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Rolling report: Effects of different levels of dietary crude protein during multiple lactations on dairy cows

PPP F4F WP6 - Long term low protein in dairy cows

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1 Introduction

The current nutritional models estimate that diets require about 15-16% CP on dry matter (DM) basis to fulfil the rumen degradable protein (RDP) and intestinal digestible protein (IDP) needs of a lactating dairy cow. Using a theoretical approach, Dijkstra et al. (2013a) calculated that levels of around 12% CP on DM basis of cows producing 40 kg/d fat- and protein corrected milk should achieve optimal N use for milk protein production, with minimal N losses. More recent work (Daniel et al., 2017) showed that protein requirements are not as fixed as previously thought. In their model-based analysis drawing on two published studies, they found that milk protein yield was quadratically related to the supply of both metabolizable protein (MP) and net energy (NE) (MP and NE expressed above maintenance requirements). Based on this relationship, it may be possible to reduce MP supply - even below the calculated requirements - without milk production losses, when properly compensated with extra NE supply.

In the past decade, several studies evaluated short term effects of lower dietary protein levels on cow metabolism (Sinclair et al., 2014). Cows were observed to adapt their metabolism in the short term when receiving low protein diets, by substantially reducing loss of N, particularly in urine, and in most studies, also by reducing milk volume (Colmenero and Broderick, 2016; Dijkstra et al., 2013b). Results from Daniel et al. (2017) indicated that for cows with different milk production levels, the response to a lower metabolizable protein content in the diet on the milk protein and milk energy yield was similar.

Long term effects (e.g., more than one lactation) of low protein diets on dairy cow production and health, have only sporadically been investigated. Reynolds (2019) investigated the long term effect of dietary CP levels of 14, 16 and 18% CP (all DM basis) for three consecutive lactations. He concluded that 16% CP level was optimal, because of the lower milk production and DM intake (DMI) observed for the 14% CP compared with 16% and 18% CP diets, and the less efficient N use for the 18% compared with 14% and 16% diets. However, Reynolds (2019) reduced both RDP and MP content of the diet, which makes it difficult to attribute the effects to either RDP or MP supply. In addition, the 16% CP diet was expected to be optimal, as RDP and MP supply was aimed to be at 100% of requirements. Law et al. (2009a,b) fed an 11.4% CP diet in comparison with 14.4% and 17.3% CP diets, and found decreased milk production during the first 150 days of lactation for 11.4% CP diet compared with both 14.4% and 17.3% CP diets, but a shorter and less severe negative energy balance. In the first 150 days of lactation, the 14.4% CP diet also resulted in lower milk production than the 17.3% CP diet, however, milk production did not differ between these CP levels in the second half of lactation (day 150 to 305). In this study, dietary CP concentrations were held constant within each period. The higher CP diets were formulated by replacing starch-rich cereal ingredients with protein- and fiber-rich feeds, resulting in substantially lower dietary starch concentrations at similar metabolizable energy supply. Law et al. (2009a,b) fed these diets for one lactation and did not investigate the effect during the next lactation. Recently, Lavery et al. (2025) fed 15%, 16%, and 17% CP diets to lactating dairy cows for a full lactation, with half of the cows in each group switching to a 14% CP diet after day 181. During the first 180 days, milk protein yield was unaffected by CP level. However, in the second half (day 181 to day 280), cows continuing on the 15% CP diet demonstrated lower milk protein yield compared with those on the 16% and 17% CP diets; cows switched to 14% CP also showed reduced milk protein yield compared to 16% and 17%.

The current study was primarily motivated by (1) what can be expected from feeding dairy cows CP levels that closely follow the changing CP requirements throughout a full lactation, and (2) what are the effects of feeding even lower CP levels than those used in previous long term studies of Reynolds (2019) and Lavery et al. (2025), but greater than the highly limiting level of 11.4% CP in Law et al. (2009a). The objective of this study was to determine the effects of diets containing 130, 145 or 160 g CP/kg DM on average during two full lactation on the performance (e.g., DMI, milk production and composition), health (e.g., disease incidence, immunologic response to vaccination), metabolism (e.g., digestibility, blood metabolites), and emissions (e.g., nitrogen excretion, methane production) in dairy cows.

2 Application for practice

The dairy sector has a substantial contribution to excretion of nitrogen (N) to the environment in the Netherlands. Harmful forms of N include ammonia (NH_3), nitrogen oxides (NO_x) and nitrous oxide (N_2O). N_2O is a greenhouse gas contributing to global warming, and both NH_3 and NO_x can cause acidification when they precipitate in excess in sensitive ecosystems and negatively impact biodiversity. Lowering the protein level in the dairy cattle diet is an effective measure to reduce urinary N excretion and subsequent NH_3 formation (Huhtanen et al., 2008). Reduction of the crude protein (CP) content of the diet, results in lower NH_3 emissions from barns as well as during the application of the manure on agricultural land. Nevertheless, in practice there are still concerns about feeding lower levels of CP, RDP, and MP than current

recommendations due to possible negative effects on dairy cow production and health, which have not been extensively evaluated under controlled experimental conditions.

3 Materials and method

3.1 Experimental Design

Sixty-five multiparous Holstein-Friesian dairy cows (parity 3.5 ± 1.27; mean ± SD) were used in a randomized complete block design and blocked per 3 cows according to parity, previous 305 d milk and protein yield, and expected calving date (Table 3.1). Within blocks, cows were randomly allocated over the 3 dietary treatments. Treatment groups were balanced for body weight, body condition score, and 305 d fat yield. The experimental diets were formulated to provide different levels of CP (including NH_3) in DM, targeting average CP levels across the entire lactation (44 wk) of 130 (LCP), 145 (MCP), and 160 (HCP) g CP/kg DM. All cows received the same partial mixed ration (PMR) and the difference in dietary CP level was achieved by feeding a low CP concentrate and a high CP concentrate in different ratios to the treatments groups (Figure 3.1). Total concentrate allowance was fixed and depended on days in milk, and was kept equal across treatment groups. Cows were monitored from calving, starting its assigned treatment diet on the first day of lactation, and received their assigned dietary treatment throughout the entire experiment. Cows were followed throughout two complete experimental lactations (L1 and L2) and into the first 4 weeks of a third lactation (L3), including the dry periods between calvings (D1 and D2).

Table 3.1: Blocking and balancing criteria and outcome (average) per treatment group

	LCP	**MCP**	**HCP**
Cows (n)	21	22	22
Parity after calving	3.8	3.8	3.9
Expected calving date	17-10-2022	21-10-2022	19-10-2022
Expected calving interval (d)	369	368	384
Previous 305 d lactation performance			
Milk yield (kg/d)	31.1	31.1	31.0
Fat content (g/100 g of milk)	4.57	4.58	4.58
Protein content (g/100 g of milk)	3.57	3.57	3.57
FPCM yield (kg/d)	33.6	33.7	33.5
Fat yield (g/d)	1,421	1,428	1,419
Protein yield (g/d)	1,108	1,111	1,106
Last measurement before dry off			
BW (kg)	741	779	760
BCS (scale 1 – 5)	3.39	3.51	3.43

BW = Body weight; BCS = Body Condition Score, from 1 = very skinny to 5 = very fat

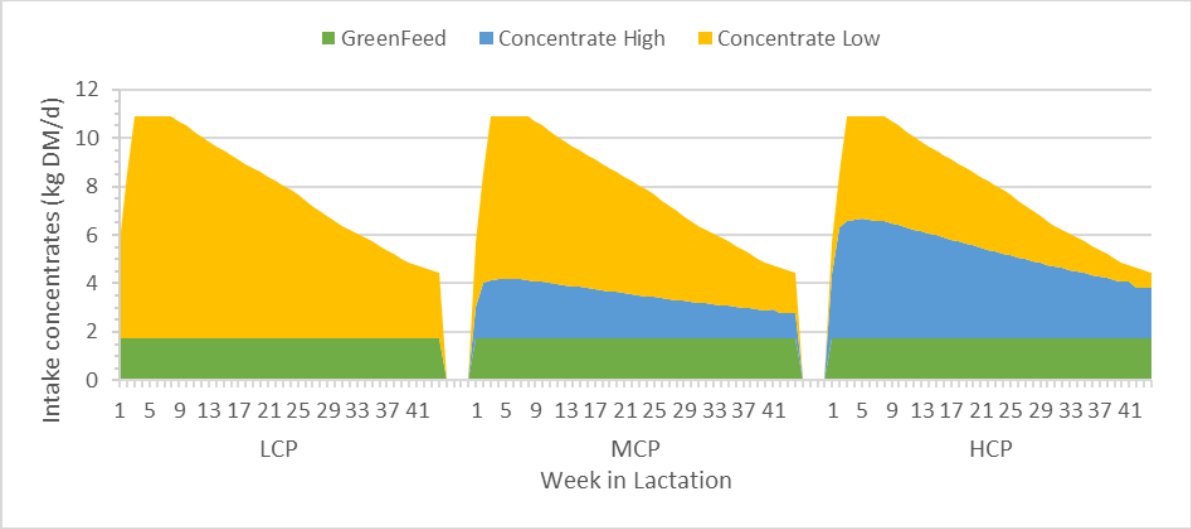


Figure 3.1. Scheduled intake of different concentrates (GreenFeed, Concentrate High and Concentrate Low) per week in lactation for cows of different treatment groups (LCP, MCP, and HCP respectively).

