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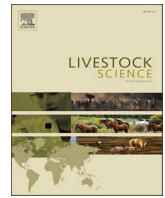
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Variation in individual milk production responses to supplementary protein feeding with two types of forages

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HIGHLIGHTS

- Production responses to supplementary protein were not related to forage type.
- Production responses were not related to animal variables during covariate period.
- Responses to supplementary protein were positively related to intake responses.
- Metabolizable protein supply per unit of intake increases with intake.
- High yielding cows may not need higher dietary protein concentration.

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ABSTRACT

The objectives of the present experiment were to investigate the effects of forage type and protein supplementation on feed intake and milk production, and between-cow variability in responses to protein supplementation. The experiment was conducted using 40 cows (28 Holstein, 12 Nordic Red, 25 multiparous, 15 primiparous). Experiment started with a covariate period of 14 d when cows received TMR (concentrate:forage, 40:60). After the covariate period four experimental diets were arranged in 2×2 factorial design with two forages and two concentrate CP levels. The forage treatment included grass silage (GS) and a mixture of whole-crop wheat/oats silage and grass silage (MIX; 40:60) on DM basis. The concentrate treatment included two concentrate CP levels [low CP (115 g CP/kg DM) and medium CP (166 g CP/kg DM)]. Concentrate CP concentration was increased by replacing barley and oats with rapeseed meal. The cows were fed the same forage throughout the 63-d experiment, whereas the CP treatments were compared in a switch-back design including three 21 d periods. Individual protein supplementation response was calculated based on switch-back periods. Feeding the MIX forage increased total DM intake by 1.3 kg/d compared with the GS diets but milk production was not influenced by forage type. Dry matter and nutrient intake increased with CP level similarly with forages. The production of milk components and milk urea concentration increased with CP level, whereas milk N efficiency decreased. Energy corrected milk (ECM) yield response to protein supplementation was not related to ECM yield measured during the covariate period whereas the ECM response was negatively related to calculated energy balance during covariate period. It is concluded that intake and production responses to moderate level of protein supplementation were highly variable, but the extent of variation could not be predicted from animal characteristics available in farm conditions. When the supply of metabolizable protein (MP) was simulated by the Karoline model for each animal, predicted MP concentration increased 0.8 g/kg increase in DM intake. This is compatible to increased MP requirement on DM basis with enhanced production level. Both the lack of the effects of initial production level on CP response and the higher predicted MP concentration with increased intake suggest that high yielding cows can deal with the higher MP requirement (on DM basis) without increasing dietary CP concentration.

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1. Introduction

The efficiency of utilization of feed N (milk N/N intake; MNE) is relatively low in milk production. According to Huhtanen and Hristov (2009) the average MNE was 0.25 in 736 North American diets and 0.28 in 998 North European diets. The MNE varied from 0.14 to 0.45 showing the possibility to improve the N efficiency (Huhtanen and Hristov, 2009). Because large amounts of N cannot be retained in body tissues, the major proportion of N intake in excess to milk N is excreted in manure, especially in urine (Tamminga 1992). Low marginal milk protein yield (MPY) responses (100–136 g/kg incremental CP intake) even to high quality protein supplements (Huhtanen et al., 2011a) suggest that the major fraction of incremental N given as protein supplements is lost in urine which is more vulnerable than faecal N for leaching and evaporative losses (Castillo et al., 2000).

Milk production and MNE responses to protein supplementation have been intensively studied, but variation in responses among the cows to protein supplementation has not received much attention. Milk yield decreases with reduced protein supplementation, but some cows may tolerate low protein diets better as they may have a higher efficiency of microbial protein synthesis. They may be metabolically more efficient in producing milk protein. By identifying responsive cows, or alternatively cows that have resilience to low protein diets (Liu and VandeHaar, 2020), greater MNE together with smaller ammonia losses could be obtained. The objective of the current study was designed to evaluate variation in production response to moderate level of protein supplementation. Heterogenous group of cows varying in feed intake, milk production, days in milk (DIM) and parity was used to determine possible factors affecting the responses. Two different basal diets based on two forages (grass silage and mixture of grass and whole-crop silage) were used to study if the CP concentration of basal diet affects on responses. The supply of MP for individual cows was predicted by the Nordic dairy cow model Karoline (Danfaer et al., 2006; Huhtanen et al., 2015b) to evaluate the effects of feed intake on dietary MP concentration. Karoline was used to test the hypothesis if increased supply of MP per unit of intake covers the higher MP requirement with increased milk yield without changing diet composition.

2. Materials and methods

2.1. Experimental feeds

The regrowth grass silage from timothy (*Phleum pratense*, cv. Tuure) and meadow fescue (*Festuca pratensis*, cv. Valtteri) sward was harvested on 27th July 2017 at the experimental farm of Natural Institute Finland (Luke) and stored in a bunker silo. The swards were cut using a mower conditioner, wilted for 12–24 h before harvesting with a self-propelled harvester and ensiled using a formic acid-based additive (AIV2 Plus Na, Eastman, Oulu, Finland) at a target rate of 5 l/t. The whole crop silage was harvested on 4th September 2017 from a mixture of oats (*Avena sativa*, cv. Bettina) and wheat (*Triticum aestivum*, cv. Puntari) sward using a direct-cut forage harvester. The crop was ensiled with formic acid-based additive (AIV Ässä Na) applied at the rate of 5 l/t and stored in a bunker silo.

Pelleted experimental concentrates (Raisioagro Ltd.) contained two levels of CP. The low CP concentrate (LCP) consisted of (g/kg on weight basis) of barley (390), oats (390), sugar beet pulp (180), vegetable oil (20) and minerals (20), respectively. The medium CP concentrate (MCP) consisted of barley (310), oats (310), sugar beet pulp (200), rapeseed meal (RSM, 140), vegetable oil (20) and minerals (20).

