Animal 17 (2023) 100695

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Dietary protein oscillation: effects on digestibility, nutrient balance and estimated microbial protein synthesis in lactating dairy cows

Rainer Rauch [a,b], Kelly Nichols [a,*], Jean-Baptiste Daniel [a], Javier Martín-Tereso [a,b], Jan Dijkstra [b]

[a] Trouw Nutrition R&D, P.O. Box 299, 3800 AG Amersfoort, the Netherlands
[b] Animal Nutrition Group, Wageningen University and Research, PO Box 338, 6700 AH Wageningen, the Netherlands

ARTICLE INFO

Article history:
Received 25 March 2022
Revised 29 November 2022
Accepted 1 December 2022
Available online 9 December 2022

Keywords:
Milk production
Nitrogen metabolism
Nitrogen utilisation efficiency
Nutrient synchrony
Protein supply

ABSTRACT

Various studies with growing ruminants report increases in nitrogen use efficiency (**NUE**) when feeding oscillating (**OS**) dietary CP, whereas limited research with lactating dairy cows demonstrates a lack of improvement in NUE when feeding OS diets. We hypothesised that a total mixed ration (**TMR**) delivering OS CP (48-h phases of 134 and 171 g CP/kg DM, respectively) compared to a static CP TMR (**ST**; 152 g CP/kg DM) would result in similar or increased urinary purine derivative excretion (as a marker of microbial protein synthesis (**MPS**)) and greater urinary nitrogen excretion in lactating dairy cows. Responses in intake, production, apparent total tract digestibility (**ATTD**), nutrient balance, and estimated MPS were evaluated using faecal and urine collection in 12 multiparous cows (172 ± 39 d in milk) in a randomised complete block design, where total urinary output was estimated indirectly. All measurements were taken during d 8 (at 1700) to d 16 (at 1700) of the 16-d study that followed a 28-d period in which cows already received their respective treatments. Dry matter intake, yields of milk, protein, fat, lactose, and fat- and protein-corrected milk were similar for ST and OS. Milk composition, BW, and body condition score also did not differ between treatments, except for a tendency for increased milk urea concentration with OS (13.7 vs 12.4 mg/dL). Feed efficiency, NUE and ATTD of organic matter, NDF, CP and gross energy did not differ, but ATTD of crude fat (658 vs 627 g/kg) and starch (980 vs 975 g/kg) increased, and ATTD of DM (702 vs 691 g/kg) tended to increase with OS. Milk energy as a proportion of digested energy tended to decrease with OS (34.6 vs 37.1%), but other energy metabolism variables were not affected by treatment. Estimated urinary nitrogen excretion increased (165 vs 144 g/d), estimated urinary nitrogen as a proportion of nitrogen intake tended to increase (25.3 vs 22.7%), and milk nitrogen as a proportion of digested nitrogen decreased (47.3 vs 51.8%) in response to OS. Estimated urinary excretion of creatinine (184 vs 165 mmol/d), uric acid (29 vs 20 mmol/d) and urea (3.1 vs 2.5 mol/d) increased, but other nitrogen metabolism parameters were not affected by OS. Overall, oscillating dietary CP content did not affect lactational performance, milk NUE, or estimated MPS. However, ATTD of some nutrients increased, postabsorptive energy use for milk synthesis tended to decrease, and estimated urinary nitrogen losses increased with OS.

Implications

Oscillating versus static dietary CP increased nitrogen efficiency in some ruminant experiments, but not in those with dairy cows. We hypothesised CP oscillations will result in similar or greater estimated microbial protein synthesis and increased urinary nitrogen losses. We evaluated this hypothesis by feeding static or oscillating CP diets (48-h phases of low- and high-CP) to 12 cows. Oscillating CP increased some nutrient digestibilities and estimated urinary nitrogen losses, but estimated microbial protein synthesis and nitrogen efficiency were unaffected. Additional research in lactating dairy cows would be valuable to further elucidate the effects of oscillating CP on nitrogen metabolism.

Introduction

Typical N utilisation efficiency (**NUE**; N in meat or milk/N intake) in ruminants ranges from 10 to 40% (Calsamiglia et al., 2010). The theoretical maximum NUE without substantial inputs of human-edible protein for a dairy cow producing 40 kg of fat- and protein-corrected milk (**FPCM**) per day is calculated as 43% (Dijkstra et al., 2013). This demonstrates the large discrepancy

* Corresponding author.
E-mail address: kelly.nichols@trouwnutrition.com (K. Nichols).

https://doi.org/10.1016/j.animal.2022.100695

between potential and realised NUE, and the opportunity to increase the anabolic use of dietary N while simultaneously reducing the environmental excretion of ammonia, nitrate, and nitrous oxide (Dijkstra et al., 2013; Montes et al., 2013). Several reviews describe the capacity of ruminants to buffer infrequent dietary CP supply through N recycling and the symbiosis between rumen microbes and the ruminant (Cabrita et al., 2006; Reynolds and Kristensen, 2008). Furthermore, several studies with growing ruminants report greater NUE in response to dietary CP oscillations (**OS**) based on 48-h phases, and relative increases in NUE ranged from 36 to 47% (Cole, 1999 (only when supplemental N came from cottonseed meal); Doranalli et al., 2011; Kiran and Mutsvangwa, 2009). Improvements in NUE may be related to upregulated urea flux to the rumen in response to OS (Archibeque et al., 2007b; Doranalli et al., 2011). In vivo observations of similar (dairy cows; Tebbe and Weiss, 2020) or greater (lambs; Kiran and Mutsvangwa, 2009; Doranalli et al., 2011) purine derivative (**PD**) excretion in response to OS points to the potential role of the ruminal microbial population in unaffected or increased NUE. Recent studies in lactating dairy cows report no effect on NUE when feeding OS diets based on 24-h (Tebbe and Weiss, 2020) or 48-h (Rauch et al., 2021) phases. Notably, in both aforementioned studies, greater apparent total tract digestibility (**ATTD**) of CP was observed with OS (significant or trend), but milk N production was not affected by treatment (Tebbe and Weiss, 2020; Rauch et al., 2021). These findings may suggest that a lack of response in NUE in dairy cows may be related to postabsorptive rather than gastrointestinal effects. Therefore, a metabolism study complementary to the production study of Rauch et al. (2021) was conducted to elucidate potential ruminal and postabsorptive responses to OS, with a specific focus on multiparous cows. The aim of this study was to evaluate responses to OS in ATTD, nutrient balance, and estimated microbial protein synthesis (**MPS**) in multiparous lactating dairy cattle. We hypothesised that oscillating dietary CP concentration (48-h phases of 134 and 171 g CP/kg DM) would result in similar or greater MPS and increased postabsorptive N losses compared with a static (**ST**) CP concentration (152 g CP/kg DM).

