutritional strategy to reduce the severity and incidence of metabolic disorders in early lactation is to help the cow maintain a positive EB in early lactation by decreasing the caloric demand of milk production. Decreasing the lipogenic-to-glucogenic nutrient ratio has been suggested to decrease the milk fat content and thereby improve the EB.

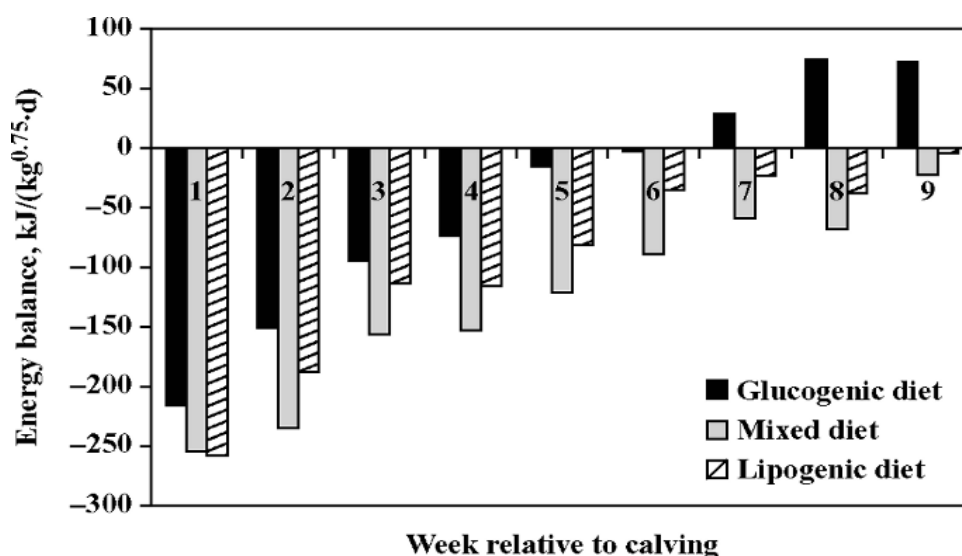
In ruminants, lipogenic nutrients originate mainly from fiber, soluble carbohydrates or dietary fat, or are derived from body reserves. Glucogenic nutrients originate mainly from starch that escapes rumen degradation or from gluconeogenesis, principally from propionic acid. Diets high in ruminal degraded starch and low in fiber typically decrease the acetate-to-propionate ratio (Bannink et al., 2006). Propionate is the major precursor for gluconeogenesis, whereas acetate is a main precursor for lipogenesis. The depression in milk fat upon feeding glucogenic diets has been explained by a shift from a high availability of fat to glucose synthesis precursors.

In a study with 79 multiparous Holstein-Friesian dairy cows Van Kneysel et al. (2007a) aimed to evaluate the effects of a mainly glucogenic or lipogenic diet and calculate the energy balance (EBc) in high-producing dairy cows in early lactation. Other objectives of the study were to evaluate plasma metabolites and metabolic hormones plasma metabolites and metabolic hormones, liver TAG content, and reproductive variables. An intermediate diet was included in the study to outline the relationship between availability of different glucogenic to lipogenic nutrients and energy metabolism. Some of the results are presented in Table 3.4. Milk yield and milk production presented as FPCM did not differ significantly between the three treatment groups. The milk fat yield did not differ between the mixed diet animals and the other two treatments, but there was a significant difference between the glucogenic and the lipogenic diet animals.

**Table 3.4** Milk production, composition and energy balance from 1 to 63 DIM in multiparous cattle (Van Kneysel et al., 2007a)

Item	Dietary treatment		
	Glucogenic	Mixed	Lipogenic
DMI (kg/d)	23.8	23.6	24.2
Milk yield (kg/d)	43.4	44.0	43.2
FPCM (kg/d)	41.5	43.8	44.1
Fat (kg/d)	1.59 <sup>a</sup>	1.76 <sup>ab</sup>	1.83 <sup>b</sup>
Energy balance (kJ/kg <sup>0.75</sup> *d)	-33 <sup>a</sup>	-125 <sup>b</sup>	-89 <sup>ab</sup>

DMI and energy intake did not differ between the different treatment groups. The calculated energy balance was less negative for glucogenic diet animals, although the difference between the glucogenic and the lipogenic group was not found to be statistically different. An overview of the calculated negative energy balance per week is presented in Figure 3.4.



**Figure 3.4** *Calculated energy balance for multiparous cows with different diets for the first 9 wk of lactation (figure derived from Van Kneegsel et al., 2007a).*

Results of Van Kneegsel et al. (2007a) show that dietary energy source affects the calculated energy balance. A glucogenic diet reduces the partitioning of energy to milk fat and decreases body fat mobilization compared with a lipogenic diet. This is in accordance with an earlier study performed by the same research group (Van Kneegsel et al., 2007b). In that study cows that were fed a glucogenic diet had a decreased milk fat production and a decrease in milk energy output which resulted in a tendency to improved energy balance.

### 3.1.5 Effects of glucogenic or lipogenic diet on milk yield persistency

In the research of Van Kneegsel et al. (2007a,b) cows were followed until week 9 in lactation, and therefore it is not possible to say anything about the effect of glucogenic diets on persistency solely based on these results. A comparable study however which did focus on persistency was the more recent research conducted by Van Hoeij et al. (2017). Van Hoeij et al. (2017) aimed to evaluate the effect of dietary energy source (glucogenic or lipogenic) and energy level (standard or low) on milk production, energy balance and lactation curve characteristics between 1 and 308 DIM in dairy cows. The results of the source of energy on milk yield, milk production and energy balance are presented in Table 3.5.

**Table 3.5** *Effect of energy source on production and energy balance for cows from 1 to 308 DIM (Van Hoeij et al., 2014).*

	Dietary treatment		P
	Glucogenic	Lipogenic	
DMI (kg/d)	21.7	20.4	<0.01
Milk yield (kg/d)	27.7	25.1	0.02
FPCM (kg/d)	29.8	28.1	0.13
Fat %	3.86	3.96	0.01
Fat (kg/d)	1.23	1.19	0.44
Energy balance (kJ/kg <sup>0.75</sup> *d)	50	48	0.89

Van Hoeij et al. (2017) compared different methods to determine persistency characteristics as a response; the initial FPCM yield, the decreasing slope of the lactation curve, lactation persistency according to Woods curve, and the relative rate of decline of FPCM yield midway between peak lactation and 305 DIM. None of these parameters turned out to be significantly different for dietary treatment or for dietary energy level, however.

### 3.1.6 Discussion and ideas for new studies

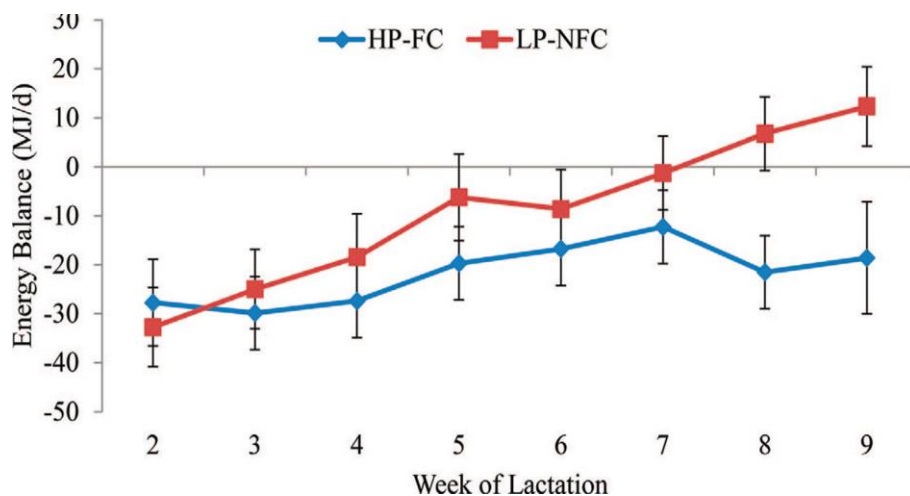
The dietary strategies discussed in previous sections aimed to reduce the peak of milk yield or milk energy output with the aim to reduce the negative energy balance during early lactation. These studies give enough leads for new ideas for experimental work and testing of dietary concepts.

Combining glucogenic diets with different dietary CP concentrations has been tested by Whelan et al. (2014) to evaluate the effects of offering diets of different CP concentration and NFC (nonfiber concentrates) content on milk production, EB and the metabolic profile in the dairy cows during early lactation. In a study with twenty Holstein-Friesian multiparous dairy cows they assigned cows to one of two isoenergetic diets; one with a high content of CP and a low content of nonfiber carbohydrates (NFC), and the other with a low CP content and a high NFC content. The results are presented in Table 3.6.