2.2. Animals, diets, and experimental design

The experiment was conducted with 40 cows (28 Holstein, 12 Nordic Red; 25 multiparous, 15 primiparous). The average (\pm SD) energy corrected milk yield (ECM) was 33.5 ± 4.60 kg, body weight (BW) $619 \pm$

72.8 kg and DIM 164 ± 58 d

The experiment started with a 14-day covariate period when the cows were fed a total mixed ration (TMR). Ingredients and nutritional values of the diets is presented in Table 1. After the covariate period the cows were blocked three blocks according to parity and DIM. The blocks were primiparous cows, mid-lactating and late-lactating multiparous cows. The forage treatment was randomly assigned to the cows within a block. After the covariate period the forages and concentrates were fed separately with the target of 400 g/kg of concentrate on DM basis. The experimental design was 2×2 factorial consisting of two concentrate CP levels and two forages. The forage treatments were grass silage (GS) and a mixture (MIX) of whole-crop silage and grass silage (400:600 g/kg on DM basis). The diets were GS + LCP, GS + MCP, MIX + LCP and MIX + MP, respectively. The cows were fed same forage through the experiment, whereas the design for concentrate treatments was a switch-back with three 21 d periods. The first 14 d of each period were used as a transition and the last 7 days were used for data collection.

The cows were kept in a loose-house dairy barn with rubber mat beds, offered TMR or experimental forages in separate compartments ad libitum with 10 % refusals and had a free access to drinking water. The daily TMR intake was measured with the Insentec feeders (Insentec BV, Marknesse, Netherlands). Concentrates were offered from automatic concentrate feeders (Pellon Ltd., Ylihärmä, Finland) which were equipped with BW scales. The cows were milked in a milking parlour twice daily starting at 6:00 and 16:00.

2.3. Sampling and chemical nutrient analysis

Milk samples were collected from 4 consecutive milking at the end of each period and stored with Bronopol in a refrigerator before analysis. The samples were analyzed for fat, protein, lactose, and urea using an infrared analyzer (Milkoscan FT6000, Valio Ltd., Seinäjoki). Milk composition was determined based on the weighted means of the a.m. and p.m. milking. Energy corrected milk (ECM) yield was calculated according to Sjaunja et al. (1990).

The DM concentration of TMR and silages were determined daily during the data collection period. Silages and concentrates for chemical analysis were sampled separately before TMR mixing during the data collection period, pooled per each experimental period, and stored at -20°C .

Table 1

Ingredients and nutritional values of experimental diets.

	Covariate period	GS ^a		MIX ^b	
		LCP ^c	MPC ^d	LCP	MCP
Grass silage	400	600	600	360	360
Whole crop silage	190			240	240
Compound concentrate		400	400	400	400
Barley	240				
Rapeseed meal	140				
Minerals	30				
g/kg DM ^e					
Neutral detergent fibre	378	408	414	402	408
Crude protein	170	147	169	134	156
Metabolizable protein	92.7	87.2	93.7	84.6	90.8
Protein balance in the rumen ^f	16.5	20.5	37.7	11.7	28.1
Metabolizable energy ^f , MJ/kg DM	11.1	11.1	11.0	10.9	10.8

^a Grass silage.

^b Grass silage and whole crop silage mixture in proportion of 60:40 as DM basis.

^c Low crude protein concentrate.

^d Medium crude protein concentrate.

^e Dry matter.

^f Luke 2023.

The DM concentration of feeds were determined by drying the samples at 105 °C for 20 h, while samples for chemical analyzes were dried at 60 °C. Dry samples were analyzed for ash (AOAC 1990, No 942.05), nitrogen (N) with the Dumas method using a Leco FP 428 analyzer, and neutral detergent fibre (NDF) according to Van Soest et al. (1991) using Na-sulphite without amylase for forages and presented ash-free. The silage samples were analyzed for pH, volatile fatty acids (VFA, Huhtanen et al. 1998), lactic acid (Haacker et al., 1983), ammonia N (McCullough 1967), water soluble N (AOAC 1990, No 984.13), water soluble carbohydrates (Somogyi 1945) and in vitro organic matter pepsin-cellulase solubility (OMS) according to Nousiainen et al. (2003). The in vitro digestibility results were calculated with correction equations to convert OMS values into in vivo digestibility using equations based on a data set comprising of Finnish in vivo digestibility trials (Huhtanen et al., 2006).

2.4. Simulation of nutrient supply

The supply of nutrients was predicted for each cow/period observations by the mechanistic dynamic dairy cow model Karoline (Danfaer et al., 2006; Huhtanen et al., 2015b). Metabolizable protein was calculated by assuming that microbial protein contained 80 % true protein and feed MP as the amount of feed protein fractions absorbed from the small intestine. In addition to Karoline, the feed MP was also calculated based on DMI according to NRC (2001).

2.5. Calculations and statistical analysis

The ME concentration of concentrates were based on the manufacturer's information. Silages ME concentration was calculated from the concentration of digestible organic matter (Luke 2023). Metabolizable protein expressed as amino acids absorbed from the small intestine and protein balance in the rumen (PBV) were calculated according to Luke (2023). Milk nitrogen efficiency was calculated as milk N / N intake. Relative forage and total DMI potential were estimated according to equations of Huhtanen et al. (2007). Individual production responses to protein supplementation within the forage were calculated as actual means of period differences: $0.5 \times (\text{Period}_1 + \text{Period}_3) - \text{Period}_2$ for the sequence MCP – LCP – MCP and as $\text{Period}_2 - 0.5 \times (\text{Period}_1 + \text{Period}_3)$ for the sequence LCP – MCP – LCP, respectively.

The regressions between the individual variables measured in the covariate period (milk production, DMI, milk yield, milk constituents, energy balance, DIM) and responses to protein supplementation during switch-back periods were tested using SAS regression analysis (SAS REG, Release 9.4, SAS Institute Inc., Cary, NC, USA). The number of observations in regression analysis for each independent variable was 40.