Material and methods

Animals, experimental design, and diet formulation

Effects of ad-libitum intake of a TMR based on static (ST; 152 g CP/kg DM) or oscillating (OS; 48-h phases each of 134 (**OS-L**) followed by 171 g CP/kg DM (**OS-H**) (DM basis)) dietary CP content were evaluated using 12 multiparous Holstein-Friesian dairy cows (172 ± 39 DIM at the start of the experiment; four cows in 2nd parity, two cows in 3rd parity, four cows in 4th parity and two cows in 5th parity) in a randomised complete block design. Cows used in the current 16-d study were a subset of cows from an experiment in a free-stall barn that used the same dietary treatments and was conducted immediately preceding the current study (Rauch et al., 2021). Cows in the current study were selected based on being multiparous and free from disease, and only complete blocks were chosen. The preceding study included a 23-d covariate period during which cows were blocked according to parity, DIM and milk N yield. In this previous study, cows were randomly assigned to one of the two experimental groups within a block and subsequently fed the respective diets for 28 d (seven oscillation periods of 4 d; Rauch et al., 2021). Adaptation of cows to their respective diets in the present study was therefore not needed, and d 1–d 8 (at 1700) of the current study served to adapt animals to the metabolic unit and d 8 (at 1700) to d 16 (at 1700) were used for data collection. Cows were fed fresh TMR at 1100 h and again during evening milking (at ~1630 h). Dietary changes to the OS treatment (i.e., at 48-h intervals) were applied at evening milking. Thus, a total of four morning milkings and four evening milkings were used to evaluate the effect of experimental diets within a 96-h period, and two complete periods of oscillation of 96 h each were used for data collection.

To isolate the effect of oscillation from any differences in the TMR composition between treatments, a common basal roughage mixture was mixed for both treatments and half of this mixture was used for each treatment. To create the final TMR for each treatment, the basal roughage mixture was mixed with the specific concentrate(s) that contained different CP concentrations: a low-CP concentrate, used for the OS-L diet, and a high-CP concentrate, used for the OS-H diet. For the ST diet, an equal proportion of the low-CP and high-CP concentrates was used. Samples of TMR ingredients were collected on days 12, 13 and 15 of the experiment and frozen immediately at −18 °C until analysis. The composition of the ST, OS-L and OS-H diets is presented in Table 1.

Housing, measurements, and sampling

Cows were housed in a tie-stall barn with free access to fresh water and individual feed bins. Leftover TMR was removed daily at evening milking, sampled and immediately frozen at −18 °C until analysis. After the removal of refused feed, fresh TMR was immediately weighed and allocated into individual feed bins. The tie-stalls were equipped with rubberised floor mats and a metal grate behind the cow covering a gutter with an automatic pulley system to remove faeces and urine. On d 9, stalls were adapted for the 8-d individual total faecal collection from each of the 12 cows by placing rubberised wooden sheets behind cows on the floor and in-between individual stalls. Each 24-h period started and ended at 1700 h, to align with daily feeding time and evening milking time. Faeces were collected regularly into buckets, and all materials used for faecal collection were designated for individual cows to avoid cross-contamination. Buckets with collected faeces were weighed at 0900, 1300 and 1700 h each day. Faeces were homogenised using an electric mixing paddle, and a 1% sample by weight was collected at each time point and immediately frozen at −18 °C. Samples collected at 1300 and 1700 h were added to the 0900 h frozen sample of each day to create a single composite sample per cow per day.

From d 8 (at 1700) to d 16 (at 1700), daily urine was collected from all cows, with each 24-h interval starting and ending at 1700 h, to align with daily feeding time and evening milking time. The experiment was designed for total urine collection based on the method of Lascano et al. (2010) to avoid potential technical and animal welfare constraints arising from the use of bladder catheters (Hristov et al., 2019). Urine collection was performed using a rubber urine collection funnel that was connected to a flexible reinforced Delphinus plastic tube of 32 mm internal diameter (Mees van den Brink, Haaksbergen, the Netherlands), which was in turn connected to a sealed plastic vessel containing 250 ml of 50% (w/w) sulphuric acid into which the excreted urine flowed. Although the experiment was designed for total urine collection, we experienced some urinary losses during the urine collection periods (i.e., urine that was excreted but not contained in the urine collection vessel) due to some technical problems with the collection set-up. The subsequent urinary N excretion calculated with the collected urine volumes resulted in calculated N retention values that were biologically unrealistic. Therefore, total urine output was estimated using dietary Na and K intakes based on the equation of Bannink et al. (1999). Additionally, we used a second approach to estimate urinary N output, in which urinary N output was calculated as the difference between digested N and milk N, assuming zero N retention. Urine vessels were weighed at 0900, 1300 and 1700 h, corrected for vessel tare weight, and replaced

Table 1
Ingredient and chemical composition of static (ST) and oscillating (OS; OS-L, low protein; OS-H, high protein) diets offered to lactating multiparous dairy cows.

Composition		ST	OS-L	OS-H
Ingredient				
	Maize silage[a]	276	276	276
	Grass silage[b]	128	128	128
	Grass hay[c]	90	90	90
	Maize[d]	158	177	139
	Soy hulls[d]	123	125	121
	Wheat[d]	107	126	88
	Soybean meal[d]	61	20	103
	Soybean meal, protected[de]	24	24	23
	Limestone[d]	9.8	9.9	9.7
	Sodium bicarbonate[d]	9.6	9.7	9.6
	Monocalcium phosphate[d]	4.4	5.3	3.5
	Vitamin/mineral premix[d]	3.5	3.6	3.5
	Sodium chloride[d]	2.0	2.0	2.0
	Magnesium sulphate, anhydrous[d]	2.7	3.1	2.2
	Magnesium oxide[d]	0.2	0.2	0.2
	Live yeast[df]	0.02	0.02	0.02
Nutrient composition				
	DM, g/kg as fed	484	483	484
	Organic matter	923	924	921
	CP	152	134	171
	Crude fat	25	25	25
	NDF	334	338	330
	ADF	191	193	188
	ADL	7	7	7
	Starch	278	303	253
	Total sugars	21	17	25
	Na	4.0	4.0	4.0
	K	13.0	12.2	13.8
	Gross energy, MJ/kg DM	18.0	17.9	18.0
	NE_L, MJ/kg DM[g]	6.89	6.89	6.89
	DVE, g/kg DM[h]	93	86	100
	OEB, g/kg DM[i]	−10	−22	2