3.2 Diet, Feeding and Housing

The experimental diets consisted of a PMR (Table 3.2), two treatment concentrates, and a bait concentrate for the GreenFeed (Table 3.3). Cows were fed ad libitum the PMR consisting of 57.7% grass silage, 36.3% corn silage, 5.2% rapeseed meal and 0.8% premix on a DM basis. The treatment concentrates (low- and high-concentrates) were fed using 3 concentrate feeders. The low- and high-concentrates differed in nutrient composition, with the low-concentrate containing less crude protein and more starch and sugars than the high-concentrate, while maintaining a similar net energy content. Additionally, cows consumed bait concentrate in two GreenFeed units (GF). Based on the formulated feeding schedules, the CP concentrations of the intended feed ingredients, and an expected feed intake standardized across all treatment groups, an expected CP concentration per week in lactation was established (Figure 3.2).

The PMR was prepared and distributed 3 times daily using a Trioliet feed mixing robot (Triomatic HP 2 300, Trioliet BV, Oldenzaal, the Netherlands) and loaded in 32 Insentec feed bins (FB; RIC system, Hokofarm Group BV, Emmeloord, the Netherlands) for automatic individual intake registration. The cows had access to every FB and the same PMR, and 5% refusals of PMR was allowed. The FB recorded visit start and end times and weights, with weekly calibration using a standard weight. The feed intake measurement system used in this study is similar to that described in detail by Van Gastelen et al. (2022). Cows had unrestricted access to clean drinking water.

Cows were housed as 1 group in a free-stall barn equipped with 64 lying cubicles fitted with rubber mats, were bedded with sawdust and cleaned daily. The barn lighting schedule ran from 0500 to 2300 h. Milking occurred twice daily approximately at 0600 and 1600 h using a milking carousel (AutoRotor PerFormer, GEA Farm Technologies, Bönen, Germany).

Table 3.2. Chemical composition of grass silages, corn silages, rape seed meal and PMR offered during the full lactation (Sep ’22 to May ’25).

		Grass silage		Corn silage		Rape seed meal		PMR	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM	g/kg product	400	40.4	408	42.4	885	5.4	431[1]	18.9
Ash	g/kg DM	113	6.8	39	3.6	107	6.4	92	2.9
CP	g/kg DM	141	13.0	66	9.6	374	34.9	125	4.2
Fat	g/kg DM	38	4.8	31	2.3	34	3.4	35	2.7
Sugar	g/kg DM	55	36.4	n.d.	n.d.	111	2.7	39	21.8
Starch	g/kg DM	n.d.	n.d.	410	29.9	24	6.3	150	12.1
NDF	g/kg DM	502	12.8	353	30.4	291	13.4	433	9.8
ADF	g/kg DM	309	11.3	193	22.5	208	6.4	260	9.3
ADL	g/kg DM	19	1.8	14	2.0	77	3.9	20	1.8

NE_L	MJ/kg DM	6.3	0.11	7.0	0.18	6.8	0.17	6.5	0.09
VEM	/kg DM	907	16.5	1019	26.4	988	25.1	944	12.6
DVE '91	g/kg DM	54	2.6	52	5.2	144	4.9	57	1.2
OEB '91	/kg DM	24	12.8	-41	4.2	180	42.4	9	3.7

n.d. = Not determined
NE_L = Net energy for lactation, calculated with the VEM system (Van Es, 1978)
[1]Calculated without added water, which was added to keep DM content of PMR around 400 g/kg product

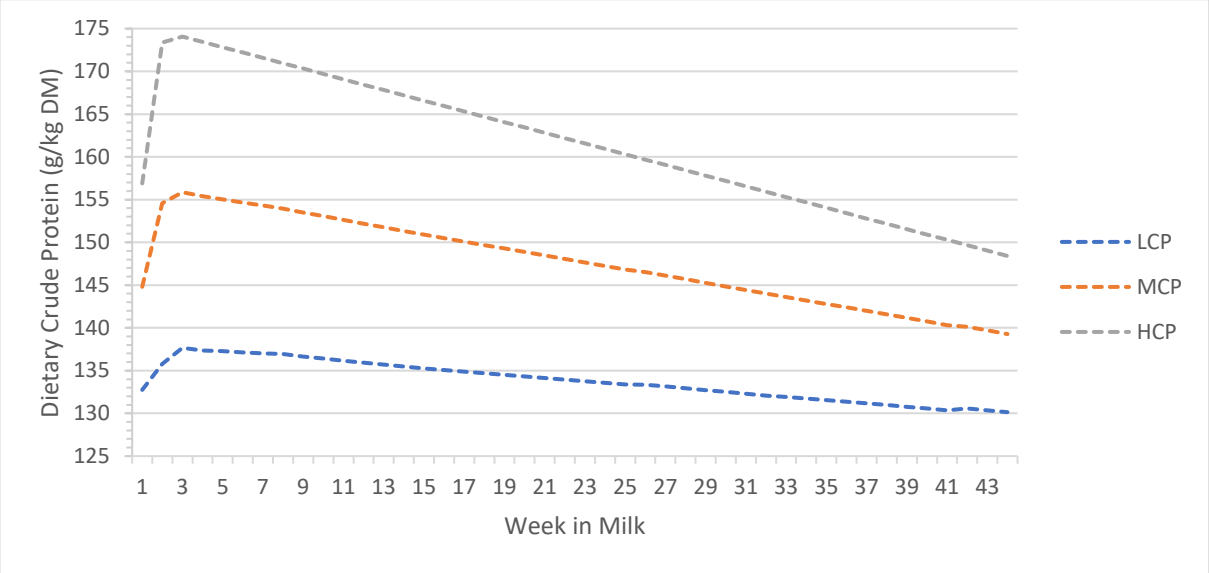


Figure 3.2. Expected crude protein levels per week in milk during the first 44 weeks of lactation for cows of different treatment groups (LCP, MCP, and HCP respectively).

3.3 General Measurements and Sample Collection

Feed composition was monitored throughout the study. Samples of all feed components were collected daily (roughages) or weekly (concentrates) and analyzed for dry matter content. In addition, a weekly sample of each feed component was collected for feed evaluation and stored at −20 °C until subsequent analysis. Samples of grass silage and corn silage were pooled per month and analyzed with NIRs (Eurofins, Wageningen, the Netherlands) to measure VEM, DVE and OEB content. Samples of all feed components (roughages, treatment concentrates, and GF bait) were pooled per 3 months and analyzed with wet chemical analysis (Eurofins, Wageningen, the Netherlands) for DM, ash, N, crude fat, sugar (except corn silage), starch (except grass silage), NDF, ADF, and ADL. Milk composition was analyzed weekly from samples collected on Tuesday afternoon and Wednesday morning. A 10 mL milk sample from each milking was collected in a tube containing 5 µL sodium azide for preservation and stored at 4°C for max 1 day.

Table 3.3. Chemical composition and ingredients of concentrates fed during full experimental period (Sep '22 to May '25).

		Concentrates					
Composition g/kg DM		Low	SD	High	SD	GF	SD
DM	g/kg	862	9.3	876	13.9	868	6.1
Ash	g/kg DM	59	2.9	62	3.9	76	2.6
CP	g/kg DM	146	4.3	304	7.2	140	3.2
Fat	g/kg DM	34	1.9	35	1.8	33	1.2
Sugar	g/kg DM	104	4.9	91	3.2	117	6.3
Starch	g/kg DM	261	6.9	190	7.5	110	6.6
NDF	g/kg DM	269	10.2	260	11.2	383	10.9
ADF	g/kg DM	157	4.6	137	7.8	234	3.0

ADL	g/kg DM	13	2.3	35	3.0	16	1.6
NE_L	MJ/kg DM	8.0	n.d.	8.0	n.d.	7.7	n.d.
VEM	/kg DM	1156	n.d.	1162	n.d.	1114	n.d.
DVE '91	g/kg DM	118	n.d.	208	n.d.	98	n.d.
OEB '91	g/kg DM	-21	n.d.	63	n.d.	-5	n.d.
Ingredients (%)							
Sugar beet pulp	%	35.30				30.75	
Soybean meal	%			29.00			
Wheat	%	23.00		12.00			
Soybean hulls	%	8.90				19.90	
Palm kernel expeller	%			15.30			
Corn	%	15.00		13.50		4.90	
Soybean meal, rumen bypass[1]	%	8.50		10.60			
Rapeseed meal, rumen bypass[2]	%			12.60			
Corn gluten feed meal	%					9.00	
Wheat middlings	%					7.50	
Alfalfa meal	%					7.00	
Sunflower seed meal	%					7.00	
Barley	%					5.00	
Glucose-rich syrup (from candy waste)	%					4.60	
Vinasse	%	4.02		4.00			
Rapeseed	%					2.10	
Sugar beet molasses	%	2.00		2.00			
Palm oil, Bergafat (C16:0 palmitic acid)	%					1.25	
Palm oil	%	1.60		0.30			
Sodium chloride	%					1.00	
Monocalcium phosphate	%	1.00					
Limestone	%			0.42			
Urea	%	0.35		0.10			
Premix	%	0.18		0.18			
Magnesium oxide 90%	%	0.15					

GF = GreenFeed; Low = low CP level; High = high CP level;
NE_L = Net energy for lactation, calculated with the VEM system (Van Es, 1978)
n.d. = Not determined
[1]Mervobest until juli 2023 and AminoGo soja from juli 2023 onwards
[2]Mervobest until juli 2023 and AminoGo raap from juli 2023 onwards

Table 3.4. Chemical composition of diets with a low, medium and high CP levels (LCP, MCP and HCP, respectively) calculated based on total daily dry matter intake and averaged for the full lactation (1-44 WIM).