A statistical comparison of production variables was performed with SAS MIXED procedure (Release 9.4, SAS Institute Inc., Cary, NC, USA). The model included treatment, sequence, CP level, forage type and CP level \times forage type interaction as fixed variables. A cow and period were used as random variables. The effect of breed on milk and feed intake was rejected from the final model due the lack of significance. The covariate measured during the last 7 days of pre-experimental period was used with production variables. Due the use of covariate, the block was rejected from the final model.

3. Results

3.1. Experimental forages

Both grass and whole-crop silages had low pH (4.18 vs. 3.93, respectively) with moderate fermentation quality in terms of acid and ammonia concentrations (Table 2). Both silages had relatively low ME concentrations. Silage intake potential expressed as SDMI-index was higher for the silage mixture than grass silage (Huhtanen et al., 2011b). Metabolizable protein balance value was negative for the LCP diet and

Table 2
Composition of experimental silages and concentrate supplements.

	Grass silage	Whole crop silage ^a	Grass-Whole crop mixture ^b	Concentrate	
				LCP ^c	MCP ^d
DM ^e , g/kg	221	321	261	882	884
Chemical composition, g/kg DM					
Ash	103	67	89	62	62
Neutral detergent fibre	518	457	494	244	257
Crude protein	171	118	150	115	166
Starch	0	161	64.3	421	341
Water soluble carbohydrates	16	28	21		
Lactic acid	67	39	56		
Volatile fatty acids	34	15	26		
Ammonium N, g/kg N	97	63	83		
Silage DM-intake index	82	99	89.0		
Feeding values					
ME ^f , MJ/kg DM	10.3	9.5	10.0	12.2	12.0
MP ^g	81	77	79	95	111
PBV ^h	51	34	44	-19	20
pH	4.18	3.93	4.08		
D-value ⁱ , g/kg DM	645	615	633		
Ammonium N, g/kg N	97	64	84		
Soluble N, g/kg N	553	614	577		

^a Oats and wheat in proportion of 40:60 as seed basis.

^b The mixture of grass silage and whole crop silage in proportion of 60:40 as DM basis.

^c Low crude protein concentrate.

^d Medium crude protein concentrate.

^e Dry matter.

^f Metabolizable energy, Luke 2023.

^g Metabolizable protein, Luke 2023.

^h Protein balance in the rumen.

ⁱ Concentration of digestible organic matter in DM.

positive for the MCP diet. The whole crop silage was typically low in NDF concentration compared with grass silage.

3.2. Feed intake and milk production

Table 3 includes the data collected during covariate period. The variation in production parameters was large due experimental design, which included different stages of lactation.

The cows fed the MIX diets had 1.1 and 1.3 kg/d ($P < 0.01$) higher forage and total DMI than the cows fed the GS diets (Table 4). Protein

Table 3
Intake and production variables during covariate period.

	Mean	SD	Min	Max
Intake, kg dry matter/d				
Silage	11.5	1.49	9.1	14.1
Concentrate	9.8	1.03	8.1	11.6
Total	21.3	2.52	17.2	25.7
Crude protein	3.12	0.365	2.53	3.76
Metabolizable protein	1.89	0.219	1.54	2.28
Metabolizable energy, MJ	237	27.9	191	286
Milk yield, kg/d	30.5	5.07	19.9	40.3
Energy corrected milk yield, kg/d	33.5	4.64	24.1	43.4
Milk composition, g/kg				
Fat	47.5	5.7	37.5	57.5
Protein	35.9	2.64	30.9	41
Lactose	46.6	1.84	41.8	49.7
Urea, mg/dl	24.7	4.28	17.2	33.8
Body weight, kg	619	73.4	472	794
Days in milk	159	58.4	83	385
Days in pregnant	38	38.0	0	130
Lactation	2.48	1.63	1	8

Table 4
Feed and nutrient intake.

	GS ^a		MIX ^b		SEM		P-values		
	LCP ^c	MPC ^d	LCP	MCP	Silage	Protein	Silage	Protein	S × P
Intake, kg/d									
Silage DM ^e	11.6	12.1	12.5	13.3	0.26	0.22	<0.01	<0.01	0.10
Concentrate DM	8.9	8.9	9.1	9.1	0.21	0.16	0.38	0.50	0.80
Total DM	20.5	21.0	21.6	22.4	0.29	0.22	<0.01	<0.01	0.10
Neutral detergent fibre	8.15	8.54	8.60	9.13	0.131	0.106	<0.01	<0.01	0.09
Crude protein	3.00	3.54	2.90	3.49	0.045	0.034	0.21	<0.01	0.13
Metabolizable protein	1.78	1.96	1.83	2.04	0.026	0.019	0.10	<0.01	0.11
PBV ^f	0.42	0.79	0.25	0.63	0.018	0.015	<0.01	<0.01	0.42
Metabolizable energy, MJ/d	227	231	235	242	3.2	2.3	0.03	<0.01	0.14

^a Grass silage.^b Grass silage and whole crop silage in proportion of 60:40 as DM basis.^c Low crude protein concentrate.^d Medium crude protein concentrate.^e Dry matter.^f Protein balance in the rumen.

supplementation increased total DMI by 0.7 kg/d ($P < 0.001$) with the effect being slightly greater with the MIX diets compared with GS diets silage (interaction $P = 0.10$). The differences in CP intake were not significant but estimated MP intake tended ($P = 0.07$) to be greater for the MIX diets. Calculated PBV was positive for all diets, and greater ($P < 0.01$) for the GS than for the MIX and it increased with protein supplementation.

Forage type did not affect milk yield, ECM yield or yield of milk components (Table 5), except milk protein concentration was higher ($P = 0.03$) in cows fed the MIX diets than in those fed the GS diets. Feed efficiency expressed as ECM/DMI was higher ($P < 0.01$) for the GS diets compared with the MIX diets. Increased protein supplementation increased ($P < 0.001$) the yields of milk, ECM, and milk components. Milk fat ($P = 0.03$) and lactose concentration ($P < 0.001$) decreased with increased protein supplementation, whereas milk protein and milk urea (MU, mg urea/dl) concentrations increased ($P < 0.01$). However, quantitatively the effects were small except for MU. Protein supplementation increased MU more ($P < 0.01$) in cows fed the MIX diets compared with those fed the GS diets.