**Table 3.6** Effect of dietary treatment on production and energy balance of 1 to 63 DIM (Whelan et al., 2014).

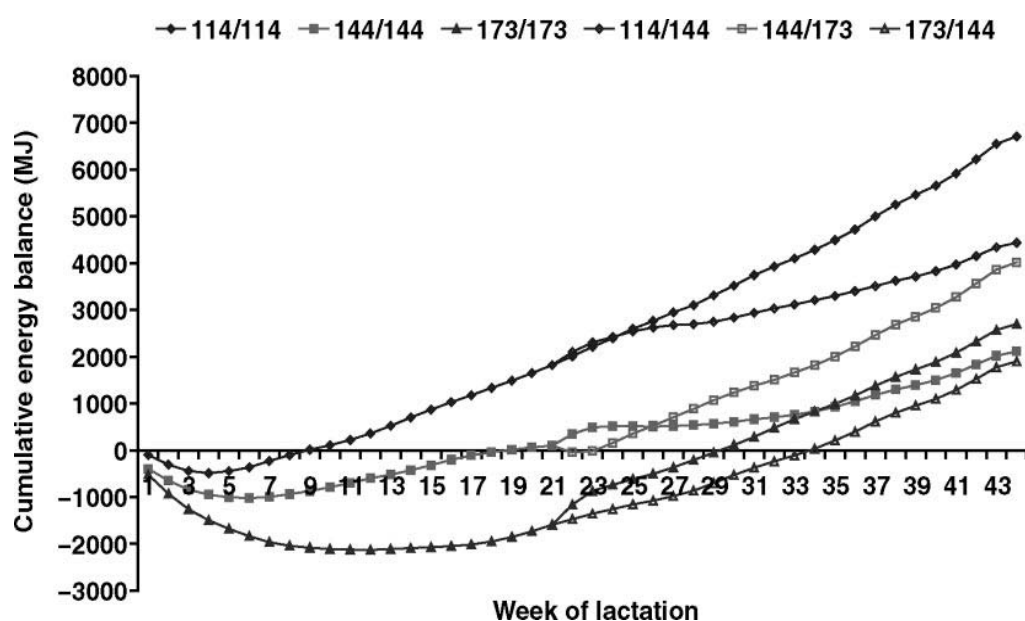
Item	Dietary treatment		P
	High CP-Low	Low CP-High	
	NFC	NFC	
DMI (kg/d)	17.0	17.6	0.35
Milk yield (kg/d)	31.3	29.6	0.01
Milk fat (kg/d)	1.29	1.19	0.08
Milk protein (kg/d)	0.92	0.85	<0.01
Energy balance (MJ/d)	-21.8	-9.6	0.01

The lower CP content was accompanied with a 1.7 kg/d lower mean milk yield. Also the milk fat output tended to be lower, and milk protein output was lower in animals offered the low CP and high NFC content which probably can be related to the differences in the energy balance between both groups. Animals on the low CP and high NFC treatment had a less pronounced negative energy balance compared to animals on the high CP and low NFC treatment. Although the numerical differences in EB remained small, the trend in the EB curve showed promising results (Figure 3.5). Larsen et al. (2017) infused casein for 29 d postpartum. DMI was not affected by infusion. As expected, the greater supply of MP from casein infusion compared with control increased milk, milk fat, and milk protein yield. In this experiment, energy balance was not reported; however BW change in those 29 d pp did not differ between treatments. Schei et al. (2005) fed cows in the first 14 wk of lactation three different diets: standard MP and NEL (CON), low MP and low NEL (LPLE), and high MP and low NEL (HPLE). Energy corrected milk did not differ between CON and HPLE, but LPLE had lower energy corrected milk production than CON and HPLE. Schei et al. (2005) did not measure or calculate energy balance; however, greater levels of nonesterified fatty acids and acetoacetate in blood and acetone in milk, and lower glucose levels in blood in HPLE compared with CON indicated greater body fat mobilization, although ketosis incidence did not differ. Amanlou et al. (2017) increased rumen undegraded protein (RUP) supply to early lactating cattle (first 3 wk after calving) to achieve diets with 16.0% (CP16), 18.7% (CP19) and 21.4% CP (CP21) (DM basis). DMI increased from CP16 to CP19, but not from CP19 to CP21. Energy corrected milk tended to increase from 16CP to 19CP, but increased only numerically from 19CP to 21CP. In this experiment, calculated energy balance (-7.1 to -8.7 Mcal/d), blood triglycerides and blood glucose did not differ between treatments. In contrast to previous experiments, this experiment with increased RUP supply improved milk production without negative effect on parameters related to NEB. This improved performance by increased RUP supply coincided with a marked decrease in milk N efficiency though (from 43% to 37% at 16CP and 21CP, respectively). All in all, it is therefore an appealing idea to think about a similar, but more comprehensive, study to further investigate the effects of combining different dietary contents of CP and type of CP and glucogenic precursors during early lactation and its effect on milk yield, energy balance and persistency of milk production.



**Figure 3.5** Effect of dietary treatment on energy balance (MJ/d) over the first 9 wk of lactation. The error bars represent the SEM for each week's LSM (figure derived from Whelan et al., 2014).

Further, altering the protein concentration during lactation can improve milk yield, but the effect seems to decrease if the CP concentration was higher during early lactation. Besides, it is important to take into consideration that dry matter intake (DMI) may respond positively to increased CP concentration (Newbold, 1994). And it is important to take into account that a part of an increase in milk yield due to increased CP concentrations can be attributed to the increased voluntary feed intake. In the research of Law et al. (2009a) this probably attributed to the positive EB for cows with an increased dietary CP concentration at day 151 in lactation (Figure 3.6). Although increasing the CP concentration in mid lactation can increase the milk production and improved the energy balance, in most cases it also leads to a decrease in nitrogen use efficiency (Wu and Satter, 2000; Law et al., 2009a) and therefore, increasing CP concentration may not contribute to reduction of N emission to the environment. Improving the adjustment of CP concentration in the diet can contribute to cow welfare and sustainability by improving the energy balance. More research on altering glucogenic diets and CP concentrations during lactation and the effect on persistency could contribute to improving the sustainability and lifespan of cows.



**Figure 3.6** Effect of maintaining or changing the dietary CP concentration on the cumulative energy balance (from 1 to 150, and from 151 to 305 DIM). After 150 DIM the CP content was either maintained or switch to a new level (figure derived from Law et al., 2009a).

Glucogenic and lipogenic nutrients may be of particular interest in supplying energy to capture N in microbial protein and in milk protein. Glucogenic nutrients may have more potential than lipogenic nutrients in this respect. For example, Cantalapiedra-Hijar et al. (2014) fed diets with 2 levels of CP (12.0 and 16.5% of DM) high in starch or high in fibre (but iso-energetic on NEL basis). Decreasing CP resulted in decreased energy corrected milk production, but increased milk N efficiency. However, compared with fibre, the starch diets improved milk N efficiency; and in contrast with fibre, starch could partially compensate for negative effects of lowering dietary CP on milk yield. Recently, Nichols et al. (2018, 2019) studied the effect of fat or glucose supplementation when feeding marginal MP levels (but not a marginal VEM level). General conclusions drawn from their work were:

- postruminal fat increased the transfer of AA into milk protein at low MP levels, and it also may improve this transfer at high MP levels
- lipogenic energy from saturated fatty acids improved N efficiency (milk N:N intake) when extra MP came from rumen-bypass soybean meal and rapeseed meal
- lipogenic energy from monounsaturated fatty acids did not affect N efficiency when extra MP came from essential AA in a casein profile
- increased circulating fatty acids did not affect mammary gland AA metabolism, in contrast to increased circulating glucose

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- postruminal fat stimulated the same level of lactose yield as postruminal protein or glucose.
  - regardless of supplemental energy source, mammary glucose uptake was not the sole regulator of milk lactose output
  - best use needs to be made of knowledge on how postabsorptive efficiencies of particular AA may change with physiological state of the cow
  - the consequence of stage of lactation and the production potential of the cow or udder (as dependent on dietary strategies and their effect on hormonal state and milk yield persistency) needs to be accounted for, as well as perhaps health status of the cow.

According to Nichols (2019) these results indicate that dairy cows display an impressive flexibility to produce milk protein, fat, and lactose from lipogenic, glucogenic, and aminogenic dietary ingredients, which at least provides opportunities to experiment how to make best use of MP with reduced dietary CP contents.

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## 4 Long term effects of low protein diets on dairy cattle performance, health and fertility

Contrary to the rather general setup of feeding trials not lasting longer than a few months (e.g. Broderick 2003, Cabrita et al., 2011; Colmenero and Broderick, 2006; Davidson et al., 2003) only few studies have been carried out to establish the long term effects (of a duration of at least one lactation) of low CP diets on cow performance (Wu and Satter, 2000; Law et al, 2009a; Law et al., 2009b; Defra project AC0122). Short term studies are performed to derive consequences of feeding measures on the relatively short term, such as the interventions discussed in previous sections but not for the long term consequences on metabolism and cow performance. Results from short term experiments may not simply be extrapolated, however, to entire lactation periods due to confounding effects such as mobilisation of body energy during early lactation and retention of energy in body reserves during mid and late lactation. For example, in the study of Garnsworthy and Jones (1987) it was observed that cows with a high BCS of around 3.5 and receiving a diet with a high proportion of rumen bypass protein had a substantially higher energy mobilisation of body energy reserves compared to cows with the same BCS receiving a diet with a low proportion of rumen bypass protein. The possibility that short term studies might yield results that may not be valid for the long term is also pointed out by Chris Reynolds in a presentation within the EU project "Smartcow" (<http://www.smartcow.eu/wp-content/uploads/2019/04/Chris-Reynolds-Protein-Efficiency-lecture-Dumfries-March-2019.pdf>) (also reviewed by Hristov et al., 2019) in which he showed that differences in plasma histidine concentrations as a result of increased rumen protected histidine supplementation were higher with increased duration of the experiment (70 d) as compared to a short duration (28 d) of the experiment. These changes in histidine levels in plasma suggests that labile protein pools exist suggesting that results from short term experiments may not be valid for the long term. Although it is not exactly known whether and how such effects occur for MP, as discussed already for oscillating dietary CP levels, it is likely that short-term and long-term trials may lead to different outcomes and have different consequences for the cow's metabolism and performance.