		LCP		MCP		HCP	
L1		Mean	SD	Mean	SD	Mean	SD
Ash	g/kg DM	82	1.1	84	0.8	84	1.0
CP	g/kg DM	133	1.1	143	1.4	154	2.3
Fat	g/kg DM	36	0.3	36	0.3	36	0.3
Sugar	g/kg DM	53	4.1	49	3.6	48	4.2
Starch	g/kg DM	170	4.0	163	3.7	158	3.8
NDF	g/kg DM	388	5.7	393	4.4	393	4.6
ADF	g/kg DM	234	3.2	236	2.6	235	2.9
ADL	g/kg DM	18	0.6	20	0.7	21	0.8

NE_L	MJ/kg DM	7.0	0.05	7.0	0.04	7.0	0.05
VEM	/kg DM	1021	7.5	1015	6.0	1014	6.7
DVE '91	g/kg DM	77	1.9	82	1.7	88	2.2
OEB '91	/kg DM	0	1.1	6	0.9	13	1.0
L2							
Ash	g/kg DM	84	3.3	85	3.0	86	3.1
CP	g/kg DM	133	3.2	144	3.7	156	4.3
Fat	g/kg DM	35	1.0	35	1.1	35	0.9
Sugar	g/kg DM	60	8.6	57	9.5	55	6.9
Starch	g/kg DM	181	5.3	174	5.5	170	5.0
NDF	g/kg DM	385	6.0	388	6.1	388	5.0
ADF	g/kg DM	229	4.8	230	5.3	228	4.2
ADL	g/kg DM	18	1.7	20	1.5	22	1.3
NE_L	MJ/kg DM	7.0	0.07	7.0	0.06	7.0	0.05
VEM	/kg DM	1017	9.8	1011	8.5	1011	7.8
DVE '91	g/kg DM	77	2.0	81	2.3	88	2.7
OEB '91	/kg DM	2	2.5	9	2.3	15	2.1

NE_L = Net energy for lactation, calculated with the VEM system (Van Es, 1978)
n.d. = Not determined

3.4 Samples, Digestibility and Balance Periods

Blood samples were collected once weekly on a fixed day during the first 4 weeks of both lactations. During the dry period between the two lactations, additional blood samples were collected 4 and 2 weeks before the expected calving date. All blood samples were obtained from the coccygeal vein and collected into two serum tubes and two EDTA tubes per sampling event.

Additionally, samples were collected during four distinct sampling periods, consisting of two consecutive lactations and two lactation stages per lactation: peak lactation (7–12 weeks in milk; WIM) and mid lactation (30–35 WIM). For each sampling period, feces and urine were collected over three consecutive days at six time points, while blood and rumen fluid were collected over two consecutive days at four time points (Figure 3.3). During each sampling event, blood collection was always performed first, followed by feces and urine collection, and finalized with rumen fluid collection.

Blood samples were taken from the coccygeal vein and collected into two serum tubes and two EDTA tubes per cow. Fecal and urine samples were collected spontaneously whenever possible, or alternatively by rectal grab (feces) or perineal stimulation (urine). Urine samples were collected in 60 ml cups and kept on ice immediately after collection. Rumen fluid was collected by inserting a sampling probe orally into the rumen and aspirating rumen contents using a manual or electric pump. The first 250-500 ml was discarded due to the risk of saliva contamination, after which uncontaminated fluid was collected in a separate container and sampled in 50 ml collection tubes and placed on ice immediately.

EDTA tubes were immediately placed on ice and centrifuged for 15 min at 5,000 × *g*; the plasma was then divided into multiple aliquots. Serum tubes were allowed to clot at room temperature for approximately 2.5 h before centrifugation for 15 min at 5,000 × g, after which serum was portioned into several aliquots. After collection, rumen fluid samples were centrifuged for 15 min at 1,500 × g and subsamples of 3 ml per sample event were put in a 15 ml collection tube, leading to a total pooled sample of 12 ml rumen fluid per cow per period. The collection tubes were stored at −20 °C between sampling events. Urine samples were stored at −20 °C per individual sample event and pooled per sample period in the lab shortly before analysis. Fecal samples were subsampled immediately after collection, with 150 g of feces added to a collection bucket at each sampling event, resulting in a total pooled sample of 1,200 g of feces per period. The collection bucket was stored at −20 °C between sampling events. All sample aliquots were stored at −20°C until further analysis.

3.5 Immune challenge by vaccination

Cows were vaccinated twice during each of two consecutive lactations using a combination vaccine against bovine herpesvirus type 1 (BHV-1) and bovine viral diarrhea virus (BVDV). Vaccinations were administered either during mid lactation (14–19 WIM) or late lactation (35–40 WIM). Blood samples were

taken immediately before vaccination (day 0) and at 1, 2, 3, 5, 14, 28, and 42 days post-vaccination. Blood samples collected on days 0, 1, 2, 3, and 5 relative to vaccination were submitted to Royal GD (Deventer, the Netherlands) for analysis of acute phase response indicators, whereas samples collected on days 0, 14, 28, and 42 were analyzed at Wageningen Bioveterinary Research (WBVR, Lelystad, the Netherlands) for determination of antibody titers.

			Sample type			
	Sample period		Blood	Feces	Urine	Rumen fluid
Lactation 1	Early lactation	Wk 1, 2, 3 and 4	1 per week			
	Digestibility and Balance	Wk 7-12	2 days, 2 per day	3 days, 2 per day	3 days, 2 per day	2 days, 2 per day
	Vaccination	Wk 14-19	1 per day Day 0, 1, 2, 3, 5, 14, 28, 42 relative to vaccination			
	Digestibility and Balance	Wk 30-35	2 days, 2 per day	3 days, 2 per day	3 days, 2 per day	2 days, 2 per day
	Vaccination	Wk 35-40	1 per day Day 0, 1, 2, 3, 5, 14, 28, 42 relative to vaccination			
Dry period 1	Dry period	Wk -4 and -2	1 sample			
Lactation 2	Early lactation	Wk 1, 2, 3 and 4	1 per week			
	Digestibility and Balance	Wk 7-12	2 days, 2 per day	3 days, 2 per day	3 days, 2 per day	2 days, 2 per day
	Vaccination	Wk 14-19	1 per day Day 0, 1, 2, 3, 5, 14, 28, 42 relative to vaccination			
	Digestibility and Balance	Wk 30-35	2 days, 2 per day	3 days, 2 per day	3 days, 2 per day	2 days, 2 per day
	Vaccination	Wk 35-40	1 per day Day 0, 1, 2, 3, 5, 14, 28, 42 relative to vaccination			
Dry period 2			No samples			
Lactation 3	Early lactation	Wk 1-4	No samples			

Figure 3.3. Sampling schedule of entire study with different sample periods during lactation in weeks (Wk) relative to calving and during dry period in weeks relative to expected calving date.

3.6 Chemical Analyses

Prior to analysis, samples of grass silage, corn silage, rapeseed meal, concentrates, and feces were thawed at room temperature, oven-dried at 60°C, ground to pass a 1-mm screen using a cross beater mill for both silages (Peppink 100AN, Olst, the Netherlands) and a ultra-centrifugal mill for all the other samples (Retsch ZM200, Retsch GmbH, Haan, Germany), and analyzed by wet chemistry [DM, ash, crude fat, starch, reducing sugars (all carbohydrates with reducing properties and soluble in 40% ethanol), NDF, ADF, ADL, and N] as described by Abrahamse et al. (2008). Bomb calorimeter (ISO 9831; International Organization for Standardization, 1998) was used to determine gross energy (GE). N was determined using the Kjeldahl method with CuSO4 as a catalyst (ISO 5983; International Organization for Standardization, 2005). The N concentrations in feces and roughages in fresh material were determined according to Klop et al. (2016). Ash insoluble in hydrochloric acid (AIA) was determined by hydrolyzing, followed by hydrochloric acid treatment, filtration, drying, incineration, and weighing of the residue (ISO, 5985; International Organization for standardization, 2002). Grass silage, corn silage, and concentrates were analyzed for DM, ash, N, crude fat, starch (except for grass silage), reducing sugars (except for corn silage), NDF, ADF, ADL, GE, and AIA. Feed residuals were analyzed for DM. Fecal samples were analyzed for DM, GE, and N. The fecal samples were analyzed for DM, ash, crude fat, starch, NDF, GE, N, and AIA. Samples of respiration acid and condensed water were analyzed for N.

Milk samples were analyzed (Qlip, Zutphen, The Netherlands) for fat, protein, lactose content, urea, and somatic cell count using mid-infrared spectroscopy (MilkoScan FT 6000, Foss, Hillerod, Denmark). A weighted average daily milk composition was calculated from the composition of the afternoon and morning milk sample.

Blood serum level were assessed at the veterinary laboratory of Royal GD (Deventer, the Netherlands) using a DxC 700 AU Chemistry Analyzer (Beckman Coulter) and all results were evaluated according to the quality control procedures of the laboratory (meeting NEN-EN-ISO 9001:2015 requirements). Colorimetric methods were used to analyze serum calcium, phosphate (ammonium-molybdate method), magnesium, total protein (Biuret method), albumin (Bromocresol Green method), and total bilirubin concentrations (all test kits were from Beckman Coulter). Haptoglobin concentrations were analyzed with a colorimetric method using the "PHASE" Haptoglobin Assay (Tridelta Development Limited). Enzymatic methods were used to analyze serum concentrations of urea (urease method; test kit from Beckman Coulter), creatinine (test kit from Beckman Coulter), BHBA (D-3-Hydroxybutyrate (Ranbut) reagent from Randox Laboratories Ltd.), and NEFA (Wako NEFA-HR(2) test kit; FUJFILM Wako Chemicals Europe GmbH). CPK, ALP, AST, and GGT concentrations were analyzed using enzymatic methods according to the IFCC reference procedures (test kits from Beckmann Coulter) and GLDH concentrations were analyzed with an enzymatic method according to the DGKC (GLDH3 test kit, Roche Diagnostics GmbH).