[a] Contained (g/kg DM): organic matter, 963; CP, 92; crude fat, 26; NDF, 370; ADF, 205; ADL, 8; starch, 352.
[b] Contained (g/kg DM): organic matter, 895; CP, 218; crude fat, 40; NDF, 443; ADF, 248; ADL, 13; sugar, 16.
[c] Contained (g/kg DM): organic matter, 915; CP, 82; crude fat, 13; NDF, 608; ADF, 340; ADL, 29.
[d] Included in the compound feeds. Low CP compound feed contained (g/kg DM): organic matter, 912; CP, 144; crude fat, 23; NDF, 246; ADF, 147; ADL, 2; starch, 407; sugar, 30. High CP compound feed contained (g/kg DM): organic matter, 906; CP, 217; crude fat, 23; NDF, 231; ADF, 138; ADL, 1; starch, 309; sugar, 46.
[e] Formaldehyde-treated soybean meal (Mervobest, Agrifirm).
[f] Levucell SC 20, containing 2×10^{10} colony forming units/g, Lallemand, Blagnac, France.
[g] Net energy (NE) for lactation. Calculated with the Dutch NE system (CVB, 2018) based on total mixed ration ingredient composition.
[h] Intestinal digestible protein. Calculated with the Dutch DVE/OEB system (CVB, 2018) based on total mixed ration ingredient composition.
[i] Rumen degradable protein balance. Calculated with the Dutch DVE/OEB system (CVB, 2018) based on total mixed ration ingredient composition.

by clean collection vessels containing 250 ml of 50% (w/w) sulphuric acid. Urine was sampled by weighing 1% of the net weight of the acidified urine and immediately frozen at −18 °C. Samples collected at 1300 and 1700 h were added to the 0900 h frozen sample to create a single composite sample per cow per day. Use of multiple sampling times within a 24-h interval was based on the importance of intermittent removal of urine during the course of a 24-h interval (Hristov et al., 2019). The pH of the acidified urine was measured at each sampling time point, and the mean pH of samples was 1.94 (±0.493). If the pH was >3, a recorded amount of additional sulphuric acid was added until the pH reached <3. The specific gravity of a vortexed urine sample was measured at each sampling time point by weighing 1.000 mL of acidified urine with a calibrated pipet. The specific gravity was used to convert urine weight to urine volume. For lab analyses of faecal and urine samples, individual samples by cow and day were combined to create a single sample per cow per each 4-d period.

Cows were milked twice daily at ∼0530 h and ∼1630 h, and milk yield was recorded manually. Milk samples were obtained via samplers connected to the milking machine that collected a fixed volume of milk per kg produced. Milk samples for analysis were collected during the morning and evening milking of the data collection period (i.e., d 8 (at 1700) to d 16 (at 1700)) of the experiment, representing two complete periods of oscillations. Samples were collected into tubes containing sodium azide, stored at 4 °C, and analysed within 3 d. Cow BW and body condition score (**BCS**) were electronically recorded at the start of the study before cows entered the metabolism unit, and when they returned to the free-stall barn at the end of the study (i.e., d 0 and 16, respectively, after evening milking). The BCS recording was done with a DeLaval (Tumba, Sweden) BCS camera system (scale 1–5). Sampling of individual feed ingredients was according to Rauch et al. (2021).

Chemical analysis

Preparation and analyses of feed and faecal samples for moisture, ash, N, NH3, NDF, ADF, ADL, starch, total sugars and crude fat, and of milk samples, were according to Rauch et al. (2021). Gross energy (**GE**) content was determined using an adiabatic bomb calorimeter (IKA-C700, Janke and Kunkel, Heitersheim, Germany; ISO 9831; ISO, 1998). Analysis of Na and K content of feed ingredients was according to NEN 6966 (2005) using atomic emission spectrometry with inductively coupled plasma. Determination of urine total N concentration was done by combustion according to the Dumas principle (ISO, 2008) and urinary urea concentration was analysed using the urea liquicolor test (HUMAN, Wiesbaden, Germany), based on measuring light absorbance at 578 nm after a modified Berthelot reaction. Analysis of creatinine, uric acid

and allantoin was performed according to Shingfield and Offer (1999) using reversed-phase high-performance liquid chromatography.

Calculations and statistical analysis

Data related to milk yield, milk composition, nutrient intake and associated variables were calculated for d 8 (at 1700) to d 16 (at 1700) of the study, thus two oscillation periods of 4 d, and corrected by cow for the related outcome observed during the covariate period (Rauch et al., 2021) in the statistical analyses. Nitrogen use efficiency (%) was calculated as (CP in milk/6.38)/(CP intake/6.25) × 100, where CP in milk and CP intake refer to total composite CP in milk and total composite CP in feed per 4-d period, respectively. Fat- and protein-corrected milk (kg/d) was calculated as [0.337 + 0.116 × milk fat (%) + 0.06 × milk protein (%)] × milk yield (kg/d) (CVB, 2018). Calculation of ATTD of DM, organic matter (**OM**), CP, NDF, crude fat, GE and starch was as the difference between total dietary intake and faecal output, by cow and 4-d period. Total PD output was calculated as the sum of allantoin and uric acid outputs. Due to the lack of fistulated cows for rumen sampling in the current experiment, a value of 11.6–100 was used for the ratio of purine N to total N in mixed rumen microbes and microbial N flow was calculated as: 0.727 × [(total PD excretion − 0.385 × $BW^{0.75}$)/0.85], according to Chen and Gomes (1992). All intake, milk production, ATTD and N balance variables were analysed as one value per cow per 4-d period. For DM intake (**DMI**) calculation, DMI was corrected for daily TMR refusal DM content. All statistical analyses were conducted with PROC MIXED in SAS (SAS 9.4M6, SAS® Studio, SAS Institute Inc., Cary, NC, USA), with cow as the experimental unit. Cow, treatment, block, and 4-d period were used as class variables. Fixed effects were treatment, period, the interaction of treatment and period, and the respective covariate value. Block was included as random effect. Period was included as a repeated statement, with cow within block as subject and a standard variance components structure as variance–covariance structure. Each period comprised the average composite output for the variable in question (thus, 48-h of OS-L and 48-h OS-H, or 96-h of ST). As BW and BCS were only recorded at the start and end of the study, statistical analysis did not include any period or treatment × period effects. If a value had a studentised residual of less than −3 or more than 3, it was classified as an outlier and removed before statistical analysis. For all statistical analyses, differences were considered significant at $P < 0.05$ and tendencies at $0.05 < P < 0.10$.