### 4.1 Long term studies available from literature

#### **Study of Law et al (2009a; 2009b)**

In the study of Law et al (2009a,b) 90 Holstein cows were divided in 6 treatment groups of various CP diets and followed during an entire lactation period (0 – 305 days). The first 3 treatments consisted of 114 (114-114), 144 (144-144) and 173 (173-173) g CP/kg DM for the entire lactation period. For the other 3 treatment at lactation day 151 the dietary CP content was changed from 114 to 144 g/kg DM (114-144) from 144 to 173 g/kg DM (144-173) and from 173 to 144 g/kg DM (173-144) until the end of the lactation period at day 305).

In Table 4.1 some of the results from this study is given which clearly show that the lower the dietary CP content the higher the N-utilisation but also the lower the milk production. Compared to treatment 114-114 the optimal treatment with the largest increase in milk production and the lowest increase in N-loss in manure per kg milk is treatment 144-144. Law et al. (2009b) furthermore found a higher incidence rate of metritis at the lowest protein diet of 114 g/kg DM compared to the other two diets. No effects of protein content on fertility parameters such as onset of luteal activity, pregnancy to first service, services per conception, 100 d in-calf rate, and calving interval were observed.

**Table 4.1** Results from a complete lactation period from the study of Law et al. (2009a).

Treatment	DMI (kg/d)	Milk (kg/d)	Milk (day 1 - 305) (kg)	Feed efficiency (kg milk/kg DMI)	N-utilization (%) Calculated	N-loss in manure (kg/cow/lactation) Calculated	N-loss (g) per kg milk Calculated	%increase relative to treatment (114-114)	
								In milk production	N-loss in manure per kg of milk
114-114	16.8	23.0	7015	1.37	40.6	55	7.8	-	-
144-144	17.8	29.8	9089	1.67	37.9	78	8.6	30	9
173-173	18.6	33.0	10065	1.77	32.5	108	10.7	43	37
114-144	17.5	26.9	8205	1.54	37.4	69	8.4	17	7
144-173	18.9	30.0	9150	1.59	34.4	97	10.6	30	35
173-144	18.9	30.7	9364	1.62	35.5	93	9.9	33	27

Interestingly, the calculated cumulative energy balance during the first 150 days of lactation was strongly negative for highest protein diet of 173 g/kg DM whereas a positive cumulative energy balance for the lowest protein diet of 114 g/kg DM was found (*Note: it is unclear how cumulative energy balance was obtained and it seems in conflict with figures in the graphs*). However, there were no significant changes in BCS between the highest and lowest protein diet which may indicate that the metabolizable energy content of feedstuffs is not fixed but dependent amongst others on the dietary protein content (possibly a reduced rumen digestion on the low protein diet due to a shortage of rumen degradable protein), or that the assumptions used for the calculation of metabolizable energy requirements for maintenance and milk production were incorrect. From a visual inspection of the graphs in which the effect of dietary protein content on milk yield and DMI was shown during lactation it was observed that a reduction in protein content from 173 to 144 g/kg DM at day 150 (week 21) of lactation did not result in a reduced milk production in the weeks following the change except during the last 6 weeks of lactation (weeks 38 – 44) compared to the group of cows that received the 173 g/kg DM protein diet during the whole lactation. However, DMI was reduced immediately after the protein content was reduced from 173 to 144 g/kg DM at day 150 of lactation. Furthermore, an increase in protein content from 114 to 144 and from 144 to 173 at day 150 of lactation resulted in an increased milk production and an increased DMI. The fact that a decrease in dietary protein content from 173 to 144 g/kg DM at day 150 (week 21) of lactation did not result in a reduced milk yield but did result in a reduced DMI during lactation weeks 21 – 38 followed by a reduced milk yield during lactation weeks 39 – 44 suggests that labile protein sources in the animal may have been used during lactation weeks 21 – 38, that these labile protein sources were depleted in week 39 resulting in a decrease in milk production during the remaining lactation period from week 39 to 44. Another interesting observation from the study of Law et al. (2009a) is the fact that the difference in daily milk yield between the 144-144 and 173-173 treatment was 3.6 kg for the period 1 – 150 days in milk but was only 1.0 kg for the period 151 – 305 days in milk. This is in contrast with the difference in daily milk yield between the 114-144 and 144-144 treatment which was 6.4 kg for the period 1 – 150 days in milk and this difference remained high (5.8 kg) for the subsequent period 151 – 305 days in milk. This suggests that the dietary protein of 114 g/kg DM clearly reduced milk production during both the first half and second half of the lactation period, whereas the dietary protein content of 144 g/kg DM clearly reduced milk production during the first half of the lactation period but not during the second half.

From the long term study of Law et al. (2009a & 2009b) it is concluded that:

1. Decreasing dietary CP content reduced milk production and DMI but increases efficiency of N-utilization for milk protein synthesis. The impact on performance depended on lactation stage. High protein diets improved DMI and milk production in early-mid lactation (up to 150 days in milk), but after that a medium protein diet is sufficient.
2. Compared to 114-114 g CP/kg DM as minimum CP allowance, the 144-144 g CP/kg DM treatment seems to deliver most increase in milk yield per unit increase in N-losses.
3. Fertility remained unaffected by dietary protein content.
4. Despite differences in dietary CP and changes in dietary CP from high to low or from low to high at week 21 of lactation, cows were able to regulate dietary energy intake and milk production in such a way that energy balance, as judged from changes in BCS, remained unaffected.



5. A change in dietary CP content from high (173 g/kg DM) to low (144 g/kg DM) in the midst of lactation (at week 21 of lactation) may have resulted in an adaptation period in which the cow utilized labile protein sources for milk production followed by a period of reduced milk production after these labile protein sources became depleted.
6. Incidence of metritis in dairy cattle may have been increased at the low CP diet with 114 g CP/kg DM compared to 144 g/kg DM and higher.

### Study of Wu and Satter (2000)

In the study of Wu and Satter et al (2000) 58 Holstein bST treated cows were divided in four treatment groups consisting of various dietary CP levels and were followed during an entire lactation period (0 – 308 days). The four treatments were as follows (the first number is the dietary CP content (g/kg DM) during the first 16 weeks of lactation and the second number is the protein content for week 17 – 44 of lactation): 154-160, 174-160, 174-179 and 193-179. In Table 4.2 some of the results from this study are given.

**Table 4.2** Results from a complete lactation period from the study of Wu and Satter (2000).

Treatment	DMI (kg/d)	Milk (kg/d)	Milk (day 1 - 308) (kg)	Feed efficiency (kg milk/kg DMI)	N-utilization (%)	N-loss in manure (kg/cow/lactation)	N-loss (g) per kg milk	%-increase relative to treatment (154-160) In milk production	N-loss in manure per kg of milk
154-160	23.0	32.6	10056	1.42	28.8	127	12.6	-	-
174-160	23.5	35.3	10832	1.50	25.9	140	12.9	8	2
174-179	24.3	35.9	11095	1.48	24.1	162	14.6	10	16
193-179	23.6	36.2	11132	1.53	24.7	161	14.5	11	15

Results from Table 4.2 shows that the lower the dietary CP content the higher the N-utilisation but also the lower the milk production. Compared to treatment 154-160, the treatment with most increase in milk production per unit increase in N-loss in manure per kg milk (compared to 154-160) is treatment 174-160. No effects of protein content on fertility parameters such as services per conception, days open, days to first AI and days to first oestrus were observed. That dietary protein content was not related to fertility is in agreement with the findings of Law et al. (2009b). There were no substantial differences in changes in BW between the dietary treatments in the first 16 weeks, although numerically BW change was negative for treatment 154-160 and positive for all other treatments, and in the period 17 – 44 weeks. Compared to results from the study of Law et al. 2009a) (excluding the lowest dietary CP treatment), milk production levels were 17% higher. However, DMI was 29% higher, kg milk per kg DMI was 9% lower, and N-loss in manure was 64% higher (or 40% higher when expressed per kg milk). This may simply be the result of the relative higher CP content of the treatments tested by Wu & Satter (2000) compared to Law et al. (2009a, 2009b), delivering more MP. It may also be related to the different diet types tested although a direct comparison remains difficult. In the study of Wu and Satter (2000) the TMR consisted of 33% alfalfa silage, 22% corn silage and 45% concentrate whereas in the study of Law et al. (2009a) this was 27% grass silage, 18% corn silage and 55% concentrate. The CVB Feed Table records total tract organic matter digestibility values based on digestibility studies with castrated sheep of alfalfa silage of 65%. In contrast, total tract organic matter digestibility values for grass silage 78% for organic matter. Furthermore, the rumen undegradable CP fraction of alfalfa silage in the CVB matrix is almost twice as high as the value for grass silage. The higher total tract organic matter digestibility of grass silage compared to alfalfa silage and in general of concentrates compared to roughages can at least in part explain the lower milk production per kg of milk produced. Furthermore, the higher rumen undegradable CP fraction of alfalfa silage compared to grass silage might also at least in part explain the 40% higher N-loss in manure when expressed per kg of milk.