At WBVR the virus neutralization test was used to determine the titer of BHV-1 antibodies and BVDV antibodies. For this purpose, serum samples were first heated at 56°C for 30 min to inactivate the complement. Two fold serial dilutions of all the test serum samples, including positive- and negative-control serum samples, were made up to 1:32768 in Eagle's Minimum Essential Medium (EMEM) with L-glutamine, containing 1x non-essential amino acids, 1mM sodium pyruvate, 1% antibiotics (penicillin-streptomycin), 1% antifungal (amphotericin B) and 5% fetal bovine serum (FBS). A total of 50 µl of undiluted (BHV-1 only) and of each dilution was put in duplicate wells in a 96 well tissue culture microtiter plate. For BHV-1 samples additionally, undiluted serum samples were also put in one well to test the toxicity of the serum to embryonic bovine tracheal (EBTr) cells. A volume of 50 µl of BHV-1 (WBVR) containing 100 TCID50 was added to each well excluding the cell control wells, and the plate was incubated at 37°C for 24 hr in a CO_2 incubator. A volume of 100 µl of EBTr cell suspension (containing about 10,000–15,000 cells) was added to all the wells. The plate was further incubated at 37°C for 72-96 hr in a CO_2 incubator and read for presence or absence of cytopathic effect (CPE). For BVDV samples, undiluted serum samples were put in one well to test the toxicity of the serum to embryonic bovine tracheal (EBTr) cells. A volume of 50 µl of BVDV (Oregon (type-1), WBVR) containing 100 TCID50 was added to each well excluding the cell control wells, and the plate was incubated at 37°C for 1 hr in a CO_2 incubator. A volume of 100 µl of EBTr cell suspension (containing about 10,000–15,000 cells) was added to all the wells. The plate was further incubated at 37°C for 5 days in a CO_2 incubator and read for presence or absence of cytopathic effect (CPE). For both BHV-1 and BVDV the wells showing CPE were considered negative, and the absence of CPE was considered positive. A toxic reaction is characterized by destruction of the monolayer in the toxicity control (to which no virus has been added) caused by components in the serum sample. The serum sample is then issued as 'sample is toxic'. In case of a toxic reaction, only sera with no or low titer could not be read. Sera with a high titer were assessed as normal.

3.7 Gas Emissions

Emissions of CH_4, H_2, and CO_2 were measured on an individual cow level for the duration of the trial by using 2 GreenFeed units (Zimmerman et al., 2011). Irrespective of treatment or lactation stage, both GreenFeed units could be visited by every cow. The GreenFeed units measured airflow as well as CH_4, H_2, and CO_2 concentrations in both the environment and exhaled air when a cow visited the system. Each GreenFeed unit was equipped with a head position sensor, and gas emission data were rejected when the head position criteria were not met. Each individual cow could visit the GreenFeed units every 5 h (until 14 July 2023) and 7 h (after July 14 2023) with a maximum of 3 visits per day, and data collection was dependent on the cows' voluntary visits to the GreenFeed units. A concentrate (Table 3.3) was offered as a feed incentive to encourage the cows to maintain a suitable head position for accurate measurements. This GreenFeed feed incentive was provided from a hopper above the GreenFeed hood using a computer-controlled rotating cup dispenser, with a maximum of 9 so-called cup drops per visit, 1 cup drop per 15 s, and 45.7 ± 2.44 g of feed per cup drop. Emissions of CH_4, H_2, and CO_2 were calculated by subtracting the recorded background gas concentration from the gas concentrations recorded during the animal's time in the GreenFeed. This difference in concentration was subsequently multiplied by the airflow to come to an emission rate in grams per day, using the ideal gas law and correcting for temperature and pressure. See van Gastelen et al. (2022) for more details regarding the procedures applied for the GreenFeed units in

the present study. The average CO_2 recovery was 99.1% (for individual GreenFeed units between 97.7% and 99.9%).

3.8 Statistical Analysis and Calculations

All daily variables were averaged over 7-d periods (week in lactation) for the first 308 days post-partum (44 weeks). Fat- and protein-corrected milk yield (FPCM) was calculated as: FPCM (kg/day) = (0.337 + 0.116 × fat % + 0.06 × protein %) × milk yield (kg/day) (CVB, 2016). Energy-corrected milk yield (ECM) was calculated as: ECM (kg/day) = (milk yield (kg/day) × 0.327) + (fat yield (kg/d) × 12.95) + (protein yield (kg/day) × 7.2) (CVB, 2016).

Feces volume was calculated using AIA as an internal marker, assuming complete recovery of ingested AIA in feces. Urine volume was calculated by dividing the estimated daily creatinine excretion, derived from body weight, by the creatinine concentration measured in urine samples.

Cow was considered the experimental unit for all variables. All statistical analyses were conducted using R (version 4.5.1, The R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was declared at $P \leq 0.05$, and trends were discussed when $0.05 < P \leq 0.10$.

Daily variables

Data were analyzed in a repeated measurements model using the *lme4* package. The model contained main effects and interaction effects of diet (LCP, MCP, and HCP) and week as fixed effects, and cow and block as random effects. Values are presented as estimated marginal means (EMM) ± pooled standard deviation (SD) or standard error of the mean (SEM). In tables with 44-wk overviews SD was reported instead of the SEM, as the large number of observations (64 cows over 44 weeks) resulted in very small SEM values that reflected the precision of the estimated means rather than the biological variation within the dataset. Comparisons between treatment means were made when a significant effect of diet was detected. When a diet × week interaction was detected comparisons between weekly treatment EMM were calculated and differences were described in the results section. The model assumed homogeneous residual variance. But for visualization in the result figures, observed standard error (SE) per week are shown. All *P*-values of pairwise comparisons of EMM were corrected with a Tukey-Kramer adjustment.

Vaccination

Statistical analyses were performed with mixed-effects models fitted using the *lme4* package and estimated marginal means obtained using the *emmeans* package. The effects of CP-level on the response variable was analyzed using a linear mixed-effects model. The model included CP-level (LCP, MCP and HCP), day (1, 2, 3, and 5; for acute phase response, and 14, 28 and 42 for titer response), and their interaction (CP × Day) as fixed effects. To account for baseline differences between cows, the pre-treatment value of the response variable (Day 0) was included as a covariate. Random intercepts were fitted for block, batch, and cow to account for the hierarchical and repeated-measures structure of the data. Model significance of fixed effects was assessed using analysis of variance (ANOVA) based on F-tests with denominator degrees of freedom estimated using the Satterthwaite method. Estimated marginal means (EMMs) were calculated to evaluate treatment effects. When a CP × Day interaction was found in the model, treatment effects were further examined within each sampling day using EMMs conditional on day. Pairwise comparisons between treatments within each day were performed using Tukey-adjusted post hoc tests to control for multiple comparisons.

Survival rate / risk of removal

Cow survival rate in the study was analyzed using a Cox proportional hazards model. Time to removal was defined as the number of days from the start of the experiment until the occurrence of the event. Cows that did not leave the study prematurely or were still present at the end of the study were treated as censored observations. Dietary treatment (LCP, MCP, or HCP), study phase (L1, D1, L2, D2, or L3), parity, and pregnancy status were included in the model as explanatory variables. Because multiple observations were available for each cow, robust standard errors were calculated with cow included as a clustering factor. Analyses were performed using the *coxph* function from the *survival* package.

Treatments for disease (or preventive) and fertility

Number of treatments, number of inseminations (for pregnancy), and in between calving interval were analyzed using a linear mixed-effects model as described above at '*Daily variables'*. The probability of treatment for disease or preventive and the pregnancy after the first insemination were analyzed using logistic regression. Outcomes were recorded as a binary response variable (0 = not pregnant/not treated,

1 = pregnant/treated). Dietary treatment was included as a fixed effect. Analyses were performed using the *glm* function with a binomial error distribution and logit link function.