3.3. Individual cow responses

The responses to protein supplementation were highly significant

Table 5
Milk yield, milk composition and feed efficiency.

	GS ^a		MIX ^b		SEM		P-values		
	LCP ^c	MPC ^d	LCP	MCP	Silage	Protein	Silage	Protein	S × P
Milk yield, kg/d	27.4	28.7	27.0	28.4	0.73	0.67	0.59	<0.001	0.76
ECM ^e yield, kg/d	29.9	30.9	29.7	31.4	0.66	0.58	0.88	<0.001	0.19
Milk composition, g/kg									
Fat	48.0	46.6	48.4	48.3	0.69	0.55	0.23	0.03	0.05
Protein	35.3	36.0	36.0	36.7	0.36	0.33	0.04	<0.001	0.94
Lactose	44.6	44.2	44.7	44.4	0.28	0.27	0.38	<0.001	0.99
Urea, mg/dl	20.0	25.9	17.1	25.7	0.77	0.68	0.06	<0.001	<0.01
Milk components, g/d									
Fat	1296	1321	1289	1357	27.6	23.2	0.61	<0.001	0.07
Protein	961	1028	963	1035	19.4	16.6	0.81	<0.001	0.76
Lactose	1214	1262	1204	1256	38.5	35.5	0.80	<0.001	0.81
Feed efficiency									
ECM/DMI ^f , kg/kg	1.45	1.47	1.37	1.39	0.019	0.017	<0.01	0.04	0.87
Milk N/N intake	0.31	0.28	0.32	0.29	0.004	0.003	0.11	<0.001	0.39

^a Grass silage.^b Grass silage and whole crop silage in proportion of 60:40 as DM basis.^c Low crude protein concentrate.^d Medium crude protein concentrate.^e Energy corrected milk.^f Dry matter intake.**Table 6**
Average responses to increased protein supplementation.

	Response	SD	P-value
DM ^a intake, kg/d	0.65	0.491	<0.001
CP ^b intake, g/d	564	109.6	<0.001
MP ^c intake, g/d	192	50.3	<0.001
Milk yield, kg/d	1.38	0.813	<0.001
Energy corrected milk yield, kg/d	1.42	1.008	<0.001
Milk composition, g/kg			<0.001
Fat	-0.7	1.92	<0.001
Protein	0.7	0.70	0.03
Lactose	-0.4	0.58	<0.001
Milk urea, mg/100 ml	7.3	3.44	<0.001
Milk protein			
g/d	69	35.4	<0.001
g per g CP /kg DM	0.12	0.062	<0.001
g per g MP/kg DM	0.37	0.182	<0.001

^a Dry matter.^b Crude protein.^c Metabolizable protein.

excluding milk protein concentration ($P < 0.001$; Table 6). Quantitatively the responses in milk composition were small except for MU concentration. The coefficient of variation (CV) in protein responses

variables was high for milk protein yield (50 %) and even higher for milk (59 %) and ECM yield (71 %). The mean and SD of responses were not related to the length of recoding period (Fig. 1) which suggests that one week measurement period was decent.

The responses of ECM to supplementary protein were not related to ECM yield (Table 7). The ECM response was greater ($P = 0.01$) in cows with negative ME balance during the covariate period compared with cows in positive ME-balance (2.64 vs. 1.46 kg/d).

Milk protein yield responses (g / incremental unit of CP or MP) were not related to ECM or protein yield during the covariate period. Calculated ME balance was negatively related to milk protein yield responses when expressed per changes in dietary CP or MP concentrations, similarly.

3.4. Modelling MP

The differences in MP supply predicted by the Karoline model reflected similar pattern to values based on Luke (2023) feed tables but were numerically about 6 % greater. Predicted increase in dietary MP concentration of the same diet with increased DMI was similar to the changes in MP requirement with increased production and intake in NRC (2001) and Luke (2023) systems (Fig. 2).

4. Discussion

4.1. Intake and production responses

The individual amount of concentrate for experimental periods was fixed according to intake measurement during covariate period. The realised concentrate proportion was little higher compared with the target of 400 g/kg in DM and it was 15 g/kg DM higher with MIX compared with GS. The difference was so small that it had minor effect on results.

Observed increase in DMI (1.3 kg/d) with MIX agreed with the predictions (1.4 kg/d) based on silage DMI index (Huhtanen et al., 2007). Grass silage was harvested from regrowth, and it had lower DM concentration and higher total acid concentration than whole crop silage, all which have negative effects on silage DMI. Feeding mixtures of whole-crop and grass silages has caused positive associative effects in intake compared with feeding a single forage (Huhtanen et al., 2007; Jaakkola et al., 2009). Increased DM intake compensated the low digestibility of MIX compared with GS, and the calculated ME intake was higher with MIX diets.

Increasing dietary CP concentration by RSM supplementation increased total DMI by 0.30 kg per 10 g/kg increase in CP which agrees with the meta-analysis of RSM supplementation studies (Huhtanen et al., 2011a). The average intake response to RSM supplementation (0.7 kg DM/d) is in line with the study of Jaakkola et al. (2009). In their

study the intake responses to RSM supplementation were not related to the proportion of whole-crop silage.

No differences were observed between the forages in milk yield or milk components although calculated ME intake was about 10 MJ/d greater for the MIX diets compared with the GS diets. Assuming 0.10 kg ECM/MJ ME production response to increased ME intake when the cows are close to zero energy balance, ECM yield should have been about 1.0 kg/d greater for cows fed the MIX diets. However, the depression in diet digestibility at production level of intake could be greater for the MIX diets compared with GS. This is supported by Ahvenjärvi et al. (2006) who suggested that the digestibility of pdNDF is decreased with increased proportion of whole-crop silage. Overall, the positive associative intake effects of feeding mixtures of grass and whole crop silages compensates for the lower digestibility of whole crop silages.