Results

Intake and performance

A trend for a treatment × period interaction for milk protein yield and a trend for increased milk urea concentration in response to OS were detected ($P < 0.10$; Table 2). Other feed intake, milk production and feed efficiency variables were not affected by treatment or period × treatment interactions ($P \geq 0.10$). Averages and changes in BW and BCS were not affected by treatment ($P \geq 0.36$).

Apparent digestibility and energy metabolism

No period × treatment interactions were detected for ATTD variables or parameters related to energy metabolism ($P \geq 0.37$; Table 3). Crude fat and starch ATTD increased ($P \leq 0.04$), and DM ATTD tended to increase ($P = 0.05$) in response to OS. The ATTD of OM, CP, NDF and GE was not affected by treatment ($P \geq 0.10$). Milk energy efficiency (% of digested energy) tended to be lower with OS ($P = 0.06$). Other energy metabolism-related parameters were not affected by treatment ($P > 0.10$).

Nitrogen metabolism

Milk N yield tended to be affected by the period × treatment interaction ($P = 0.05$), but no other period × treatment interaction was detected for variables related to N metabolism ($P > 0.23$; Table 4). Milk N (% of digested N) was decreased by OS ($P = 0.01$). Urinary N content tended ($P = 0.07$) to be greater with OS than ST. Urinary N excretion according to predicted urine volumes using the equation of Bannink et al. (1999) and according to the difference between digestible N intake and milk N output (assuming zero N balance) increased in response to OS ($P < 0.04$).

Table 2
Intake and performance of lactating multiparous dairy cows (n = 6 per treatment) receiving total mixed ration based on either static (ST; daily 152 g CP/kg DM) or oscillating (OS; 134 g CP/kg DM for 48 h, followed by 171 g CP/kg DM for 48 h) dietary CP content.

Item	Treatment			*P*-value		
	ST	OS	SEM	Treatment	Period	Treatment × Period
DM intake (kg/d)	26.5	26.5	0.85	0.98	0.42	0.82
CP intake (kg/d)	4.03	4.04	0.129	0.91	0.33	0.98
Yield						
Milk (kg/d)	35.6	36.1	1.60	0.69	0.79	0.75
Fat (kg/d)	1.61	1.59	0.079	0.80	0.48	0.98
CP (kg/d)	1.28	1.29	0.069	0.85	0.25	0.05
Lactose (kg/d)	1.62	1.62	0.069	0.92	0.94	0.81
Fat- and protein-corrected milk (kg/d)[a]	38.3	38.3	1.68	0.99	0.59	0.89
Milk composition						
Fat (%)	4.52	4.39	0.120	0.33	0.39	0.83
CP (%)	3.64	3.55	0.048	0.10	0.94	0.64
Lactose (%)	4.53	4.51	0.046	0.45	0.53	0.78
Urea (mg/dL)	12.4	13.7	0.67	0.08	0.03	0.49
Body condition score[b]	2.84	2.88	0.039	0.45		
Body condition score change (units/30 d)[c]	0.04	0.16	0.103	0.37		
BW (kg)[b]	678	688	7.5	0.41		
BW change (kg/d)[c]	−0.84	−0.50	0.449	0.54		
Feed efficiency (kg fat- and protein-corrected milk/kg DMI)	1.45	1.42	0.039	0.41	0.04	0.44

[a] Fat- and protein-corrected milk calculated using the formula: [0.337 + 0.116 × milk fat (%) + 0.06 × milk protein (%)] × milk yield (kg/d) (CVB, 2018).
[b] As at end of experiment, i.e., day 16.
[c] Change between start and end of experiment.

Table 3
Apparent total tract digestibility of nutrients and energy metabolism in lactating multiparous dairy cows (n = 6 per treatment) receiving total mixed ration based on either static (ST; daily 152 g CP/kg DM) or oscillating (OS; 134 g CP/kg DM for 48 h, followed by 171 g CP/kg DM for 48 h) dietary CP content.

	Treatment			*P*-value		
Component	ST	OS	SEM	Treatment	Period	Treatment × Period
Digestibility (g/kg)						
DM	691	702	4.7	0.05	0.01	0.48
Organic matter	710	719	4.6	0.14	0.01	0.84
CP	626	633	6.4	0.35	0.30	0.38
Crude fat	627	658	11.1	0.04	0.04	0.89
NDF	508	509	10.0	0.86	<0.01	0.37
Starch	975	980	1.8	0.02	0.02	0.66
Gross energy (kJ/MJ)	685	696	4.6	0.10	0.01	0.78
Energy metabolism						
Gross energy intake (MJ/d)	475	480	13.6	0.62	0.41	0.88
Digestible energy intake (MJ/d)	325	334	10.1	0.36	0.14	0.99
Milk energy output (MJ/d)	119	118	5.1	0.92	0.60	0.91
Milk energy content (MJ/kg)[a]	3.32	3.28	0.066	0.71	0.16	0.78
Milk energy efficiency (% of energy intake)	25.4	24.0	0.84	0.11	0.26	0.77
Milk energy efficiency (% of digested energy)	37.1	34.6	1.27	0.06	0.08	0.82

[a] Milk energy content = (0.0929 × milk fat % + 0.0547 × milk CP % + 0.0395 × milk lactose %) × 4.184 (NRC, 2001).

Table 4
Nitrogen (N) metabolism and urinary excretion of nitrogenous metabolites in lactating multiparous dairy cows (n = 6 per treatment) receiving total mixed ration based on either static (ST; daily 152 g CP/kg DM) or oscillating (OS; 134 g CP/kg DM for 48 h, followed by 171 g CP/kg DM for 48 h) dietary CP content.