4 Feed intake and milk production in lactation 1

4.1 Results (L1)

- Three cows were removed from the study because of chronic disease before they reached 308 DIM (Appendix 3). Data from these animals was included until the week they were removed from the study. Data from cows with health issues were retained in the analysis, as animal health was a central theme of the study and contributed directly to the interpretation of outcomes.
- The realized overall CP levels were 133, 143 and 154 g/kg DM for LCP, MCP and HCP, respectively (Table 4.1; Figure 4.1). CP level showed a diet × week interaction ($P < 0.01$), also showing differences between treatments during all 44 weeks in lactation. HCP was higher than MCP and LCP in week 1 to 44, and MCP was higher than LCP in week 1 to 44. The mean CP concentrations, particularly for HCP and to a lesser extent MCP, were lower than the targeted CP concentrations (Figure 3.1).
- Different dietary CP levels influenced total DMI ($P < 0.01$), with cows fed HCP and MCP diets having higher total DMI compared with cows fed the LCP diet (Figure 4.2). Intake of the PMR was also higher for HCP and MCP diets compared with LCP ($P < 0.01$). Additionally, there was a trend ($P = 0.07$) toward CP level influencing concentrate intake, which seemed to be caused by a difference in intake of GreenFeed concentrates ($P = 0.03$) rather than a different intake in treatment concentrates (P = 0.89).
- Milk yield (Figure 4.3) showed an interaction between diet and week ($P = 0.01$). Milk yield did not differ between HCP and MCP diets overall or in any week. Milk yield was higher for HCP compared with LCP in weeks 3 to 10 and weeks 14 to 44. Similarly, milk yield was higher for MCP compared with LCP in weeks 3 to 29, weeks 31 to 33, and in week 40.
- Both milk fat and protein contents showed diet × week interactions ($P < 0.01$), indicating that the temporal patterns differed among diets. However, no overall differences between HCP, MCP, and LCP diets were present for fat or protein content, with only in week 9 showing a tendency for a diet effect on protein content.
- Milk urea concentration (Figure 4.4) showed a diet × week interaction ($P < 0.01$). Milk urea concentration was higher for HCP compared with MCP in weeks 2 to 21 and weeks 23 to 44, and higher compared with LCP in weeks 2 to 44. Additionally, MCP had higher urea concentrations compared with LCP in weeks 3 to 9, weeks 11 to 18, weeks 20 and 21, weeks 23 to 28, weeks 30 to 39, and week 44.
- Fat- and protein-corrected milk yield (Figure 4.5) exhibited a diet × week interaction ($P < 0.01$). FPCM yield did not differ between HCP and MCP diets (overall or in any week). FPCM yield was higher for HCP compared with LCP in weeks 3 to 12 and weeks 14 to 44. Similarly, FPCM yield was higher for MCP compared with LCP in weeks 3 to 5, weeks 7 to 34, week 36 to 37, and week 39.
- Fat yield (Figure 4.6) also showed a diet × week interaction ($P < 0.01$). Fat yield did not differ between HCP and MCP diets in any week. Fat yield was higher for HCP compared with LCP in weeks 4 to 44, and higher for MCP in weeks 3 to 5, weeks 7 to 34, and week 37 compared with LCP.
- Protein yield (Figure 4.7) showed a diet × week interaction ($P = 0.05$). Protein yield was higher for HCP and MCP compared with LCP ($P < 0.01$) independent of week, whereas protein yield did not differ between HCP and MCP. Protein yield was higher for HCP compared to LCP in week 3 to 12 and week 14 to 44. Protein yield was higher for MCP compared with LCP in week 4 and 5, week 8 to 40 and week 42 to 44. No differences were found between HCP and MCP overall or in any week.
- Feed efficiency, calculated as milk or FPCM yield per kilogram of DMI, exhibited a diet × week interaction ($P < 0.01$), indicating diet-dependent changes over time.
- N use efficiency for milk (Figure 4.8) showed a diet × week interaction ($P < 0.01$), with LCP being higher than MCP in weeks 1 to 11 and weeks 14 to 15. LCP being higher than HCP in weeks 2 to 21, and weeks 24 and 27. Whereas, MCP was higher than HCP in week 2 and 4, weeks 6 to 13, and week 15 to 17.
- Body weight (Figure 4.9) differed among CP levels (P = 0.02), with cows fed the LCP diet having lower body weight than those fed the MCP and HCP diets, whereas no difference was observed between the MCP and HCP diets. BCS was not affected by dietary CP level.
- Lastly, all analyzed variables were affected by week in lactation ($P < 0.01$).

Table 4.1: Effect of dietary crude protein (CP) level (133, 143, and 154 g/kg DM for LCP, MCP, and HCP, respectively) and week in milk (WIM; week 1 to 44), on dry matter intake (DMI), milk yield and composition, feed efficiency, BW and BCS of dairy cows, during the first lactation of the study.

		Diet (CP)				*P*-value		
		LCP	MCP	HCP	SD	CP	WIM	CP × WIM
CP	g/kg DM	133	143	154	10.4	<0.01	<0.01	<0.01
DMI total	kg/d	21.7[b]	23.9[a]	23.9[a]	2.65	<0.01	<0.01	0.26
DMI PMR	kg/d	13.9[b]	16.2[a]	16.4[a]	2.37	<0.01	<0.01	0.11
DMI conc.	kg/d	7.78	7.73	7.51	2.19	0.07	<0.01	0.23
DMI CP	kg/d	5.74	5.73	5.73	2.12	0.89	<0.01	0.99
DMI GF	kg/d	2.05	2.00	1.78	0.45	0.03	<0.01	0.01
Milk yield	kg/d	29.9	33.8	34.2	8.08	<0.01	<0.01	0.01
Fat	g/100 g milk	4.41	4.48	4.60	0.565	0.21	<0.01	<0.01
Protein	g/100 g milk	3.48	3.50	3.57	0.348	0.19	<0.01	<0.01
Lactose	g/100 g milk	4.47	4.48	4.49	0.187	0.90	<0.01	0.96
Urea	mg/dL	9.2	12.1	16.3	4.57	<0.01	<0.01	<0.01
SCC	× 1,000 cells/ml	253	317	291	597.9	0.83	<0.01	0.59
SCC(log10)		2.04	2.04	1.89	0.599	0.34	<0.01	0.58
FPCM yield	kg/d	31.3	35.7	36.7	7.78	<0.01	<0.01	<0.01
ECM yield	kg/d	34.0	38.8	39.9	8.42	<0.01	<0.01	<0.01
Fat yield	g/d	1,300	1,496	1,550	342	<0.01	<0.01	<0.01
Protein yield	g/d	1,025	1,163	1,201	235	<0.01	<0.01	0.05
FE Milk	Milk/DMI	1.37	1.40	1.42	0.290	0.48	<0.01	<0.01
FE FPCM	FPCM/DMI	1.44	1.49	1.53	0.296	0.10	<0.01	<0.01
N intake	g/d	462	548	590	84.3	<0.01	<0.01	<0.01
N secretion milk	g/d	161	182	188	36.8	<0.01	<0.01	0.05
N use efficiency		0.348	0.332	0.319	0.061	<0.01	<0.01	<0.01
Body weight	kg	679[b]	720[a]	716[a]	72.0	0.02	<0.01	0.36
BCS		3.1	3.1	3.2	0.38	0.48	<0.01	0.77

[ab] Values with different superscript are significantly different ($P < 0.05$)
GF = GreenFeed; SCC = Somatic cell count; FE = Feed efficiency; BCS = Body Condition Score

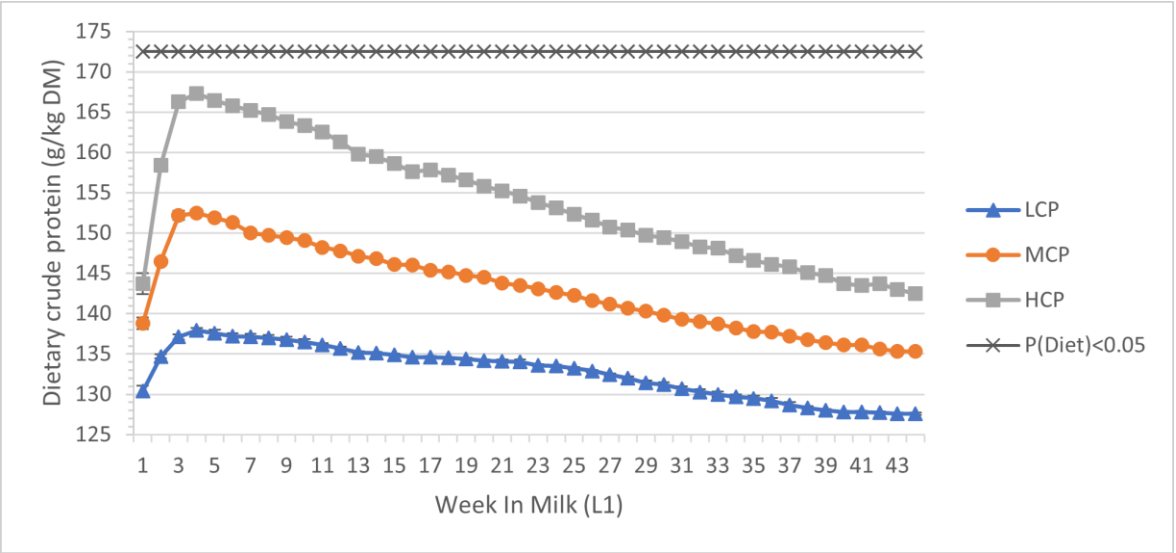


Figure 4.1. Realized crude protein level per week in milk during the first lactation (L1) for cows fed different dietary crude protein levels of 133, 143 and 154 g CP/kg DM (LCP, MCP, and HCP respectively) and the significant differences of dietary treatment per week (P(Diet)<0.05).

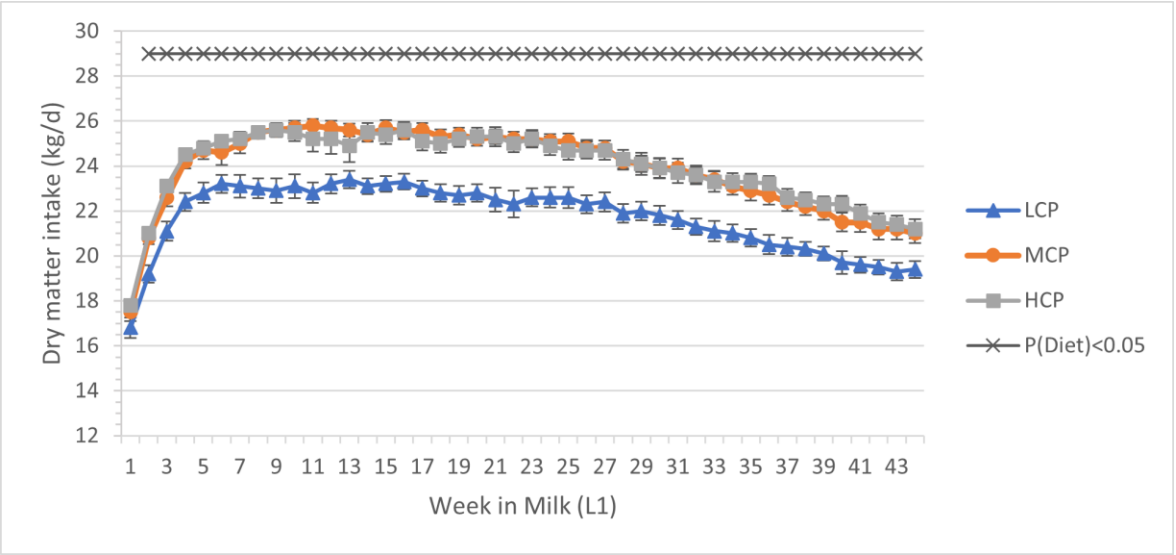


Figure 4.1. Dry matter intake per week in milk for cows fed different dietary crude protein (CP) levels of 133, 143, and 154 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the first lactation of the study (L1).