From the long term study of Wu and Satter (2000) it is concluded that:

1. Decreasing dietary CP content reduced milk production and DMI but increased efficiency of N-utilization for milk protein synthesis. The impact on performance depended on lactation stage. The protein content of 174 g/kg DM resulted in maximal performance in the first 30 wks; after 30 wks in lactation, protein could be reduced to 160 g/kg DM without negative impact on performance.
2. Compared to the treatment of 154 going to 160 g CP/kg DM as minimum CP allowance, the 174 going to 160 g CP/kg DM treatment appears to result most increase in milk yield per unit of increase in N-losses.
3. Fertility remained unaffected by dietary CP content.

#### Study of Tolkamp et al. (1998)

In the study of Tolkamp et al. (1998) 38 Holstein cows were divided in 3 treatment groups consisting of a low CP diet (LP; 131 g CP per kg DM; 10 animals), a high protein diet (HP; 185 g CP per kg DM; 12 animals) and a choice diet in which cows were able to select both from the LP and the HP diet (C; 16 animals). Animals were followed during the first 22 weeks of lactation. It appeared for the choice diet that cows had a preference for HP feed by selecting HP over LP feed, its consumption being 68.7% of total feed intake and resulting in a CP content of the choice diet of 170 g/kg DM. In Table 4.3 some of the results of this study are given in detail.

**Table 4.3** Results from the first 22 weeks of lactation from the study of Tolkamp et al. (1998).

Treatment (CP content: g/kg DM)	DMI (kg/d)	Milk (kg/d)	Feed efficiency (kg milk/kg DMI)	N- utilization (%)  Calculated	N-loss in manure (kg/cow/ lactation) Calculated	N-loss (g) per kg milk	%increase relative to treatment (135)	
							In milk production	N-loss in manure per kg of milk
131	20.8	28.3	1.36	36.1	110	10.7	-	-
170	22.7	36.5	1.61	29.3	160	12.0	29	12
185	23.4	36.6	1.56	21.8	189	14.1	29	32

The results show that the lower the dietary CP content the higher the efficiency of N-utilisation. Furthermore, a reduction of dietary CP content from 185 to 170 g/kg DM did not result in reduced milk production whereas lowering the dietary CP content from 170 to 131 g/kg DM substantially reduced milk production. There were no substantial differences in changes in BW between the three treatments. It is noted that compared to the HP diet the choice diet resulted in a similar milk production with a lower DMI resulting in more milk per kg dry matter intake. Tolkamp et al. (1998) explained this outcome from cows on the choice diet being able to select for the HP diet on the basis of the rumen degradable protein content in the feed as other nutrient differences between the LP and HP diets were small except for CP, and from the fact that for the LP diet the provision of MP for milk production was insufficient.

From the study of Tolkamp et al. (1998) it is concluded that:

1. Decreasing dietary CP from 170 to 131 g/kg DM, but not from 185 to 170 g/kg DM, reduced milk production and DMI; decreasing dietary CP always increased efficiency of N-utilization.
2. Cows, when given a choice, were able to select for a high CP diet of 170 g/kg DM.

In a recent experiment at University of Reading 215 heifers enrolled at calving and were fed one of 3 diets with protein contents of either 140, 160 or 180 g/kg DM for a period of 3 lactations. Diets consisted of 51% roughage (15% grass silage, 35% maize silage, 1.5% barley straw) and 49% concentrate feedstuffs. In Table 4.4 some of the results from this study are given.

**Table 4.4** Results from 3 complete lactations from the study Defra AC0122. Different superscript letters indicate significant differences (Reynolds et al., 2021; Crompton et al., 2021).

[https://www.smartcow.eu/wp-content/uploads/2021/11/EAAP21\\_Session72\\_Reynolds\\_Poster.pdf](https://www.smartcow.eu/wp-content/uploads/2021/11/EAAP21_Session72_Reynolds_Poster.pdf);

<https://www.landbruksinfo.dk/->

[/media/landbruksinfo/public/8/4/8/fd19\\_chris\\_reynolds\\_protein\\_efficiency.pdf](/media/landbruksinfo/public/8/4/8/fd19_chris_reynolds_protein_efficiency.pdf)

Lactation	Treatment (CP content: g/kg DM)	DMI (kg/d)	Milk (kg/d)	Feed efficiency (kg milk/kg DMI)	N-utilization (%)	N-loss in manure (kg/cow/lactation) Calculated	N-loss (g) per kg milk Calculated	% -increase relative to treatment 140 CP	
								In milk production	N-loss in manure per kg of milk
1	140	21.3	28.1	1.32	31.5	122	11.8	-	-
	160	21.3	29.6	1.39	29.5	143	13.2	5	12
	180	22.0	29.7	1.35	25.5	175	16.1	6	36
2	140	24.8	32.1	1.29	30.7	143	12.2	-	-
	160	25.5	34.5	1.35	28.4	172	13.7	7	12
	180	26.2	34.3	1.31	24.1	211	16.8	7	38
3	140	25.9	34.5	1.33	31.1	148	11.8	-	-
	160	26.5	37.0	1.40	28.4	179	13.3	7	13
	180	27.3	36.1	1.32	24.3	219	16.6	5	41

Results from Table 4.4 shows that the lower the dietary CP content the higher the N-utilisation. Furthermore, a reduction of dietary CP content from 180 to 160 g/kg DM did not result in reduced milk production whereas lowering the dietary CP content from 160 to 140 g/kg DM did result in reduced milk production although not substantially. There were no substantial and significant differences in BCS between the three treatments although numerically cows receiving the 180 g/kg DM protein diet had higher BCS compared to the other two treatments. Fertility as judged from days from calving to conception was not affected by dietary protein content. Furthermore, it was observed that the planned CP concentrations of TMR concentrations (before adjusting with soy bean meal) were extremely variable and deviated sometimes with more than 10 g/kg DM from the planned dietary CP concentration as a consequence of variation in CP contents of feedstuffs used. Furthermore, it was observed that variation in efficiency of N-utilization varied substantially between animals with differences between lowest and highest efficiencies within treatment of roughly an 10% units. Although part may be related to estimation of N intake as well, this variation appears enormous considering the average efficiency being close to 30%. With respect to economic impact it was concluded that feed costs were highest for the 180 treatment group, that veterinary and medicine costs and costs due to milk dumping were highest for the 140 treatment group and also that replacement costs were highest for the 140 treatment group resulting in the conclusion that the 160 treatment resulted in the highest margin.

From the long term study Defra AC0122 it is concluded that:

1. Decreasing dietary CP from 160 to 140 g/kg DM generally reduced milk production and DMI, but milk production and DMI were unaffected when decreasing dietary CP from 180 to 160 g/kg DM; a decrease in dietary CP always increased efficiency of N- utilization.
2. The optimal dietary CP content relating increase in milk yield with increase in nitrogen losses in manure appears to be within the range of 140 – 160 g CP/kg DM.
3. Fertility of dairy cattle remained unaffected by dietary CP content.
4. Health of cows seems impaired for cows receiving the 140 g CP/kg DM treatment compared to the other two higher CP treatments.

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5. The large variation in efficiency of N-utilization between animals within treatments suggests that personalized nutrition for dairy cows is a promising way to further increase this efficiency.
  6. In making diets for dairy cattle the variation in CP is substantial which might be problematic when feeding diets with CP-contents close to the minimum MP or tamen degradable protein requirements of dairy cattle, and thereby allowing a small margin of error. In this respect, it is of interest to examine the possible advantages of allowing diets to oscillate in CP content and make cows more robust in handling variation in CP-intake and in balancing protein allowance by correcting a deviation in planned protein supply on one day by increasing or decreasing the protein supply the other day.