	Treatment			*P*-value		
Component	ST	OS	SEM	Treatment	Period	Treatment × Period
N metabolism						
N intake (g/d)	645	647	20.6	0.91	0.33	0.98
N digested (g/d)	403	413	11.2	0.44	0.26	0.70
Milk N (g/d)	200	203	10.8	0.85	0.25	0.05
Faecal N (g/d)	240	239	9.0	0.75	0.96	0.35
Urinary N (g/d)[a]	144	165	5.0	<0.01	0.11	0.75
Urinary N (g/d)[b]	194	218	7.5	0.03	0.12	0.84
Urinary N concentration (g/kg)	4.58	5.10	0.228	0.07	0.29	0.76
N retained (g/d)[a]	49	53	7.2	0.71	0.65	0.68
Milk N (% intake)[c]	31.5	30.6	0.61	0.21	0.06	0.24
Milk N (% digested N)	51.8	47.3	1.75	0.01	0.19	0.92
Urinary N (% intake)[a]	22.7	25.3	1.13	0.07	0.32	0.71
Urinary N (% intake)[b]	30.2	33.4	1.31	<0.01	0.11	0.84
Urinary N (% digested N)[a]	36.3	39.9	1.75	0.16	0.50	0.61
Urinary N (% digested N)[b]	48.2	52.7	1.72	0.01	0.19	0.92
N retained (% intake)[a]	7.5	8.2	1.04	0.65	0.78	0.63
Urinary excretion and related variables[a]						
Urine mass (kg/d)	31.9	32.3	0.91	0.60	0.39	0.92
Allantoin excretion (mmol/d)	357	365	8.7	0.43	0.01	0.83
Allantoin concentration (mmol/kg)	11.27	11.35	0.229	0.82	0.08	0.85
Uric acid excretion (mmol/d)	20	29	2.4	0.01	0.01	0.43
Uric acid concentration (mmol/kg)	0.64	0.89	0.068	0.02	0.02	0.49
Total purine derivative excretion (mmol/d)[d]	377	394	10.4	0.17	0.01	0.70
Total purine derivative concentration (mmol/kg)[d]	11.91	12.24	0.270	0.41	0.03	0.74
Creatinine excretion (mmol/d)	165	184	6.7	<0.01	0.84	0.61
Creatinine concentration (mmol/kg)	5.24	5.76	0.310	0.01	0.75	0.55
Urea excretion (mol/d)	2.5	3.1	0.16	0.01	0.33	0.96
Urea concentration (mol/kg)	0.080	0.097	0.0065	0.03	0.50	0.95
Microbial N flow (g/d)[e]	279	292	9.2	0.22	0.01	0.71
EMPS (g/kg digestible organic matter in the rumen)[f]	156	158	4.3	0.71	0.12	0.82
Total purine derivatives (mmol/kg organic matter digested)[g]	21.9	22.2	0.53	0.70	0.16	0.81

[a] Calculation based on urine volumes using the formula: urine volume (kg/d) = 0.1153 × Na intake (g/d) + 0.0577 × K intake (g/d) (Bannink et al., 1999).
[b] Calculation based on urinary N excretion using the difference between digestible N and milk N output, assuming zero N retention.
[c] Milk nitrogen efficiency (%) = [(milk protein output/6.38)/(dietary CP intake/6.25)] × 100.
[d] Sum of allantoin excretion and uric acid excretion.
[e] Calculated as: 0.727 × [(total purine derivative excretion − 0.385 × $BW^{0.75}$)/0.85], according to Chen and Gomes (1992).
[f] Efficiency of microbial protein synthesis (g/kg organic matter digested in rumen), assuming that 65% of total tract digestion of organic matter occurred in the rumen (ARC, 1984).
[g] Using apparent total tract organic matter digestibility.

Urinary N (% intake) tended to increase ($P = 0.07$) using predicted urine volumes according to Bannink et al. (1999) and increased ($P < 0.01$) according to the difference between digestible N intake and milk N output in response to OS. Urinary N (% of digested N) according to the difference between digestible N intake and milk N output increased ($P = 0.01$) in response to OS. Urinary

concentrations of uric acid, creatinine and urea were greater ($P \leq 0.03$) with OS than ST. Excretion of uric acid, urea and creatinine increased in response to OS ($P < 0.02$). No other urinary excretion and related variables were affected by a treatment or treatment × period interaction ($P \geq 0.16$).

Discussion

We hypothesised that cows would respond to oscillating dietary CP concentration (48-h phases of 134 and 171 g CP/kg DM) with similar or greater MPS and an increase in urinary N losses. Results showed that ATTD of some nutrients increased with OS but intake, performance and predicted MPS of cows were not affected by the treatment. However, estimated urinary N losses were increased in response to OS. All parameters related to urinary excretion were based on estimates of total urinary output, rather than on direct measurements of urinary output, and results should therefore be interpreted accordingly.

Intake and performance

The lack of treatment effect on DM and CP intake in the present metabolism study is consistent with the production study of Rauch et al. (2021; 48-h oscillation phases). However, these results contrast with the lower CP intake in cows offered an oscillating CP diet (119 and 162 g CP/kg DM, 24-h basis) compared with a static CP diet (141 g CP/kg DM) in Tebbe and Weiss (2020), suggesting that length of the oscillation phase may have influenced the DMI responses across these studies. The trend for increased milk urea content in response to OS in the current study is consistent with the greater milk urea content reported by Rauch et al. (2021). The lack of treatment effect on milk protein content in the current study is not consistent with the reduced milk protein content in response to OS observed by Tebbe and Weiss (2020) but agrees with the lack of response in milk protein concentration to OS reported by Rauch et al. (2021). The absence of treatment effects for other variables related to feed efficiency, milk production, milk composition, and milk component yields are in line with Tebbe and Weiss (2020) and Rauch et al. (2021). Consistent with our observations of a lack of treatment effect on averages and changes in BW and BCS, Tebbe and Weiss (2020) and Rauch et al. (2021) showed that BW and BCS did not respond to OS. However, the loss in BW of both OS and ST cows is not in line with the positive body N retention. Considering the variable nature of BW measurements, a limitation in the current study is that only two BW measurements were recorded per cow (at the start and at the end of the study). For future studies, it is advisable to measure BW at least over 3–4 d to increase the reliability of measurements. As reviewed by Hristov et al. (2019), measurements of body N balance are prone to substantial measurement error, especially in terms of volatile N losses during collection, processing, and analysis of samples, which may result in the overestimation of body N accretion. The current study's average estimated N retention value (as proportion of N intake) of 7.9% is similar to the values of 8.3 and 6.7% reported in the meta-analyses of N balance experiments of Spanghero and Kowalski (1997) and Spanghero and Kowalski (2021), respectively. Furthermore, average N excretion at zero N balance is underestimated by ~10% (Spanghero and Kowalski, 2021), which is consistent with the N retention values in the current study (Table 4), considering BW losses observed.

Apparent digestibility and energy and nitrogen metabolism

The increases or tendency for increases in DM, starch and crude fat ATTD with OS are consistent with increases in ATTD of DM and starch reported by Rauch et al. (2021), but crude fat ATTD in Rauch et al. (2021) was not affected by treatment despite similar numerical differences between treatments as in the current study. Tebbe and Weiss (2020) observed no treatment effect on ATTD of DM, starch or long-chain fatty acids. A possible explanation may be the difference in length of oscillation phase (48 h in the current study and Rauch et al. (2021) vs 24 h in the study of Tebbe and Weiss, 2020), the different methodologies related to the analysis of crude fat vs long-chain fatty acids, or the replacement of CP with mainly starch (current study) vs starch and fibre (Tebbe and Weiss, 2020), considering otherwise similar experimental designs, diets, and animal characteristics between studies.