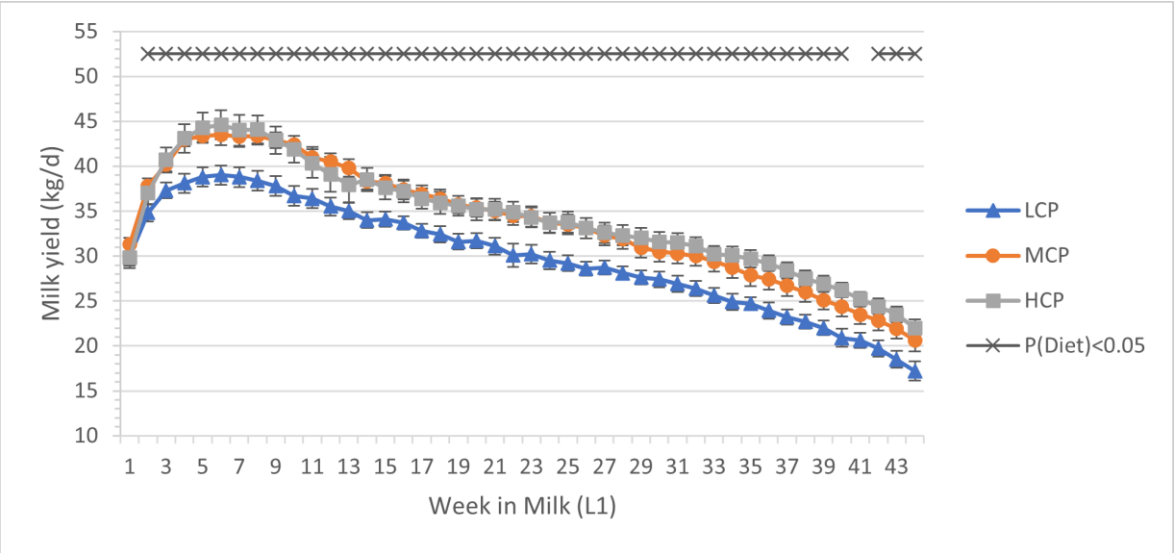


Figure 4.3. Milk yield per week in milk for cows fed different dietary crude protein (CP) levels of 133, 143, and 154 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the first lactation of the study (L1).

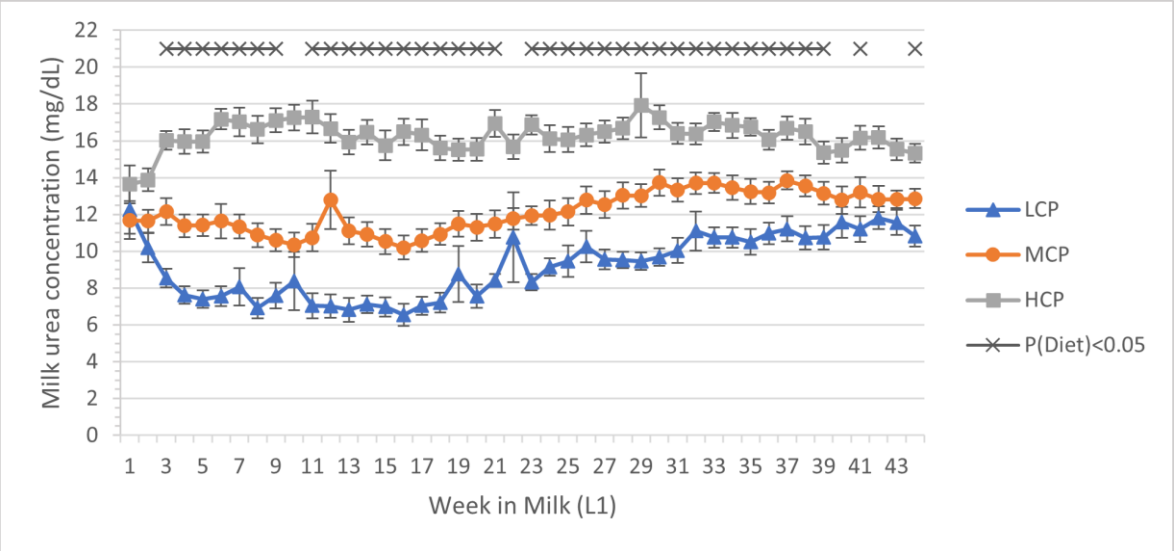


Figure 4.4. Milk urea concentration per week in milk for cows fed different dietary crude protein (CP) levels of 133, 143, and 154 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the first lactation of the study (L1).

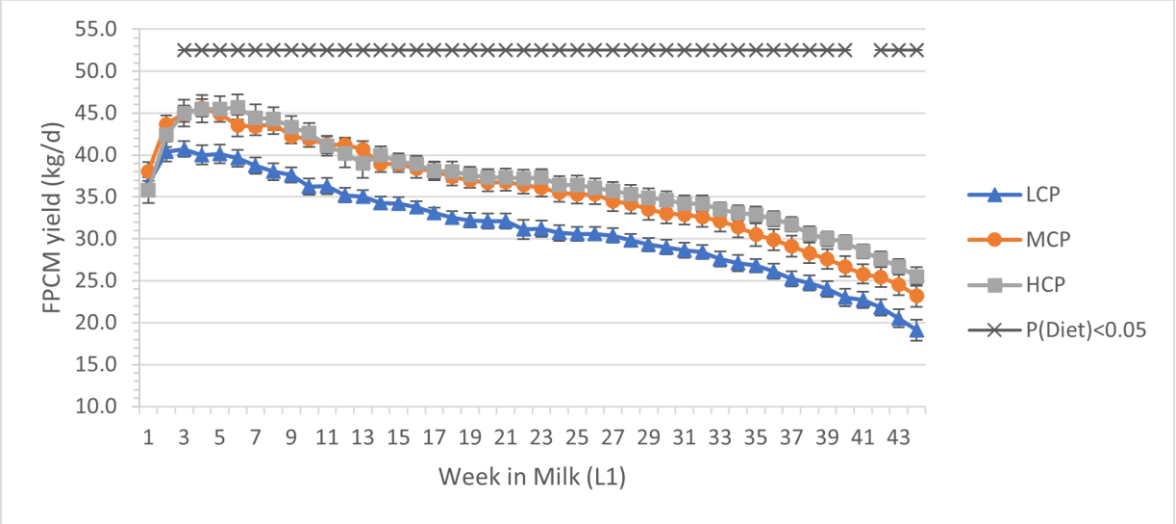


Figure 4.5. Fat and protein corrected milk (FPCM) yield per week in milk for cows fed different dietary crude protein (CP) levels of 133, 143, and 154 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the first lactation of the study (L1).

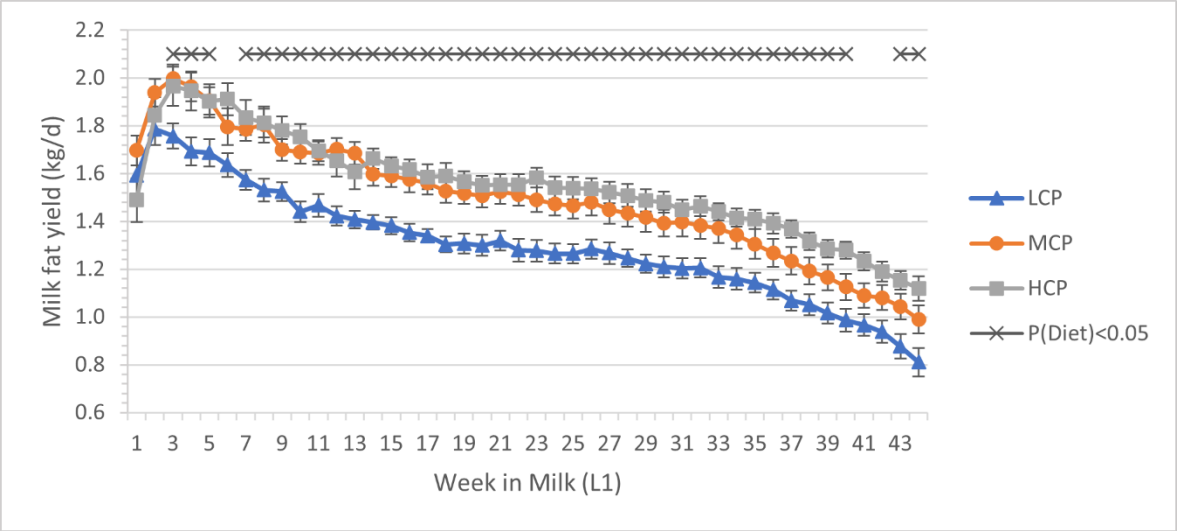


Figure 4.6. Fat yield per week in milk for cows fed different dietary crude protein (CP) levels of 133, 143, and 154 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the first lactation of the study (L1).