## 4.2 Overall conclusions from long-term studies on low CP nutrition

Overall, results from the 4 long term studies of Law et al. (2009a,b), Wu and Satter (2000), Tolcamp et al. (1998) and Defra AC0122 (Reynolds et al., 2021; Crompton et al., 2021) it is concluded that:

1. Decreasing dietary CP content is a very effective way to increase efficiency of nitrogen utilization but it may decrease DMI and milk production. The effect of dietary CP content on milk production appears curvilinear and is most pronounced during the first half of lactation.
2. Changes in dietary CP content only moderately alter energy balance of the cow as judged from reported changes in BW or BCS.
3. There were no clear indications of fertility of dairy cattle to be affected by lower dietary CP content.
4. Increasing dietary CP content above 17% in DM in fresh cows will result in unnecessary losses of nitrogen with excreta (predominantly in urine).
5. For full lactations, the optimal average dietary CP concentration at which milk production is maximized, allowing a minimal increase in N-loss in manure above minimum values, seems to be in the range of 140 to 160 g/kg DM for cows producing roughly 10,000 kg of milk per year.
6. Health of cows may be compromised when feeding low CP diets during the first half of lactation lower than 140-150 g/kg DM. However, reports studied did not demonstrate this and perhaps a larger number of measurements in dairy cows receiving low CP diets are required to reach statistically significant conclusions. On the other hand, also from Defra AC0122 with feeding a 14% CP diet throughout three lactations did not demonstrate detrimental effects on health. It may be that health effects of low CP diets become apparent only with even lower CP levels. It should be noted that the 14% CP diet in Defra AC0122 was deficient in rumen degradable protein. Protein characteristics (decrease in rumen degradable protein; decrease in rumen undegradable protein) may impact the results (milk production and N excretion) in different ways.
7. Further studies are required to fine-tune the optimal digestible protein concentrations at the various stages of lactation, also taking into account potential carry-over effects of dietary CP or digestible protein levels fed prior to the start.
8. Considering the large variation between individual animals, personalized nutrition of individual cows seems a promising way to improving efficiency of N-utilization without compromising too much on production and cow performance.
9. In making diets for dairy cattle substantial variation may occur in the dietary CP content, which may be problematic when intending to feed diets with CP contents close to the minimum requirement of dairy cattle. This means that feeding low CP diets probably have a smaller margin of error that can be accepted, increasing to importance to become accurate in feed formulation and mixing. Feeding oscillating diets may help in making cows more robust in handling day-to-day variation in CP-intake. Alternatively, protein allowance in principle may be balanced by correcting a deviation from the planned protein allowance on a day-to-day basis, by increasing or decreasing protein supplementation.

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## 5 Low protein allowance and capacity of visceral organs

After parturition there is a rapid increase in feed intake, which creates the need for a much greater capacity of the gastrointestinal tract to digest feed and of visceral organs in intermediary metabolism. Organs such as the rumen, the intestine and the liver rapidly grow in size. Gibb et al. (1992) reported a liver protein increase of 0.1 kg, and gut protein increase of 0.3 kg, in the first 2 wks post-partum. Measurements by Reynolds et al. (2004) indicate that during the last week of pregnancy and the first three weeks of lactation there is a total growth in visceral organ mass of 5 kg. Assuming a protein content of 25% of tissue mass (dry matter being constituted of protein mainly), this means that 1,3 kg protein needs to be deposited in the organs which is an average 45 g of protein per day during the first three weeks of lactation. Adopting a fractional liver protein turnover rate of 2.5 /d and 60% efficiency in such protein synthesis (the viscera are metabolically highly active and fractional re-synthesis of tissue protein is high and also not 100% efficient) this means at least 75 g of protein per day is required which accounts for about 0.25 kg of rumen-bypass soybean meal or 1.5 kg grass silage. It must be noted however that these calculations indicate average value for the first three weeks; there may be variation in daily protein requirement for viscera growth leading to incidentally even higher requirements to sustain viscera growth.

Even though these protein requirements for viscera growth appear rather small, with the onset of lactation the cow is temporarily in negative protein balance, produces large amounts of milk protein, and feed intake lacks behind compared to the onset of milk synthesis and udder capacity partly fed by mobilization of fat reserves. On top of this, extra protein requirements may be needed to overcome immune challenges although detailed information on this is lacking in literature (F4F3 project Protein nutrition and immune response). Hence viscera growth and protein requirements to overcome immune challenges may pose an extra challenge to meet protein requirements of fresh cows with low CP diets.

It is concluded that keeping MP to the minimum requirements in fresh cows perhaps also has to take into account the extra protein requirements for viscera development, next to those for other maintenance purposes and milk synthesis. This may not immediately become apparent from results on the negative protein balance in fresh cows. For example, Gibb et al. (1992) observed a decline in empty body protein mass up to wk 5-8 postpartum. Van Knegsel et al. (2007) found a positive nitrogen balance in the third week of lactation again in contrast to negative values in the second week, which is a shorter period than that of visceral growth dynamics as described by Reynolds et al. (2004). No specific research was found on the aspect of visceral growth and protein requirements for this. It is perhaps of importance to at least consider the possibility of detrimental effects on viscera development (or related disorders) during this particular stage of lactation when reducing dietary CP content to reduce N losses to the environment.

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## 6 Reduced dietary CP content and rumen function

### 6.1 Modelling studies

#### 6.1.1 Rumen function

Previously, simulations have been performed (Bannink et al., 2016) with a predecessor of the current Tier3 model predicting enteric methane emission and apparent fecal N digestibility in dairy cows (Bannink et al., 2018). Consequences for rumen function were simulated with this dynamic model when substituting maize silage for grass silage at the same proportion of roughage in dietary DM (80%) and without changing concentrates. The same calculations were performed according to the concepts adopted in a static model such as the DVE/OEB system.

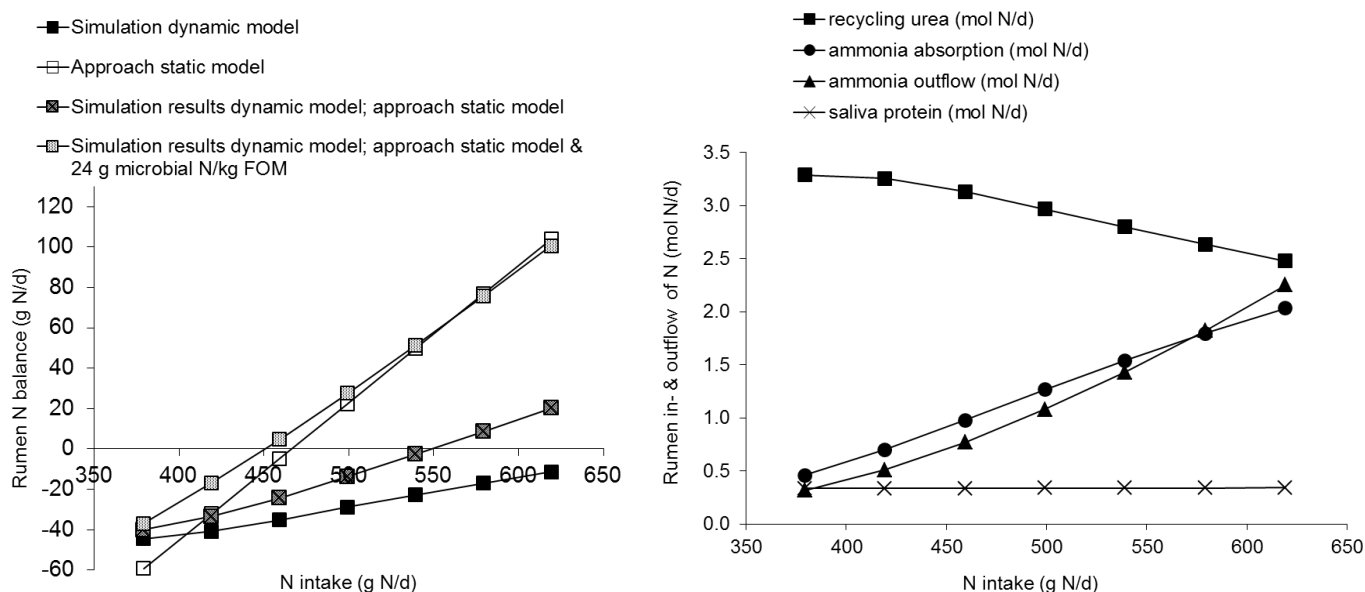
The left graph in Figure 6.1 demonstrates the differences between the dynamic and static approach. Open symbols indicate that with increment of the proportion of maize silage in the diet the rumen N balance calculated with the current DVE/OEB system (as static model) declines and eventually become negative when increasing maize silage up to 70% of dietary DM. The rumen N balance simulated with the dynamic model indicated by the black symbols in the left figure (N inflow with feed minus N outflow to the intestine) simulates much lower rumen N balance (N inflow with feed minus microbial and feed N outflow to the intestine). This means that under all conditions N inflow with feed is less than outflow of feed and microbial N to the intestine, and a net non-feed N inflow to rumen is simulated (depicted in the right graph in Figure 6.1). Simulated values with dynamic model also decline with increase of dietary proportion of maize silage, but the decline starts to saturate when maize silage goes beyond 60% of dietary DM in contrast to outcomes of the static model which keep declining linearly according to the concept of OEB calculations.

In the right graph in Figure 6.1 the individual N flows predicted by the dynamic model, underlying the rumen N balance, are shown, i.e. ammonia outflow, ammonia absorption, saliva protein inflow and urea N recycling from blood to the rumen. With decrease of N intake (through increase of dietary maize silage proportion) there a drastic decline in ammonia absorption and outflow but simultaneously a strong increase in the inflow of recycled urea.

Recalculating rumen N balance with the dynamic model by adopting the same concept as used in the static model (i.e. OEB in the DVE/OEB system) delivered comparable simulated values (see hatched and crossed symbols in the left graph). These results indicate that the rumen N balance as calculated in the current DVE/OEB system cannot be taken as being identical to the physiological N balance of the rumen as an organ compartment.