The average MPY responses of 125 g/kg incremental CP intake agrees with the values of 136 and 133 g/kg CP for untreated and heat-treated RSM in the meta-analysis of Huhtanen et al. (2011a). The positive effect of RSM on milk and protein yield was similar in cows fed diets based on GS or MIX despite lower CP concentration in MIX. When forage CP concentration was increased by earlier harvesting of grass (Rinne et al., 1999), higher N fertilization of grass sward (Shingfield et al., 2001) or increasing replacement of grass silage with red clover silage (Gidlund et al., 2017) production responses to increased protein supplementation were not related to forage CP concentration.

The efficiency of N utilization of GS was in accordance with published reviews (Castillo et al., 2000; Huhtanen and Hristov, 2009). Typically, the utilization decreases with increasing diet CP concentration, but forage type had no significant effect despite of 12 g/kg DM difference between MIX and GS. Increased intake of MIX increased CP intake which compensated low CP content. Using treatment mean values MNE decreased 1.28 g/kg N ($R^2 = 0.92$) per 1 g/kg DM increase in dietary CP concentration. This value agrees with Huhtanen and Hristov (2009) who reported corresponding decreases of 1.21 and 1.40 for typical North European and North American diets, respectively.

4.2. Individual cow responses

Milk yield response remained stable irrespective of the length of measurement period suggesting that full response to protein supplementation was obtained in one week (Fig. 1). With post-ruminal casein infusion changes over time for key response variables indicated that a 4-day adaptation period was appropriate (Ardalan et al., 2022). Choung et al. (1993) reported that maximum response to abomasal casein infusion was reached in 3 days. Even shorter period reaching the complete response was reported by Whitelaw et al. (1986). Although reaching the full production response with protein supplementation can take longer compared with protein infusions, stable responses (Fig. 1) indicate that the length of adaptation or measurement period did not affect the results. Neither between-cow variability in production was not influenced by the length of measurement periods

There was considerable variability among cows in ECM (coefficient of variation, CV = 0.70) and milk protein yield (CV = 0.50) responses to increased CP supplementation. High variability in ECM responses can partly be related to random sampling errors in milk fat composition. Numerically ECM and MPY responses were positive for 38 and 39 out of 40 cows. The two cows with negative ECM responses had unexpected low milk fat concentration with MCP diet. Ideally, protein supplements should be given to the cows that respond better to optimize economy and to reduce environmental emissions. The practical problem is how to identify the cows responding better to increased CP intake and/or less to decreased CP intake. To be useful the response predictor should be easily available in farm conditions, such as current milk yield and composition, DIM, and parity. Intake of efficiency variables are not practical due to difficulties in measuring DMI on farm conditions.

The proportion of RSM in dietary DM was constant, i.e., high yield

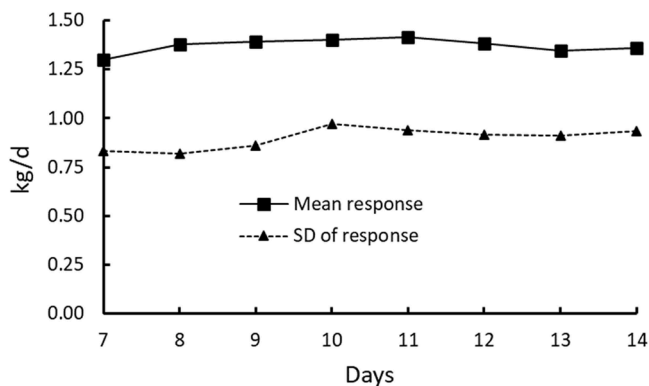


Fig. 1. The effect of the length of recording period on mean and standard deviation (SD) of milk yield responses to supplementary protein feeding.

Table 7

The effects of covariate period variables on production responses to protein supplementation.

Covariate	Intercept Estimate	SE	P-value	Slope Estimate	SE	P-value	RMSE	R ²
ECM ^a response, g /kg MP intake								
Milk, kg/d	4.48	3.484	0.21	0.03	0.112	0.75	3.57	0.003
ECM, kg/d	-0.34	4.066	0.93	0.17	0.120	0.15	3.48	0.054
DMI ^b , kg/d	5.14	4.939	0.30	0.02	0.229	0.93	3.58	0.000
Body weight, kg	4.26	4.909	0.39	0.00	0.008	0.79	3.57	0.002
Days in milk	6.45	1.649	<0.001	-0.01	0.010	0.57	3.56	0.008
Milk fat, g/kg	-4.74	4.506	0.30	0.21	0.094	0.03	3.35	0.123
ME ^c balance, MJ/d	25.6	17.52	0.15	-0.73	0.366	0.05	13.0	0.095
Milk protein/fat	42.2	19.74	0.04	-38.8	14.85	0.01	12.6	0.152
MU ^d	3.69	3.35	0.28	0.07	0.133	0.57	3.56	0.008
Protein yield response, g/kg CP intake								
ECM, kg/d	61	73	0.41	1.9	2.16	0.38	62.5	0.02
Protein yield, g/d	80	71	0.27	0.04	0.065	0.52	62.8	0.011
DMI, kg/d	155	87	0.08	-1.4	4.04	0.73	63.0	0.003
MU, mg/100 mL	92	59.1	0.13	1.3	2.35	0.58	62.9	0.008
ME balance, MJ/d	110	11.3	<0.001	-1.7	0.70	0.02	58.9	0.131
Protein yield response, g/kg MP intake								
ECM, kg/d	254	215	0.24	3.5	6.36	0.59	184.1	0.008
Protein yield g/d	299	208.7	0.30	0	0	0.82	184.5	0.003
DMI, kg/d	510	254.1	0.05	-6.5	11.81	0.58	184.1	0.008
Days in milk	344	85.4	0	0.2	0.51	0.74	184.6	0.003
MU, mg/100 mL	274	173	0.12	3.9	6.89	0.57	184.1	0.008
ME balance, MJ/d	329	33.3	<0.001	-4.6	2.06	0.03	173.9	0.115