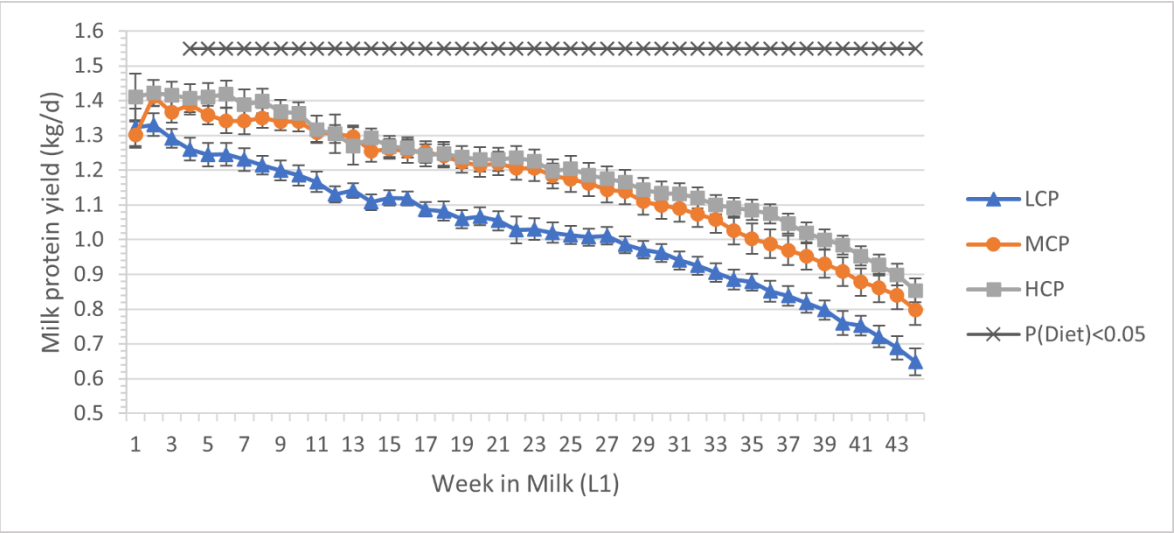


Figure 4.7. Protein yield per week in milk for cows fed different dietary crude protein (CP) levels of 133, 143, and 154 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the first lactation of the study (L1).

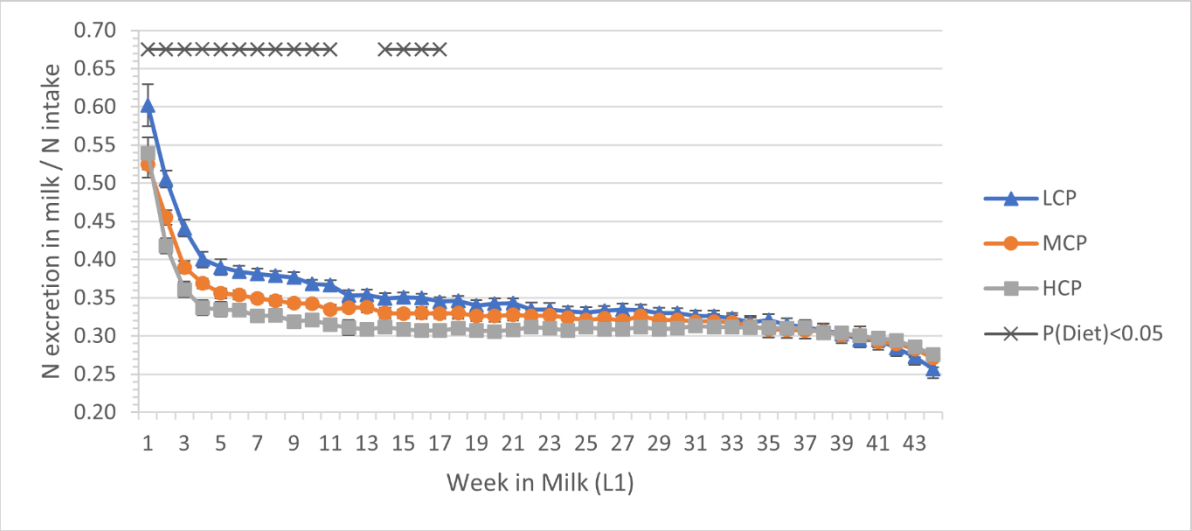


Figure 4.8. Nitrogen use efficiency for milk per week in milk for cows fed different dietary crude protein (CP) levels of 133, 143, and 154 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the first lactation of the study (L1).

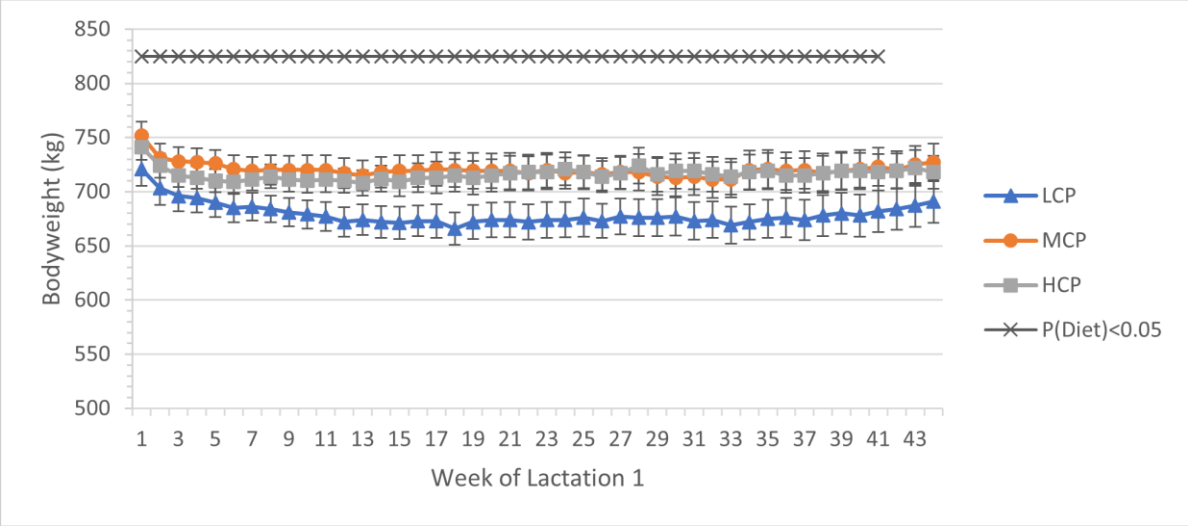


Figure 4.9. Body weight per week in milk for cows fed different dietary crude protein (CP) levels of 133, 143, and 154 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the first lactation of the study (L1).

4.2 Interpretation

During this study we successfully applied differences in dietary CP levels, with 133, 143 and 154 g CP / kg DM for treatments LCP, MCP and HCP respectively. The actual CP levels deviated from the proposed CP levels of 130, 145 and 160 g CP/kg DM for treatments LCP, MCP and HCP respectively. In contrast to previous long-term studies (Law et al., 2009; Reynolds, 2022; Lavery et al., 2025), where CP levels were kept constant throughout lactation, we varied CP levels within each treatment group (133, 143, and 154 g/kg DM) in accordance with the general lactation curve, aiming to match the higher CP requirements associated with increased milk production during peak lactation.

Feeding multiparous lactating dairy cows MCP over a 44-week lactation did not reduce milk yield or milk composition compared with cows fed HCP. Cows fed LCP had lower DMI (21.7 kg/d) and milk yield (29.9 kg/d) compared with MCP (23.9 kg/d intake; 33.8 kg/d milk yield) and HCP (23.9 kg/d intake; 34.2 kg/d milk yield) cows ($P < 0.01$).

One potential explanation for the reduced DMI observed in LCP compared with MCP and HCP is the prolonged negative OEB during early and mid-lactation (weeks 2–26; Appendix 1). During this period, inadequate rumen degradable protein supply may have constrained rumen microbial protein synthesis, thereby limiting nutrient availability and depressing intake and milk production. In contrast, the OEB of MCP and HCP was positive throughout lactation, suggesting that rumen nitrogen supply was not limiting in these treatments. However, despite OEB becoming positive in LCP during the second half of lactation, DMI remained lower than in MCP and HCP, indicating that OEB alone is unlikely to fully explain the sustained differences in intake. This suggests that other factors, such as a lower DVE supply may also have contributed to the reduced DMI observed in LCP. In contrast, the difference in DVE between MCP and HCP did not result in differences in either DMI or milk yield, indicating that the threshold of DVE requirements for observed milk yield may have been met in both diets despite varying CP levels. However, this outcome does not fully align with the results of Daniel et al. (2016) who indicated that at a constant NE level additional MP supply above maintenance would still have a positive impact on milk production. In the present study, however, DVE increased only modestly from 82 to 88 g/kg DM between MCP and HCP, placing this comparison within the flatter portion of the DVE–milk response curve described by Daniel et al. Consequently, the limited numerical response observed in milk protein yield (~40 g/d) may still be consistent with their findings, reflecting a diminishing marginal response to additional DVE once requirements are largely met.

Evaluation of energy and protein balances further supports this interpretation. The DVE balance increased progressively (91.8, 92.9, and 97.9% for LCP, MCP, and HCP, respectively), indicating a more adequate protein supply at higher CP levels. In contrast, the VEM balance was highest for LCP (112.1%) and declined with increasing CP level (109.3 and 107.0% for MCP and HCP, respectively), which would suggest a greater surplus of available energy in LCP and, consequently, greater body fat accretion. However, this was not reflected in body condition score, which did not differ between treatments (3.1, 3.1, and 3.2 for LCP, MCP, and HCP). One possible explanation is that the prolonged negative OEB in LCP impaired rumen fermentation

efficiency, thereby limiting nutrient digestion and absorption and reducing the effective energy available for body reserve deposition. As a result, the higher calculated VEM balance in LCP may not have translated into increased body condition.