It may be concluded that conceptually, the dynamic model simulates very different N inflows and outflows and rumen N balance than represented in the static model. This may be important when evaluating low CP diets and establishing levels where rumen microbial synthesis becomes limited or not.

Although these are theoretical calculations, the differences between the static and dynamic model indicates that the concept of rumen N balance as applied in the DVE/OEB system has not to be taken to reflect directly the in vivo rumen N balance as it does not take N recycling and ammonia N flows into account.



**Figure 6.1** Effect of substitution of maize silage for grass silage (up to 70%) in a diet containing 80% roughages and 20% concentrates on a DM basis, assuming a DM intake of 20 kg DM/d, simulated by the model of Dijkstra et al. (1992) as well as by a current protein evaluation system (Van Duinkerken et al., 2011), as examples of a dynamic and a static rumen model, respectively (results derived from Bannink et al., 2006a). Step of increase in N intake reflect 10% increments of % maize silage in dietary DM (from 70% at the left to 10% at the right).

Left: Simulated rumen nitrogen balance (g N/d) according to the static model and with the dynamic model; three different calculations are shown for the dynamic model, 1) calculations according to the same concept of rumen protein balance as used in the static model, 2) calculations according to the same concept of rumen protein balance as used in the static model applying 24 g microbial N synthesized per kg rumen fermented OM as assumed in the static model, and 3) simulated outcome of inflow of feed N minus outflow of N to the intestine.

Right: Simulated non-feed and non-microbial N inflows and outflows for the rumen compartment with the dynamic model (urea recycling, ammonia outflow, ammonia absorption, and saliva protein inflow). These flows are not as such represented in the static model.

The lowest dietary CP content simulated for 70% maize silage (and 10% grass silage) in dietary DM corresponded with 12% CP in dietary DM. This level is far lower than used in most experiments, although it is similar to the lowest CP level tested by Law et al. (2009a) and by Cantalapiedra-Hijar et al. (2014) as discussed in previous sections. Feed intake and milk yield were much lower for this CP level, which may have its cause at the digestive level (rumen fermentation was limited) or at the metabolic level (shortage of MP), or both. The exact cause cannot be derived from the study of Law et al. (2009a).

Also Spek et al. (2013) studied the effect of such low CP levels in a study on the effects and interaction of urine volume and dietary CP level on milk urea content in a balance trial including the measurement of digestibility. The dietary OEB values were slightly negative (-9 g OEB/kg DM) and in combination with less than 12% CP in dietary DM. Compared to the higher CP treatments (15-16% CP) the DM digestibility was 3 to 5 %units lower, which seems to indicate that in this particular trial there was a marked trade-off with a compromised feed digestibility. Although the low CP treatments was achieved by replacing 13% of DM of protected soybean meal with soybean hulls which have a lower digestibility, it seems unlikely this fully can entirely explain the 3-5% lower DM digestibility of the whole diet. The DM digestibility of soybean hulls would have to be more than 30% lower than that of the protected soybean meal, which does not correspond to table values indicating a less than 10% lower value. Milk urea content on the low protein diet was only 8 – 11 mg/dL, indicating an N shortage, whereas on the high protein diet it was 16 to 20 mg/dL.

More recently, indications were obtained from grazing studies by Zom et al. (2019) where unintentionally the dietary treatments with the low rumen degradable protein content ended up at 12% CP in one of the measurement periods (2016 experiment). This led to milk urea levels lower than 10 mg/dL which are generally thought to be an indication of N shortage. The calculated N use efficiency was even 40% which is rather high, also compared to the results obtained in the recent work of Nichols (2019) with a maximum N use efficiency of 36% achieved with a diet of marginal MP allowance and abomasally infusing essential AA in proportion of their presence in casein. In contrast with the study of Spek et al. (2013), no or minor N limitation on rumen function and digestion seems to have taken place as OM digestibility only tended ( $P = 0.08$ ) to be greater for the high CP dietary treatments, the difference only being 1-2 percentage units. It seems that effects on milk production have to be attributed mainly to the lower feed intake that was achieved or to a limitation of nutrient supply to the mammary gland, but not so much to a reduced feed digestibility due to low rumen nitrogen availability. This corresponds to the findings of Ahvenjärvi and Huhtanen (2018) that fiber digestion is reduced at dietary CP levels below 10%. The experiment of Spek et al. (2013) indicates this did happen at a CP content of 12% already, however. In experiments of Nichols et al. (2019) and Nichols et al. (2020) with dietary CP contents of 14% and 13% of the basal diet, resp., there was no clear effect of abomasal infusion of AA on fecal digestibility of NDF, OM or DM whereas these AA infusions likely have contributed to N recycling. Less than 40% of infused AA is retained in extra milk protein synthesis, meaning that about 60% of this will have been oxidized and may have delivered urea entering the blood urea pool and become available for recycling to the rumen.

These results indicate that a CP content below 12% is likely to become limiting for feed digestibility, although the precise CP level at which such a limitation occurs will depend on DM intake, OM fermentability, N intake and N digestibility.

### 6.1.2 Excreta composition

Based on work of Reijs (2007), the Dijkstra et al. (1992) model was extended with calculations on intestinal digestion and fermentation, and inclusion of calculations for the type of compounds excreted with urine and faeces (Dijkstra et al., 2018). These concepts have meanwhile also been introduced in the Tier3 model used for enteric methane emission estimates in the Dutch emissions inventory (Bannink et al., 2019). Simulation results of Dijkstra et al. (2018) clearly indicate the strong impact of nutritional N mitigation measures on N excretion with urine and faeces, with urea-like components (urea, uric acid, allantoin) in urine being most sensitive to lowering of dietary CP content. Furthermore, the predicted apparent faecal N digestibility appears to be very sensitive to the contribution of undigested rumen-synthesized microbial N and of undigested endogenous N (Bannink et al., 2018). As a result, simulated faecal N digestibility declines with decline of dietary CP content, in contrast to fixed CP digestibility values reported in feeding tables. It is concluded that the aforementioned elements become highly important to consider when predicting consequences of N mitigating feeding measures on the site and type of N excreted. This may be relevant when aiming to predict consequences for volatilization of excreted ammoniacal N, or predict the distribution of excreted N over urine and faeces.

## 6.2 Improving efficiency of microbial protein synthesis and of N recycling to the rumen

### 6.2.1 N recycling to the rumen

Aspects of N recycling from blood to the rumen have been represented in the model of Dijkstra et al. (1992) and in other models. The Baldwin et al. (1997) and Dijkstra et al. (1992) equations represent the effect of blood urea or dietary N supply and rumen ammonia concentration based on physiological principles. However, in an independent evaluation (EU-REDNEX project; Dijkstra, Bannink, France and Ellis, unpublished) the absolute transfer of urea-N into the rumen appears not predicted well by the Baldwin equation and much better by the Dijkstra equation.



The CNCPS/NRC (1985) and Danfær et al. (2006) equations predict urea recycling from DMI alone or DMI and dietary CP content in an empirical way, but prediction accuracy of absolute urea entry flux rates appears to be low. Based on the limited evaluation data available, the mechanistic prediction approach of Dijkstra et al., representing a stimulatory effect of blood urea concentration (indirectly, via dietary N intake) and an inhibitory effect of rumen ammonia concentration on urea recycling into the rumen, appears realistic and is recommended. The increased permeability represented with reduced rumen ammonia concentrations remains insufficient if the amount of urea formed is limited (i.e. a low dietary CP content). With further decrease of N intake, the amount of urea in blood become the limiting factor to let the cow recycle more urea to the rumen. The available blood pool of urea hence determines at whether a dietary CP content is too limited to sustain optimal rumen microbial synthesis from fermented OM or not. The cow is hence only partly effective to regulate increase of blood urea recycling to the rumen with progressive decline in dietary CP content.

From the EU-REDNEX project (2013) it was concluded that urea transport appears rather unaffected by even major changes in dietary CP content, which means that with high CP contents urea recycles almost just as well as with low CP contents, as modelled by Dijkstra et al. (1992) and simulated by Bannink et al. (2016; Figure 6.1).

### 6.2.2 Efficiency of microbial N synthesis

Concepts of representing and quantifying microbial N synthesis have been discussed for decades. Fractional rate of passage of feed particles and rumen fluid both appear important determinants of apparent efficiency of microbial N synthesis (review Hristov et al., 2019), together with the fractional rate of substrate degradation and utilization. Various modelling efforts have captured these concepts, and dynamic models (e.g. Dijkstra et al., 1992) are likely more capable to do represent the variation in efficiency of microbial N synthesis. Static approaches are inherently incapable of predicting such variation, and variation in efficiencies will have to be represented based on assumptions (see reviews of Bannink et al., 2016, and Dijkstra et al., 1998) or will have to be made functions of for example fractional passage rate. The latter approach has also been adopted by Van Duinkerken et al. (2011) in the DVE/OEB+ system. It is currently unclear what further aspects could be taken into account in order to further improve efficiency of microbial N synthesis.