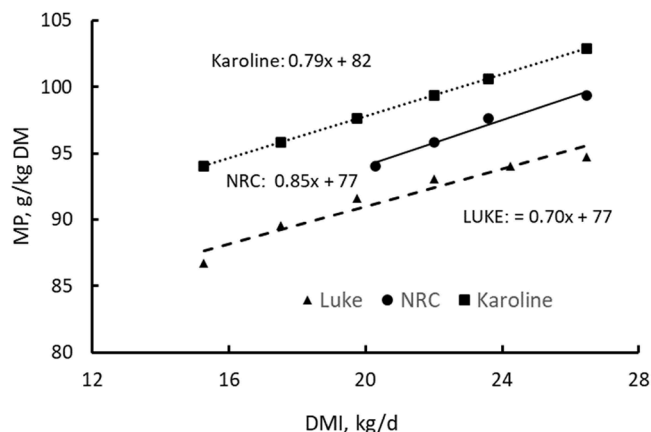
^a Energy corrected milk.^b Dry matter intake.^c Metabolizable energy.^d Milk urea.

Fig. 2. The effect of dry matter intake (DMI) on MP requirement (g/kg DMI) according to NRC (2001) and Luke (2023) systems and predicted changes in MP concentration (g/kg DM) according to Karoline simulations when fed experimental diets.

cows with higher DMI also consumed more RSM. When the ECM response to protein supplementation was expressed per kg RSM intake (or incremental CP intake) the effect of covariate ECM yield on the response was not significant. Overall, the effects of covariate ECM yield on protein responses were quantitatively small. Reallocating RSM from low yielding cows to high yielding cows would result in 0.11 kg increase in average ECM yield / d.

Negative relationship between calculated ME balance during the covariate period and ECM response could be expected. This phenomenon agrees observations from energy metabolism. In a respiration chamber study (Kirkland and Gordon, 2001) early lactation cows in negative energy balance partitioned more energy to milk than late lactation cows in positive energy balance. It is also possible that the cows in negative ME balance were more efficient than cows in positive ME balance, since ME balance was calculated from maintenance and

production coefficients. The calculation of ME balance also requires data on DMI that is not available in practical farms.

Milk protein yield response was not related with ECM or protein yields during the covariate period when expressed per kg CP or MP intake (Table 7). In agreement with the present study, responses to increased MP supply were not related to average milk production level in the study (Huhtanen and Nousiainen 2012). The lack of DIM effects on MPY agrees with Liu and VandeHaar (2020) who reported 0.14 and 0.12 kg/d higher MPY for high vs low protein diets in early and late lactation, respectively.

Milk urea concentration is positively related to dietary CP concentration and negatively to MNE (Broderick and Clayton, 1997; Nousiainen et al., 2004) when dietary effects are considered. Therefore, it could be expected that cows with high MU have lower MNE than the cows with low MU when fed the same diet. In the present study MU was highly variable between the cows during covariate period (CV = 0.17), but it was not related to MNE. Neither the response to supplementary protein was related to MU during the covariate period or to the difference in MU between LP and MP treatments. The changes in MU between low and high CP diets were similar among high and low protein resilience cows in both early and late lactation (Li and VandeHaar, 2020). In the meta-analysis of individual cow data (Huhtanen et al., 2015a) MNE decreased significantly with increased MU concentration, but the effects were quantitatively too small for reliable ranking of the cows according to MNE.

The intake response (mean 0.65 kg DM/d) was highly variable among the cows (CV = 0.75, range -0.4 -1.8 kg/d), but it was insignificantly related to any animal (BW, DIM), feed intake, milk production and composition variables or calculated MP and ME balances during the covariate period.

Observed ECM responses to supplementary protein were more closely ($P = 0.001$, $R^2 = 0.25$) related to observed DMI response during the experiment than to DMI or ECM levels during the covariate period (ECM response (kg/d) = $0.75 \pm 0.23 + 1.02 \pm 0.29 \times$ DMI response (kg/d)). Both regression coefficient and intercept were significant ($P < 0.01$). Assuming ME concentration of 11 MJ/kg DM the ECM response was 0.093 kg ECM per MJ incremental ME that agrees with marginal ME intake responses in cows at different production levels in meta-analysis

of Huhtanen and Nousiainen (2012). Positive intercept could be interpreted as a specific protein effect.

Liu and VandeHaar (2020) reported greater decreases in DMI and ECM yield in low protein resilience cows compared with high protein resilience cows when dietary CP concentration was decreased. The same effects were observed in both early and late lactation. Their results indicated that there was significant variation in DMI responses among cows when fed low protein diet. Different DMI responses to supplementary protein could be explained by minimizing discomfort (Forbes, 2007). The animal must balance the problems arising from insufficient amino acids with those arising from excess energy. Greater intake responses to duodenal protein infusion compared with ruminal protein or glucose infusions (Faverdin et al., 2003) and to high quality (fish meal, RSM) protein supplements compared with low quality (feather meal, wheat gluten meal) protein supplements (Chamberlain et al., 1992; Shingfield et al., 2001) support this theory. Improved AA/ME balance increased milk production that “pulled” intake. It could be expected that there are between-cow differences in the MP supply even at the same intake due to differences in digesta passage rate.

4.3. Between-cow variation in MP supply

Although calculated MP/ME ratio is constant within a diet, difference in feed intake and passage rate can lead to substantial differences in the efficiency of microbial protein synthesis and escape of rumen undegraded protein. In the study of Volden (1999) the efficiency of microbial N synthesis per kg digested organic matter (OM) was on average 19 % greater for cows at high feeding level compared to those at low level of feeding (18 vs. 9 kg OM/d, respectively). Similarly, Broderick et al. (2010) reported increased microbial N efficiency with increased DMI. Most likely these effects are related to increased digesta passage rate and to reduced ATP requirement for microbial maintenance. Substantial between-animal variation in digesta passage kinetics (Pinares-Patiño et al., 2003; Cabezas-Garcia et al., 2017) suggest that MP/ME ratio can differ between the cows even when fed the same diet at same intake. The increase in the efficiency of microbial N synthesis with increased DMI was similar (0.29 vs. 0.34 g/kg OM truly digested per kg increase in DMI) in Karoline simulations and in the analysis of omasal flow data (Broderick et al., 2010). Whether the variation in the MP supply even at the same intake is related to variation in production responses to supplementary protein needs further investigations.