The lack of treatment effect on ATTD of CP and NDF contrasts the increases in ATTD of these nutrients reported by Rauch et al. (2021) and the tendency for an increase in CP ATTD by Tebbe and Weiss (2020) in response to OS. Possible explanations for the differences in NDF and CP ATTD of the current study and that with Rauch et al. (2021) despite identical dietary treatments may be the different housing systems (free-stall vs tie-stall housing) resulting in possible differences in feed intake behaviour (e.g., rate and spread of intake through a day), different methodologies used to calculate ATTD (titanium dioxide as a marker vs total collection), or a smaller number of animals. Variable outcomes in ATTD parameters appear typical of studies with infrequent CP supply, where CP ATTD increased (Archibeque et al., 2007a; Kiran and Mutsvangwa, 2009, Doranalli et al., 2011; Tebbe and Weiss, 2020 (trend); Rauch et al., 2021), decreased (Ludden et al., 2002a, restricted feeding) or remained unaffected (Collins and Pritchard, 1992; Cole, 1999; Ludden et al., 2002b, restricted feeding; Cole et al., 2003; Menezes et al., 2019). This may be related to differences in diet composition between studies (e.g., the use of rumen degradable protein vs rumen undegradable protein to vary dietary CP content; see Collins and Pritchard, 1992), but this is unlikely to explain the differences in CP ATTD responses to CP oscillations between the dairy cow studies (Tebbe and Weiss, 2020; Rauch et al., 2021; current study) as diet characteristics were rather similar among these studies, except that CP was replaced mainly by starch in the current study, whereas CP was replaced by starch and fibre in the study of Tebbe and Weiss (2020; discussed previously).

Measured urine mass excretion for ST and OS groups was 71 and 70%, respectively, of the predicted urine mass according to the equation of Bannink et al. (1999) reported in Table 4. Assuming that the technical problems in quantitatively collecting urine were similar for both treatment groups, the difference between predicted and measured urine mass supported the observations that not all urine output was collected, and results related to calculated urinary output should therefore be interpreted with this in consideration. All raw data related to actual measured urine collection were analysed statistically to validate results based on predicted urine mass. Results showed trends and significant differences for treatment effects that were similar to those reported in Table 4 (according to the equation of Bannink et al., 1999), except for creatinine excretion, which displayed a tendency for a difference between OS and ST. These results support the use of the Bannink et al. (1999) equation in calculating urinary excretion and related variables. Thus, despite the differences between measured and estimated urine output, similar treatment differences were observed for several variables with both approaches.

None of the CP oscillation dairy cow studies published to date (Tebbe and Weiss, 2020; Rauch et al., 2021; current study) observed treatment differences in NUE between ST and OS. This contrasts several growing ruminant studies in which N retention or NUE was improved with OS (Cole, 1999; Archibeque et al., 2007b; Kiran and Mutsvangwa, 2009; Doranalli et al., 2011). A possible explanation for differences between studies may be that urea recycling did not increase in response to OS in the current study,

that urea recycling to the rumen increased but that microbes did not benefit from the additional N (discussed in more detail in next section), or that rumen efficiency was affected. Another possible reason for a lack of improvement in NUE in the current study is related to postabsorptive N metabolism. Consistent with this is the reduced milk N (as % of digested N) and greater estimated urinary N excretion (according to both estimation methods) with OS in the current study. Similarly, Rauch et al. (2021) reported a greater CP ATTD in response to OS despite equal milk N output between treatments. Estimated urinary N as a proportion of digested N (according to the difference between digestible N intake and milk N output, assuming zero N balance) increased with OS in the current study and is consistent with Tebbe and Weiss (2020). A potential role of the mammary gland or other tissues in the observed reduction in estimated postabsorptive N efficiency in response to OS seems likely but requires further evaluation. Apart from a reduced estimated postabsorptive N efficiency in response to OS, and based on actually observed intake, digestion, and output in milk of energy, we observed a trend for a lower postabsorptive milk energy efficiency. This reduced energetic efficiency, together with increased milk urea content and increased estimated urinary urea excretion, is consistent with the positive association between excess N and heat production in cattle, and may be indicative of increased hepatic energy demands for ureagenesis (Reed et al., 2017) in response to OS.

Urinary purine derivatives and creatinine excretion

As with parameters related to urinary N excretion discussed above, the values related to purine derivative and creatinine excretion relied on estimated rather than on measured urinary output, and should be interpreted accordingly. The observation of increased estimated daily uric acid excretion with OS in the absence of treatment effects on estimated total PD excretion, estimated microbial N flow and estimated efficiency of MPS may suggest that OS had limited effects on rumen fermentation, although more robust experimental evidence (e.g., based on total urine collection) is required to state this conclusively. In the study of Tebbe and Weiss (2020), authors did not report the effects of OS on PD excretion or total PD excretion per kg of OM digested. These results contrast several studies with growing ruminants in which microbial fermentation positively responded to 48-h CP oscillations. Kiran and Mutsvangwa (2009) reported increases in excretion of allantoin and uric acid in response to OS in sheep, and lambs in a study by Doranalli et al. (2011) responded to OS with increased ruminal VFA concentrations, MPS and EMPS. Modulation of urea flux to the rumen is one of the mechanisms responsible for improvements in production or efficiency parameters in response to OS (Doranalli et al., 2011; Archibeque et al., 2007b). Differences in ruminal responses to OS between studies may be related to contrasting diets fed and associated effects on ruminal ammonia N, regardless of possible increases in urea flux to the rumen with OS. Based on the current study's rumen protein balance values, predicted rumen ammonia N levels were 5.2 and 7.7 mmol/L for the OS-L and OS-H diets of the OS treatment, respectively (INRA, 2018), which generally is consistent with reported optimal ammonia N levels for microbial growth and MPS, i.e., 3.6 mmol/L (Satter and Slyter, 1974) to 6.1 mmol/L (Kang-Meznarich and Broderick, 1980). In contrast, in the study of Doranalli et al. (2011), microbial non-ammonia N supply increased by 10% with OS, but rumen ammonia N levels of the ST (4.5 mmol/L) and the low protein OS (3.7 mmol/L) diets were marginally low compared to reported optimal levels. If ruminal ammonia N levels during OS-L and ST feeding in the current study were not sufficiently low to considerably disadvantage MPS compared to optimal ruminal ammonia N levels, any increases in ruminal ammonia N availability due to putative increases in urea flux would likely have been inconsequential for MPS and metabolisable protein supply. Different dietary characteristics between studies (e.g., CP or fermentable energy levels) which result in differences in ruminal ammonia N levels may be among the reasons for the contrasting outcomes in response to OS between studies. Consistent with this, CP content of the ST diets in dairy cow studies ranged from 141 to 152 g/kg DM (Tebbe and Weiss, 2020; Rauch et al., 2021; current study) whereas those in studies that reported positive effects in NUE or N retention in response to OS ranged from 122 to 127 g/kg DM (Doranalli et al., 2011; Kiran and Mutsvangwa, 2009; Archibeque et al., 2007b; Cole, 1999, cottonseed meal as sole supplementary N source). Within-study contrasts by Cole (1999) and Collins and Pritchard (1992) showed that the response to infrequent CP supply is enhanced when using proportionally more dietary rumen undegradable protein relative to rumen degradable protein sources. These conditions are associated with reduced ruminal ammonia N concentrations, which may stimulate urea recycling to the rumen (Kennedy and Milligan, 1980; Li et al., 2019), and may support the notion that diets lower in CP or rumen degradable protein may be most likely to result in improvements in NUE when oscillating CP, but further research is warranted.