Apart from available energy for microbial N synthesis, rumen N availability and N losses occurring also determine whether microbial growth and protein synthesis will be optimal. A theoretical consideration by Dijkstra et al. (2013) delineated the various aspects involved (Textbox 1). Due to inevitable N losses the minimum N concentrations found in vitro do not apply to the in vivo situation where 6 to 18 mM seems necessary (Reynal and Broderick, 2005). Dijkstra et al. (2013) argued there will be a minimal loss of N from the rumen of 105 g N/d whereas 75 g N/d is recycled. Ammonia is not the sole N source for micro-organisms and microbial N synthesis is about 20% higher per unit of OM fermented when originating from the use of preformed AA and peptides (Dijkstra et al., 1992). Such an increase reaches an optimum however and declines with excessive supply of preformed AA and peptides, basically because a high supply of AA and peptides leads to a large proportion of these being fermented to provide ATP, whereas the amount of ATP gained per gram of fermented AA or peptides is significantly lower than that of fermented carbohydrates. There seems to be only limited scope for improving efficiency of microbial N synthesis through manipulation of the non-protein-N and protein-N proportion in diets low in CP as these generally will not contain a high NPN content. One may consider whether supplementing a soluble protein source offers a benefit over supplementing feed NPN, as demonstrated in some experiments (e.g., Broderick and Reynal, 2009).

Presence and activity of rumen protozoa affects the apparent efficiency of microbial N synthesis at the whole rumen level as modelled by Dijkstra et al. (1994). Extensive experimental work has been conducted to study the effect of removal of protozoal activity on rumen function and microbial N synthesis. Meta-analysis of studies on the effect of rumen defaunation indeed indicate that duodenal flow of microbial N increases about 10% (Eugene et al., 2004). However, protozoa also contribute to OM and NDF digestion and in the study this indeed became apparent as reduced apparent faecal OM (2 %units) and NDF digestibility (about 6 %units). This indicates that there is perhaps limited scope for defaunation feeding strategies to improve efficiency for microbial N synthesis and protein supply to the host, due to the trade-offs towards digestibility.

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## 7 Targeted supplementation of MP allowance with rumen-bypass essential amino-acids

MP supply is the sum of protein contribution from digestible microbial protein and feed protein not degraded in the rumen (RUP) but digestible in the intestine, with minor contributions of reabsorbed endogenous protein. The profile of amino acids (AA) in the MP may vary widely. The major contributor to MP is microbial protein. Although the AA profile of microbial protein in protein evaluation systems is generally assumed to be constant, factors influencing the variation in AA composition of microbial protein are largely unknown. However, the composition of microbial protein may well vary significantly. Marked differences in AA profile between protozoal and bacterial protein have been reported (e.g., 10.9 g Lys/100 g total AA in protozoa vs. 7.6 g Lys/100 g total AA in bacteria), with some further differences between solid associated and liquid associated bacteria (Sok et al., 2017). Given that diet composition and intake level impact on the relative proportion of these microbial groups in the microbial biomass flowing to the duodenum, variation in AA profile of microbial protein available for digestion in the intestine likely occurs. Similarly, only limited data are available on the AA composition of RUP. Usually, it is assumed that the AA composition of RUP is identical to that of the original feed protein; however, the actual AA composition of RUP may differ from that in feed protein (e.g., Gonzalez et al., 2000), and furthermore differences in intestinal digestibility of AA from RUP may occur (e.g., Ceresnakova et al., 2002). All in all, the profile of AA absorbed from the small intestine may vary widely, but quantification of this variation is difficult.

A variable proportion of the absorbed AA is captured in milk protein. As discussed in previous paragraphs in this review, this N capture depends on characteristics of the diet including energy and protein content, stage of lactation of the cow, and so on. The profile of AA may be an important factor in this capture of absorbed AA-N into milk protein-N, and thus in milk N efficiency and urinary N excretion. In any diet fed to dairy cattle, certain AA will be absorbed in limited quantities relative to other AA, or relative to their requirement for synthesis of a certain level of milk protein. In the dairy sector worldwide, the principle of Von Liebig's Law of the Minimum is then often used in assuming that milk protein synthesis in mammary gland functions according to the expectations of a limiting AA system. However, the problem lies when this theory is translated into the assumption that the addition of a single, first limiting essential AA will stimulate protein synthesis (Nichols, 2019). Several AA have been proposed to be first limiting in dairy cattle production. Lysine and methionine are considered the main limiting essential AA in dairy cattle (e.g., NRC, 2001). In particular in high-forage diets, histidine is often considered first limiting due to the generally low levels of rumen escape protein and greater reliance on microbial protein (Korhonen, 2000; Lee et al., 2012). When microbial protein supply is limited, leucine (one of the major AA in microbial protein) is thought to be deficient (Allison and Garnsworthy, 2002).

However, several attempts to identify which AA is most limiting for milk protein yield have shown that milk protein yield is not limited by supply of a single AA (review Cant et al., 2018). Evidence against the single limiting AA response in dairy cattle includes stimulation of milk protein yield by mutually exclusive sets of essential AA (Schwab et al., 1976), equal losses in milk protein yield when Met, Lys, His, Phe, or Leu are subtracted from the duodenal essential AA supply (Weekes et al., 2006; Doelman et al., 2015a,b), and equal stimulation of milk protein yield in the absence of so-called group 2 AA profiles (Nichols et al., 2019). The mammary gland in dairy cattle may regulate sequestration of those essential AA that may be in limited quantities relative to requirements under a range of nutritional and physiological states. Besides, intramammary metabolism allows certain AA to compensate for low circulating levels of other AA. Moreover, the mammary gland 'pull' of essential AA may reduce hepatic catabolism of certain essential AA in coordination with mammary set points for protein synthesis (discussed by Nichols, 2019). Milk protein synthesis in mammary cells requires absorption of AA from arterial blood, and uptake is considered to be a function of AA concentrations in arterial blood and the mammary blood flow rate.

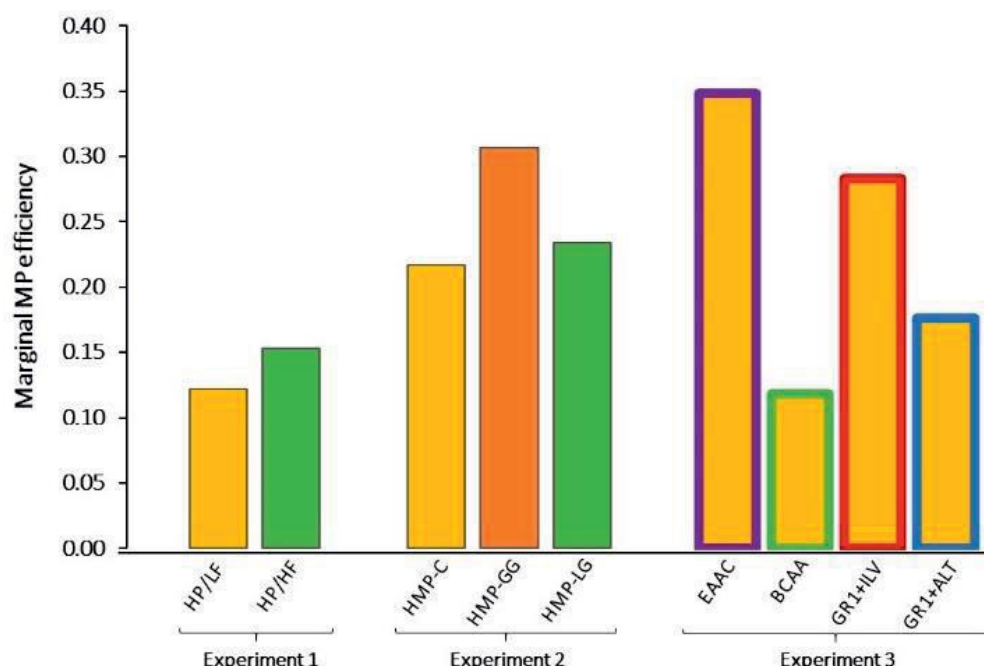
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Thus, milk protein yield may not respond according to a limiting AA phenomenon because of the effect of extra-mammary organs on arterial amino acid concentrations and local regulation of milk blood flow. For example, in lactating goats, histidine addition to an abomasal infusate of essential AA lacking histidine decreased mammary blood flow, and the net uptake of histidine by the mammary gland was not affected (Bequette et al., 2000). Nichols (2019) proposed that as an alternative to first-limiting AA theory, the more accurate statement is that any essential AA can be limiting when the bounds of the mammary gland's capacity to adapt to varied AA supplies has been reached.

In practice, supplementing single rumen-protected AA such as methionine and lysine may give positive responses in some cases, but not in all. The return on that response (marginal response) with respect to milk N efficiency will vary depending on the adaptation of the gland to derive other essential AA from the diet. Detrimental effects of single AA supplementation in dairy cows are also possible. These may arise from reduced net transport of individual AA into mammary cells resulting from increased competition for limited transport capacity via countertransport systems (Maas et al., 1998), and from induced metabolic imbalances resulting in energy-costly functions for catabolism and excretion of excess AA (Reed et al., 2017).