Calculated MP requirement per kg DMI increases with increased production level (NRC, 2001; Luke, 2023) and therefore dietary MP concentration should increase with milk yield to meet the calculated requirements. However, because of the faster digesta passage rate with increased dietary MP concentration increases with intake. According to the Karoline simulations predicted dietary MP concentration increased by 0.8 g/kg DMI with increased intake. The model simulations suggested that the MP supply from the same diet enhanced with DMI to cover increased MP requirements per kg DMI with increased intake and production (Fig. 2). This suggests greater MP flow per kg DMI covers the need for higher dietary MP concentration with milk production. In line with this, in the analysis of data from 21 milk production studies evaluation responses to supplementary protein feeding only in one study the blocks of high yielding cows responded better than low yielding cows (Sairanen et al., unpublished report)

5. Conclusions

Moderate level of protein supplementation increased feed intake and milk production independently of forage type. Intake and production responses varied largely among cows. The responses of ECM and milk protein to protein supplementation were negatively related to calculated energy balance but poorly related to production level during the covariate period. However, ECM response was significantly related to DMI response, but DMI response was not related to any variables measured

during the covariate period. Because MU during covariate period and MU responses to the changes in dietary CP concentration were poorly associated with intake or production responses, we suggest that using MU to rank cows for protein efficiency or responses to protein may be misleading. It is concluded that intake and production responses to moderate level of protein supplementation were highly variable, but the extent of variation could not be predicted from available animal characteristics. More work is needed to examine whether the protein responses are repeatable across other types of basal diets and different number of lengths of time periods.

CRedit authorship contribution statement

A. Sairanen: Funding acquisition, Project administration, Data curation, Resources, Validation, Investigation, Writing – original draft. **P. Huhtanen:** Conceptualization, Methodology, Formal analysis, Supervision, Validation, Writing – original draft.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Auvo Sairanen reports financial support was provided by Valio Ltd., Raisioagro Ltd.

References

- Ahvenjärvi, S., Joki-Tokola, E., Vanhatalo, A., Jaakkola, S., Huhtanen, P., 2006. Effects of replacing grass silage with barley silage in dairy cow diets. *J. Dairy Sci.* 89, 1678–1687. [https://doi.org/10.3168/jds.S0022-0302\(06\)72235-4](https://doi.org/10.3168/jds.S0022-0302(06)72235-4).
- AOAC, 1990. Official Methods of Analysis. Association of Official Analytical Chemists, Inc., Arlington, VA, USA. ISBN 0-935584-42-0.
- Ardalan, M., Hussein, A.H., Titgemeyer, E.C., 2022. Effect of post-ruminal casein infusion on milk yield, milk composition, and efficiency of nitrogen use in dairy cows. *Dairy* 3, 163–173. <https://doi.org/10.3390/dairy3010013>.
- Broderick, G.A., Huhtanen, P., Ahvenjärvi, S., Reynal, S.M., Shingfield, K.J., 2010. Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle - A meta-analysis. *Dairy Sci* 93, 3216–3230.
- Broderick, G.A., Clayton, M.K., 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy Sci.* 80, 2964–2971.
- Cabezas-Garcia, E., Krizsan, S.J., Shingfield, K.J., Huhtanen, P., 2017. Between-cow variation in digestion and rumen fermentation variables associated with methane production. *Dairy Sci.* 100, 4409–4424.
- Castillo, A., Kebreab, E., Beever, D., France, J., 2000. A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. *J. Anim. Feed Sci.* 9, 1–32.
- Chamberlain, D.G., Choung, J.J., Robertson, S., 1992. Protein nutrition of dairy cows receiving grass silage diets: effects of feeding a protein supplement of unbalanced amino acid composition. *J. Sci. Food Agric.* 60, 425430.
- Choung, J.J., Chamberlain, D.G., 1993. The effects of abomasal infusions of casein or soya-bean-protein isolate on the milk production of dairy cows in mid-lactation. *British J. of Nutr.* 69, 103–115. <https://doi.org/10.1079/bjn19930013>.
- Danfer, A., Huhtanen, P., Udén, P., Sveinbjörnsson, J., Volden, H., 2006. The Nordic dairy cow model, Karoline - description. *Nutrient Digestion and Utilization in Farm Animals: Modelling approaches.* CAB International, pp. 383–406.
- Faverdin, P., M'hamed, D., Vérité, R., 2003. Effects of metabolizable protein on intake and milk production of dairy cows independent of effects on ruminal digestion. *Anim. Sci.* 76, 137–146.
- Forbes, M.J., 2007. A personal view of how ruminant animals control their intake and choice of food: minimal total discomfort. *Nutr. Res. Rev.* 20, 132–146.
- Gidlund, H., Hetta, M., Huhtanen, P., 2017. Milk production and methane emissions from dairy cows fed a low or high proportion of red clover silage and an incremental level of rapeseed expeller. *Livestock Sci.* 197, 73–81. <https://doi.org/10.1016/j.livsci.2017.01.009>.
- Haacker, K., Block, H.J., Weissbach, F., 1983. Zur kolorimetrischen Milchsäurebestimmung in Silagen mit p-Hydroxydiphenyl. (On the colorimetric determination of lactic acid in silages with p-hydroxydiphenyl). *Arch. Tierernähr.* 33, 505–512. <https://doi.org/10.1080/17450398309425704>.
- Huhtanen, P., Blauwiekel, R., Saastamoinen, I., 1998. Effects of intraruminal infusions of propionate and butyrate with two different protein supplements on milk production and blood metabolites in dairy cows receiving grass silage-based diet. *J. Sci. Food Agric.* 77, 213–222.
- Huhtanen, P., Nousiainen, J., Rinne, M., 2006. Recent developments in forage evaluation with special reference to practical applications. *Agric. Food Sci.* 15, 293–323. <https://doi.org/10.2137/145960606779216317>.