Estimated daily excretion of creatinine, a biomarker for muscle mass, increased by 12% in response to OS. This is broadly consistent with Tebbe and Weiss (2020), where OS resulted in a 20% increase (trend) in creatinine excretion. Tebbe and Weiss (2020) reported that treatment did not affect absolute levels or changes in body protein mass. Similarly, in the current study, treatment did not affect absolute BW or BCS values, or changes therein. The use of a daily urinary volume prediction equation to calculate total creatinine excretion in the current study together with limited research on creatinine excretion in response to OS precludes a clear interpretation of the apparent increase in creatine excretion with OS in the current study. Further research would be valuable to elucidate any potential effects of OS on creatinine metabolism.

Conclusion

In summary, cows responded to OS with increased ATTD of some nutrients, similar estimated MPS, and an increase in estimated urinary N losses. Intake, milk component production, NUE, feed efficiency, and changes in BW and BCS were not affected by treatment. Additional research is warranted to evaluate the effects of CP oscillation in lactating dairy cows on microbial protein synthesis and postabsorptive metabolism, and to determine if alternative approaches may be successful to improve NUE.

Ethics approval

The study complied with the Dutch Act on Animal Experiments in accordance with European Directive 2010/63/EU and was approved by the Central Committee of Animal Experiments (the Hague, the Netherlands), under the project licence number AVD2040020173426.

Data and model availability statement

All data were deposited in the Trouw Nutrition repository (Ruminant Research Centre). The data/models that support the study findings may be made available upon request to the authors.

Author ORCIDs

Rainer Rauch: https://orcid.org/0000-0002-0513-0643.
Kelly Nichols: https://orcid.org/0000-0001-6062-7460.

Jean-Baptiste Daniel: https://orcid.org/0000-0001-7627-3708.
Javier Martín-Tereso: https://orcid.org/0000-0002-6757-4629.
Jan Dijkstra: https://orcid.org/0000-0003-3728-6885.

Author contributions

Conceptualisation: **R.R., K.N., J.B.D., J.M.T, J.D.**; Methodology: **R.R., K.N., J.B.D., J.M.T, J.D.**; Formal analysis: **R.R., J.B.D., J.M.T, J. D.**; Investigation: **R.R., K.N., J.B.D.**; Data curation: **R.R., J.B.D.**; Writing – original draft: **R.R.**; Writing - review and editing: **R.R., K.N., J.B.D., J.M.T, J.D.**; Supervision: **J.M.T, J.D.**; Project administration: **R.R.**; Funding acquisition: **J.M.T**.

Declaration of interest

All authors except J.D. are employed by Trouw Nutrition (Amersfoort, the Netherlands), a company with commercial interests in ruminant nutrition. Trouw Nutrition R&D adheres to the principles of the European Code of Conduct for Research Integrity (Drenth, 2012).

Acknowledgements

The authors wish to thank Paul van Kempen, Frans van Kempen, Chantal van den Hoven, Akke ten Berge and the research farm staff (all Kempenshof, Boxmeer, the Netherlands) for their efforts and contributions in conducting the experiment. Gratitude is expressed to Christian Winkel (Wageningen University) for assisting with experimental procedures and data collection.

Financial support statement

This study was funded by Trouw Nutrition (Amersfoort, the Netherlands; no relevant grant number).