The AA profile of protein supplements impacts milk N efficiency. A clear example is presented in Figure 6.2. The marginal MP efficiency upon supplementing rumen protected soybean meal plus rapeseed meal is 12% (no added fat) or 16% (added fat) (Experiment 1 in Figure 2). In comparison to abomasal infusion of casein only (marginal MP efficiency 22%; Experiment 2 in Figure 2), this marginal MP efficiency of 12 to 16% is rather low, and is likely related to the fact that the AA profile of soybean meal and rapeseed meal varies more from the profile of milk protein, than casein. As discussed in a previous section in this review, supplemental energy (in particular glucogenic energy) may help to improve milk N efficiency. Indeed, the marginal MP efficiency increased to 24% when both casein and fatty acids were infused in the abomasum, and this efficiency increased further to 31% when casein and glucose were infused into the abomasum. Infusion of all 10 essential AA resulted in a marginal MP efficiency of 35% (Experiment 3 in Figure 2). Deleting the branched-chain AA from this mixture of essential AA decreased marginal MP efficiency to 28%, and deleting arginine, lysine and threonine from this mixture of essential AA decreased marginal MP efficiency further to 18%. Abomasal infusion of the branched-chain AA only resulted in the lowest marginal MP efficiency (12%).

The differences in marginal MP efficiency (i.e., capture of extra N absorbed into milk protein N) depicted in Figure 6.2 will have a major impact on excretion of N in urine, with lower marginal MP efficiency resulting in greater extra urinary N excretion. These series of experiments of Nichols (2019) and co-workers clearly indicate a positive impact of a well-balanced AA profile on milk N efficiency, and offers opportunities to reduce N excretion. According to these results, supplementing low (12-14%) CP diets with approximately 0.5 kg/d of rumen-bypass essential AA in the profile of casein may stimulate milk protein yield to levels that are at least as high as when 16% CP diets are fed. Such low CP diets with supplemental essential AA have the advantage that there is no loss in milk protein production compared with a 'normal' CP diet, and milk N efficiency can be increased to levels around 35%, whereas feeding a 16% CP diet may result in similar milk protein production but milk N efficiencies of ~25% are expected. This would offer appreciable benefits in terms of reducing N excretion to the environment (in particular in reducing urinary N excretion) with no negative impact on milk protein production levels. Currently, no rumen protected protein product or individual rumen-bypass essential AA exist that allow supplementation of the EAA profile of casein. In practice, this is a major limiting factor in the ability to implement such a feeding scheme. In the absence of this technology, efforts should be made to formulate rations with protein sources that contribute favourably to MP supply as a proportion of CP (Nichols, 2019).



**Figure 6.2** Marginal metabolizable protein (MP) efficiency calculated as the marginal milk protein yield arising from the supplemented protein relative to the marginal MP intake between each protein supplemented experimental treatment and experimental control. Experiment 1, supplementation with soybean meal and rapeseed meal (source of essential and non-essential AA) (HP) without (LF) or with (HF) supplementation with saturated fatty acids (C16:0 and C18:0). Experiment 2, infusion of essential AA (HMP; in the profile of casein) only (HMP-C) or together with glucose (HMP-GG) or saturated (C16:0) and mono-unsaturated (*cis*-9 C18:1) fatty acids (HMP-LG). Experiment 3, infusion of all essential AA (in the profile of casein) (EAAC), branched-chain AA (Ile, Leu, Val; BCAA), group 1 AA (His, Met, Phe, Trp) plus branched-chain AA (GR1+ILV), or group 1 AA plus arginine, leucine and threonine (GR1+ALT). Adopted from Nichols (2019).

With respect to AA profile and its impact on improving milk N efficiency and reducing N excretion, the major conclusions are:

- There is generally no single limiting AA. Any essential AA may be limiting when the bounds of the mammary gland's capacity to adapt to varied essential AA supplies has been reached
- Fundamental research into aspects of AA metabolism in dairy cattle should continue, with focus placed on optimizing ideal AA profiles and their use through the postabsorptive system, in relation to energy supply
- Further research and data on the requirements of AA in various stages of lactation and under various physiological states is needed to reduce N excretion
- Focus on the development of a wider profile of rumen-bypass essential AA products is a critically important avenue for future innovation

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## 8 Conclusions and research recommendations

The present review provides an overview of aspects of protein metabolism generally receiving less attention in protein evaluation systems, but which may prove to become more relevant under conditions of reduced metabolizable protein allowance to dairy cows in order to minimize nitrogen losses to the environment and reduce ammonia and nitrous oxide emissions. When decreasing safety ranges in the allowance of digestible protein (DVE) and rumen protein balance (OEB), or when underfeeding cows according to current requirements, cow performance may become more sensitive to these aspects.

Our main **conclusions** include the following.

- In the Netherlands, currently some 25% of N consumed by dairy cattle is secreted as milk protein-N; virtually all of the remaining 75% is excreted in faeces and urine. This milk N efficiency is well below the theoretical maximal efficiency of 42 – 43% for cattle producing 25 – 40 kg FPCM/d, and indicates major improvements are possible to reduce N excretion in faeces and urine.
- Oscillating dietary protein supply with alternating high and low dietary protein contents does not improve N efficiency in dairy cattle, in contrast to improved N efficiency in beef cattle and sheep.
- Based on results of protein oscillation experiments, cows are able to cope with temporary (~48-h) periods of N deficiency, and imbalances in formulated diets resulting in suboptimal dietary protein level can be corrected within a window of opportunity period of 48 h.
- Evidence on impact (negative or positive) of level of dietary crude protein on energy balance related characteristics in early lactation is ambiguous.
- Increasing energy supply may help to reduce dietary protein content and N excretion (particularly in urine) without reducing milk production. Glucogenic nutrients have more potential than lipogenic nutrients in this respect, and when compared with fibre, starch can better compensate for negative effects of lowering dietary crude protein on milk yield.
- Long-term studies (1 full lactation or more) on impact of dietary protein level on cow performance and N efficiency are limited. Decreasing dietary protein content is an effective way to improve milk N efficiency and to reduce N losses in faeces and urine, but it may have negative impact on feed intake and milk production in a curvilinear way. The impact of lowering dietary protein content on cow performance is greater in the first than in the second half of lactation.
- Fertility of dairy cattle remains unaffected by long term lowering of dietary crude protein content, but very limited data indicate health of cows may be compromised if low protein diets are fed for extended periods of time.
- Significant variation in N efficiency between cows in the same lactation stage and fed the same dietary protein level occurs.
- The concept of rumen N balance as applied in the current DVE/OEB protein evaluation system may not reflect the true rumen N balance in dairy cattle, and may not be accurate to define limits to N availability maintaining microbial activity.
- Opportunities to increase urea recycling to the rumen to improve N efficiency are limited or absent.
- To evaluate the consequences of N mitigating feeding strategies in terms of losses of N after excretion (during storage or upon application), amount and type of N in faeces and urine has to be considered.
- Dairy cows display an impressive flexibility to produce milk protein, fat, and lactose from lipogenic, glucogenic, and aminogenic dietary ingredients. Besides, recent experimental results exemplify the metabolic flexibility of dairy cattle to deal with different essential AA profiles at the whole-body and mammary gland level.
- There is generally no single limiting AA in milk protein production of dairy cattle. Any essential AA may be limiting when the bounds of the mammary gland's capacity to adapt to varied essential AA supplies has been reached.

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Our main **recommendation for further research** include the following.

- Research efforts should strive to decrease N excretion and increase milk N efficiency, with a view to ultimately close the gap between current N efficiency of 25% and maximal theoretical N efficiency of 42-43%. This research should focus on response of cows to dietary protein levels, but equally important to levels and type of dietary energy, rather than on establishing requirements of dairy cattle for these nutrients.
- Dairy cattle tolerate some temporal shortages in protein allowance without negative effects on milk N efficiency; further data/research is required to evaluate if an impact of temporary shortages may depend on CP level of the diet and/or on length of such intermittent shortages
- Severe negative energy balance and associated metabolic problems in early lactation may be affected by dietary protein level. Further research is needed to evaluate impact of protein level and in particular type of protein (rumen degradable vs rumen bypass) on improving length and severity of negative energy balance and consequently on decreased N losses in urine and faeces during full lactation, in combination with level of glucogenic nutrient supply. Such research may also help to improve welfare and longevity of cattle.
- Further research is needed as to the long term consequences of low protein diets (multiple lactations) on production and health of cattle. Such studies should take into account optimal levels required at various stages of lactation and include potential carry-over effects of dietary crude protein levels to the next lactation or lactation phase.
- Major losses of N occur due to common safety margins applied for rumen degradable N balance. Research on responses in cow performance to different levels of rumen degradable N balance are required, to finetune / lower current safety margins and ultimately to reduce losses from inefficient N metabolism at rumen level.
- Considering the large variation between individual cows in N efficiency in response to change in dietary crude protein level, personalized nutrition of individual cows seems a promising way to improve efficiency of N utilization. Research is required to find suitable, easy indicators of individual cow variation, as well as to practical means to feed cows according to individual needs.
- Fundamental research into aspects of AA metabolism in dairy cattle should continue, with focus placed on optimizing ideal AA profiles and their use through the postabsorptive system, in relation to energy supply at low protein diets. Further research and data on the responses to variation in AA supply in various stages of lactation and under various physiological states is needed to reduce N excretion.
- At present, only rumen protected lysine and methionine, and in near future rumen protected histidine, are commercially available. Focus on the development of a wider profile of rumen-bypass essential AA products is a critically important avenue for future innovation.

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To explore  
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