- Huhtanen, P., Rinne, M., Nousiainen, J., 2007. Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. *Animal* 1, 758–770. <https://doi.org/10.1017/S175173110773673X>.
- Huhtanen, P., Hristov, A.N., 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. *J. Dairy Sci.* 92, 3222–3232. <https://doi.org/10.3168/jds.2008-1352>.
- Huhtanen, P., Hetta, M., Swenson, C., 2011a. Evaluation of canola meal as a protein supplement for dairy cows: a review and meta-analysis. *Can. J. Anim. Sci.* 91, 529–543.
- Huhtanen, P., Rinne, M., Mäntysaari, P., Nousiainen, J., 2011b. Integration of the effects of animal and dietary factors on total dry matter intake of dairy cows fed silage-based diets. *Animal* 5, 691–702. <https://doi.org/10.1017/S1751731110002363>.
- Huhtanen, P., Nousiainen, J., 2012. Production responses of lactating dairy cows fed silage-based diets to changes in nutrient supply. *Livest. Sci.* 148, 146–158.
- Huhtanen, P., Cabezas-Garcia, E.H., Krizsan, S.J., Shingfield, K.J., 2015a. Evaluation of between-cow variation in milk urea and rumen ammonia nitrogen concentrations and the association with nitrogen utilization and diet digestibility in lactating cows. *J. Dairy Sci.* 98, 3182–3196. <https://doi.org/10.3168/jds.2014-8215>.
- Huhtanen, P., Ramin, M., Udén, P., 2015b. Nordic dairy cow model Karoline in predicting methane emissions: 1. Model description and sensitivity analysis. *Livest. Sci.* 178, 71–80.
- Jaakkola, S., Saarisalo, E., Heikkilä, T., 2009. Formic acid treated whole crop barley and wheat silages in dairy cow diets: effects of crop maturity, proportion in the diet, and level and type of concentrate supplementation. *Agric. Food Sci.* 18, 234–256. <https://doi.org/10.2137/145960609790059569>.
- Kirkland, R.M., Gordon, F.J., 2001. The effects of milk yield and stage of lactation on the partitioning of nutrients in lactating dairy cows. *J. Dairy Sci.* 84, 233–240. [https://doi.org/10.3168/jds.S0022-0302\(01\)74473-6](https://doi.org/10.3168/jds.S0022-0302(01)74473-6).
- Liu, E., VandeHaar, M.J., 2020. Low protein resilience is an indicator of the relative protein efficiency of individual cows. *J. Dairy Sci.* 103, 11401–11412.
- Luke, 2023. Feed Tables and Nutrient Requirements. Natural Resources Institute Finland, Jokioinen, Finland. www.luke.fi/feedtables. Accessed on 1 April 2023.
- McCullough, H., 1967. The determination of ammonia in whole blood by direct colorimetric method. *Clin. Chim. Acta* 17, 297–304. [https://doi.org/10.1016/0009-8981\(67\)90133-7](https://doi.org/10.1016/0009-8981(67)90133-7).
- Nousiainen, J., Rinne, M., Hellämäki, M., Huhtanen, P., 2003. Prediction of the digestibility of the primary growth of grass silages harvested at different stages of maturity from chemical composition and pepsin-cellulase solubility. *Anim. Feed Sci. Tech.* 103, 97–111. [https://doi.org/10.1016/S0377-8401\(02\)00283-3](https://doi.org/10.1016/S0377-8401(02)00283-3).
- Nousiainen, J., Shingfield, K.J., Huhtanen, P., 2004. Evaluation of milk urea nitrogen as a diagnostic of protein feeding. *J. Dairy Sci.* 87, 386–398.
- NRC, 2001. Nutrient Requirements of Dairy Cattle, 7th ed. National Academy Press, Washington, DC.
- Pinares-Patiño, C.S., Ulyatt, M.J., Lassey, K.R., Barry, T.N., Holmes, C.W., 2003. Rumen function and digestion parameters associated with differences between sheep in methane emissions when fed chaffed lucerne hay. *J. Agric. Sci.* 140, 205–214.
- Shingfield, K.J., Jaakkola, S., Huhtanen, P., 2001. Effects of level of nitrogen fertilizer application and various nitrogenous supplements on milk production and nitrogen utilization of dairy cows given grass silage-based diets. *Anim. Sci.* 73, 541–554.
- Sjaunja, L.O., Baevere, L., Junkkarinen, L., Pedersen, J., Setälä, J., 1990. A Nordic proposal for an energy corrected milk (ECM) formula. Gaillon, P. & Chabert, Y. (eds.). In: Proceedings of the 27th session of International Committee of Recording and Productivity of Milk Animal. Paris, France, pp. 156–157.
- Somogyi, M., 1945. A new reagent for the determination of sugars. *J. Biol. Chem.* 160, 61–68. [https://doi.org/10.1016/S0021-9258\(18\)43097-9](https://doi.org/10.1016/S0021-9258(18)43097-9). The equipment used was Shimadzu double-beam UV-VIS spectrophotometer UV-1800 (Shimadzu Co., Kyoto, Japan).
- Tamminga, S., 1992. Nutrition management of dairy cows as a contribution to pollution control. *J. Dairy Sci.* 75, 345–357.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fibre and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2).
- Volden, H., 1999. Effects of level of feeding and ruminally undegraded protein on ruminal bacterial protein synthesis, escape of dietary protein, intestinal amino acid profile, and performance of dairy cows. *J. Anim. Sci.* 77, 1905–1918.
- Whitelaw, F.G., Milne, J.S., Ørskov, E.R., Smith, J.S., 1986. The nitrogen and energy metabolism of lactating cows given abomasal infusions of casein. *Br. J. Nutr.* 55, 537–556. <https://doi.org/10.1079/bjn19860061>.