References

Agricultural Research Council (ARC), 1984. The nutrient requirements of ruminants, Suppl. No.1. Commonwealth Agricultural Bureaux, Farnham Royal, Slough, UK.
Archibeque, S.L., Freetly, H., Cole, N., Ferrell, C., 2007a. The influence of oscillating dietary protein concentrations on finishing cattle. II. Nutrient retention and ammonia emissions. Journal of Animal Science 85, 1496–1503.
Archibeque, S.L., Freetly, H., Ferrell, C., 2007b. Net portal and hepatic flux of nutrients in growing wethers fed high-concentrate diets with oscillating protein concentrations. Journal of Animal Science 85, 997–1005.
Bannink, A., Valk, H., Van Vuuren, A.M., 1999. Intake and excretion of sodium, potassium, and nitrogen and the effects on urine production by lactating dairy cows. Journal of Dairy Science 82, 1008–1018.
Cabrita, A.R.J., Dewhurst, R.J., Abreu, J.M.F., Fonseca, A.J.M., 2006. Evaluation of the effects of synchronising the availability of N and energy on rumen function and production responses of dairy cows–a review. Animal Research 55, 1–24.
Calsamiglia, S., Ferret, A., Reynolds, C.K., Kristensen, N.B., Van Vuuren, A.M., 2010. Strategies for optimizing nitrogen use by ruminants. Animal 4, 1184–1196.
Chen, X.B., Gomes, M.J., 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: An overview of technical details. Int. Feed Res. Unit, Occasional Publ. Rowett Research Institute, Aberdeen, United Kingdom.
Cole, N.A., 1999. Nitrogen retention by lambs fed oscillating dietary protein concentrations. Journal of Animal Science 77, 215–222.
Cole, N.A., Greene, L., McCollum, F., Montgomery, T., Mc-Bride, K., 2003. Influence of oscillating dietary crude protein concentration on performance, acid-base balance, and nitrogen excretion of steers. Journal of Animal Science 81, 2660–2668.
Collins, R.M., Pritchard, R., 1992. Alternate day supplementation of corn stalk diets with soybean meal or corn gluten meal fed to ruminants. Journal of Animal Science 70, 3899–3908.
CVB (Centraal Veevoederbureau), 2018. CVB Table Ruminants 2018. CVB, The Hague, The Netherlands.
Dijkstra, J., Reynolds, C.K., Kebreab, E., Bannink, A., Ellis, J.L., France, J., Van Vuuren, A.M., 2013. Challenges in ruminant nutrition: towards minimal nitrogen losses in cattle. In: Oltjen, W.J., Kebreab, E., Lapierre, H. (Eds.), Energy and protein metabolism and nutrition in sustainable animal production. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 47–58.
Doranalli, K., Penner, G.B., Mutsvangwa, T., 2011. Feeding oscillating dietary crude protein concentrations increases nitrogen utilization in growing lambs and this response is partly attributable to increased urea transfer to the rumen. The Journal of Nutrition 141, 560–567.
Drenth, P.J.D., 2012. The European code of conduct for research integrity. In: Mayer, T., Steneck, N. (Eds.), Promoting Research Integrity in a Global Environment. World Scientific Publishing Co Pte Ltd, Singapore City, Singapore, pp. 161–168.
Hristov, A.N., Bannink, A., Crompton, L.A., Huhtanen, P., Kreuzer, M., McGee, M., Nozière, P., Reynolds, C.K., Bayat, A.R., Yáñez-Ruiz, D.R., Dijkstra, J., Kebreab, E., Schwarm, A., Shingfield, K.J., Yu, Z., 2019. Invited review: Nitrogen in ruminant nutrition: A review of measurement techniques. Journal of Dairy Science 102, 5811–5852.
INRA, 2018. INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, The Netherlands.
ISO (International Organization for Standardization), 1998. Animal feeding stuffs, animal products, and feces or urine. Determination of gross calorific value—Bomb calorimeter method. International Standards Organization (ISO), Geneva, Switzerland.
ISO (International Organization for Standardization), 2008. 16634-1: 2008: Food Products—Determination of the Total Nitrogen Content by Combustion According to the Dumas Principle and Calculation of the Crude Protein Content—part 1: Oilseeds and Animal Feeding Stuffs. International Standards Organization (ISO), Geneva, Switzerland.
Kang-Meznarich, J.H., Broderick, G.A., 1980. Effects of incremental urea supplementation on ruminal ammonia concentration and bacterial protein formation. Journal of Animal Science 51, 422–431.
Kennedy, P., Milligan, L., 1980. The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: A review. Canadian Journal of Animal Science 60, 205–221.
Kiran, D., Mutsvangwa, T., 2009. Nitrogen utilization in growing lambs fed oscillating dietary protein concentrations. Animal Feed Science and Technology 152, 33–41.
Lascano, G.J., Zanton, G.I., Heinrichs, A.J., Weiss, W.P., 2010. A noninvasive urine collection device for female cattle: Modification of the urine cup collection method. Journal of Dairy Science 93, 2691–2694.
Li, M.M., Titgemeyer, E.C., Hanigan, M.D., 2019. A revised representation of urea and ammonia nitrogen recycling and use in the Molly cow model. Journal of Dairy Science 102, 5109–5129.
Ludden, P.A., Wechter, T.L., Hess, B.W., 2002a. Effects of oscillating dietary protein on nutrient digestibility, nitrogen metabolism, and gastrointestinal organ mass in sheep. Journal of Animal Science 80, 3021–3026.
Ludden, P.A., Wechter, T.L., Hess, B.W., 2002b. Effects of oscillating dietary protein on ruminal fermentation and site and extent of nutrient digestion in sheep. Journal of Animal Science 80, 3336–3346.
Menezes, A.C.B., Valadares Filho, S.C., Pacheco, M.V., Pucetti, P., Silva, B.C., Zanetti, D., Paulino, M.F., Silva, F.F., Neville, T.L., Caton, J.S., 2019. Oscillating and static dietary crude protein supply. I. Impacts on intake, digestibility, performance, and nitrogen balance in young Nellore bulls. Translational Animal Science 3, 1205–1215.
Montes, F., Meinen, R., Dell, C., Rotz, A., Hristov, A.N., Oh, J., Waghorn, G., Gerber, P.J., Henderson, B., Makkar, H.P.S., Dijkstra, J., 2013. SPECIAL TOPICS—mitigation of methane and nitrous oxide emissions from animal operations: II. A review of manure management mitigation options. Journal of Animal Science 91, 5070–5094.
National Research Council (NRC), 2001. Nutrient requirements of dairy cattle, 7th edition. National Academy Press, Washington, DC, USA.
NEN, 2005. Environment – Analyses of selected elements in water, eluates and destruates – Atomic emission spectrometry with inductively coupled plasma. Dutch Normalization Institute (NEN), Delft, The Netherlands.
Rauch, R., Martín-Tereso, J., Daniel, J.B., Dijkstra, J., 2021. Dietary protein oscillation: Effects on feed intake, lactation performance, and milk nitrogen efficiency in lactating dairy cows. Journal of Dairy Science 104, 10714–10726.
Reed, K.F., Bonfá, H.C., Dijkstra, J., Casper, D.P., Kebreab, E., 2017. Estimating the energetic cost of feeding excess dietary nitrogen to dairy cows. Journal of Dairy Science 100, 7116–7126.
Reynolds, C.K., Kristensen, N.B., 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: an asynchronous symbiosis. Journal of Animal Science 86 (suppl_14), E293–E305.
Satter, L.D., Slyter, L.L., 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. British Journal of Nutrition 32, 199–208.
Shingfield, K.J., Offer, N.W., 1999. Simultaneous determination of purine metabolites, creatinine and pseudouridine in ruminant urine by reversed-phase high performance liquid chromatography. Journal of Chromatography B: Biomedical Sciences and Applications 723, 81–94.
Spanghero, M., Kowalski, Z.M., 1997. Critical analysis of N balance experiments with lactating cows. Livestock Production Science 52, 113–122.
Spanghero, M., Kowalski, Z.M., 2021. Updating analysis of nitrogen balance experiments in dairy cows. Journal of Dairy Science 104, 7725–7737.
Tebbe, A.W., Weiss, W.P., 2020. Effects of oscillating dietary crude protein concentrations on production, nutrient digestion, plasma metabolites, and body composition in lactating dairy cows. Journal of Dairy Science 103, 10219–10232.