The lower FPCM yield in LCP cows (31.5 kg/d) and their lower DMI ($P < 0.01$) suggest a close relationship between milk production and feed intake. Although intake and production between HCP and MCP were not different, HCP cows had numerically higher FPCM yield (37.0 kg/d) than MCP cows (35.9 kg/d). This difference of approximately 1.0 kg FPCM/d can be perceived to be of a relevant size of effect for farming practitioners. However, it is important to note that the variation in individual cow response to either MCP or HCP diets was considerable. Figure 4.4 shows weekly averages of FPCM yield of HCP and MCP to slightly deviate more from each other in the second half of the lactation, but differences did not reach statistical significance.

The lower BW observed in LCP cows was likely largely driven by pre-existing differences among treatment groups, as LCP cows already had the lowest BW at the start of the experiment. Their lower DMI may have further contributed to this difference through reduced rumen and gastrointestinal fill rather than differences in body fat reserves, as reflected by the absence of treatment effects on BCS.

Moreover, the HCP level of 154 g/kg DM is already considerably lower than the 2023 Dutch average dietary CP level of approximately 164 g/kg DM. While extrapolation should be approached with caution, the non-linear response observed in the current study — combined with supporting evidence from the literature — indicates that a reduction in dietary CP content to the HCP level is feasible without compromising animal performance, but with large advantage of reductions in N excretion and NH_3 emissions.

These results clearly present how dietary CP level affects DMI and milk production variables in lactating dairy cows during a 44-week lactation.

4.3 Conclusion

A reduction in dietary CP level from 154 to 143 g/kg DM over a 44-wk lactation period did not affect feed intake or overall milk performance in multiparous dairy cows, whereas a further reduction to 133 g/kg DM strongly reduced both intake and performance, possibly due to insufficient rumen degradable nitrogen supply and/or insufficient metabolizable protein supply.

5 Feed intake and milk production in lactation 2

5.1 Results (L2)

- The second lactation started with 61 cows, with 19, 22, and 20 cows for LCP, MCP and HCP respectively, because one non-pregnant cow was removed after L1. In total 9 cows were removed from the study during the second lactation of the experiment before they reached 308 DIM. Cows were removed because of traumatic injury, severe or chronic illness (Appendix 3). Data from these animals was included until the week they were removed from the study. Data from cows with health issues were retained in the analysis, as animal health was a central theme of the study and contributed directly to the interpretation of outcomes. Additionally, 2 cows were removed after 308 DIM in L2, because of abortion and not being pregnant.
- The realized overall CP levels were 133, 144 and 156 g/kg DM for LCP, MCP and HCP, respectively (Table 5.1; Figure 5.1). CP level showed a diet × week interaction ($P < 0.01$), also showing differences between treatments during all 44 weeks in lactation. HCP was higher than MCP and LCP in weeks 1 to 44, and MCP was higher than LCP in weeks 1 to 44. The mean CP concentrations were comparable to those of L1 (Figure 4.1), but were lower than the targeted CP concentrations particularly for HCP and to a lesser extent for MCP (Figure 3.1). In addition, the CP concentration curve exhibited a less pronounced peak at the beginning of the lactation and a less marked decline during the mid-lactation period compared with L1 and the targeted CP curve.
- Different dietary CP levels influenced total DMI ($P < 0.01$), with cows fed HCP and MCP diets having higher total DMI compared with cows fed the LCP diet (Figure 5.2). Dry matter intake was higher for HCP compared with LCP in weeks 3 to 4, 6 to 7, 9 to 11, week 13, 18 to 21, 23 to 26, 28 to 29, and weeks 33 and 36. Similarly, DMI was higher for MCP compared with LCP in weeks 5 to 7 and weeks 9 to 41. Intake of the PMR was also higher for HCP and MCP diets compared with LCP ($P < 0.01$). Additionally, there was a trend ($P = 0.06$) toward that dietary treatment influenced concentrate intake, which seemed to be caused by a difference in intake in GreenFeed concentrates ($P = 0.04$), rather than a difference in intake of treatment concentrates ($P = 0.46$).
- Milk yield (Figure 5.3) was affected by an interaction between diet and week ($P < 0.01$). Milk yield did not differ between HCP and MCP diets. Milk yield was higher for HCP compared with LCP in weeks 5 to 6, weeks 24 to 26, weeks 28 to 29 and weeks 33 to 37. Similarly, milk yield was higher for MCP compared with LCP in weeks 3 to 10, weeks 12 to 15, weeks 18 to 26, week 29, and in weeks 35 to 36.
- Milk protein content was affected by an interaction between diet and week ($P < 0.01$), indicating that the pattern of change over time differed among diets. Differences were found between MCP and HCP in week 12 to 14 and week 16, with MCP being lower compared with HCP.
- Milk urea concentration (Figure 5.4) showed a diet × week interaction ($P < 0.01$). Milk urea concentration was higher for HCP compared with MCP in weeks 3 to 32 and weeks 34, 36 and 37, and higher compared with LCP in weeks 2 to 42, and week 44. Additionally, MCP had higher urea concentrations compared with LCP during weeks 2 to 11, week 17 to 32, weeks 34 to 36, weeks 39 to 41, and week 44.
- Fat- and protein-corrected milk yield (Figure 5.5) was affected by a diet × week interaction ($P < 0.01$). FPCM yield did not differ between HCP and MCP diets (overall or in any week). FPCM yield was higher for HCP compared with LCP in weeks 3 to 7, weeks 9 to 10, weeks 18 to 29, week 31, and weeks 33 to 37. Similarly, FPCM yield was higher for MCP compared with LCP in weeks 3 to 29, and weeks 34 to 36.
- Fat yield (Figure 5.6) also showed a diet × week interaction ($P = 0.01$). Fat yield was higher for HCP compared with LCP in weeks 4 to 7, week 9 to 10, weeks 18 to 28, and weeks 33 to 36. Fat yield was also higher for MCP compared with LCP in weeks 2 to 14, weeks 16 to 27, and week 36.
- Protein yield (Figure 5.7) was higher for HCP and MCP compared with LCP ($P < 0.01$), whereas protein yield did not differ between HCP and MCP.
- Feed efficiency, calculated as milk or FPCM yield per kilogram of DMI, was affected by a diet × week interaction ($P < 0.01$), indicating diet-dependent changes over time.
- N intake showed a diet × week interaction ($P < 0.01$). The differences in N intake and N secretion in milk led to a diet × week interaction for N use efficiency for milk ($P < 0.01$). LCP was higher than MCP in weeks 9, 11, 13 and 14. LCP was higher than HCP in weeks 1 to 23. MCP was higher than HCP in weeks 2 to 13, week 17, and weeks 19 to 21 (Figure 5.8).

- Body weight (Figure 5.9) was not affected by dietary CP level (P = 0.49), but there was an interaction of CP × WIM for BCS (P < 0.01), with LCP being higher in weeks 37 and 38 compared with HCP.
- Lastly, all variables were affected by a week effect ($P \leq 0.04$).

Table 5.1: Effect of dietary crude protein (CP) level (133, 144, and 156 g/kg DM for LCP, MCP, and HCP, respectively) and week in milk (WIM; week 1 to 44), on dry matter intake (DMI), milk yield and composition, feed efficiency, BW and BCS of dairy cows, during the second lactation of the study.

		Diet (CP)				*P*-value		
		LCP	MCP	HCP	SD	CP	WIM	CP × WIM
CP	g/kg DM	133	144	156	10.1	<0.01	<0.01	<0.01
DMI total	kg/d	23.1[b]	25.1[a]	24.6[a]	3.00	<0.01	<0.01	0.08
DMI PMR	kg/d	15.2	17.1	17.0	2.60	<0.01	<0.01	0.03
DMI conc.	kg/d	7.91	7.92	7.57	2.07	0.06	<0.01	0.20
DMI CP	kg/d	5.94	5.94	5.88	2.03	0.46	<0.01	0.52
DMI GF	kg/d	1.97	1.98	1.69	0.45	0.04	<0.01	0.04
Milk yield	kg/d	31.4	35.7	35.2	8.58	0.03	<0.01	<0.01
Fat	g/100 g milk	4.62	4.59	4.64	0.603	0.88	<0.01	0.77
Protein	g/100 g milk	3.57	3.57	3.60	0.357	0.84	<0.01	<0.01
Lactose	g/100 g milk	4.54	4.56	4.57	0.178	0.85	<0.01	0.13
Urea	mg/dL	10.9	14.1	19.3	5.38	<0.01	<0.01	<0.01
SCC	× 1,000 cells/ml	527	335	290	841.2	0.31	0.04	0.01
SCC(log10)		2.15	2.08	1.97	0.604	0.50	<0.01	<0.01
FPCM yield	kg/d	33.8	38.3	37.9	8.25	0.02	<0.01	<0.01
ECM yield	kg/d	36.7	41.6	41.3	8.92	0.02	<0.01	<0.01
Fat yield	g/d	1,429	1,616	1,604	363	0.03	<0.01	0.01
Protein yield	g/d	1,105[b]	1,250[a]	1,250[a]	247	0.01	<0.01	0.08
FE (milk/DMI)		1.36	1.42	1.42	0.295	0.43	<0.01	<0.01
FE (FPCM/DMI)		1.47	1.53	1.53	0.303	0.31	<0.01	<0.01
N intake	g/d	492	577	615	87.0	<0.01	<0.01	<0.01
N secretion milk	g/d	173[b]	196[a]	196[a]	38.7	0.01	<0.01	0.08
N use efficiency		0.353	0.340	0.318	0.062	<0.01	<0.01	<0.01
Body weight	kg	681	701	696	74.9	0.46	<0.01	0.97
BCS		3.2	3.1	3.1	0.35	0.29	<0.01	<0.01

[ab] Values with different superscript are significantly different (P < 0.05)
GF = GreenFeed; SCC = Somatic cell count; FE = Feed efficiency; BCS = Body Condition Score

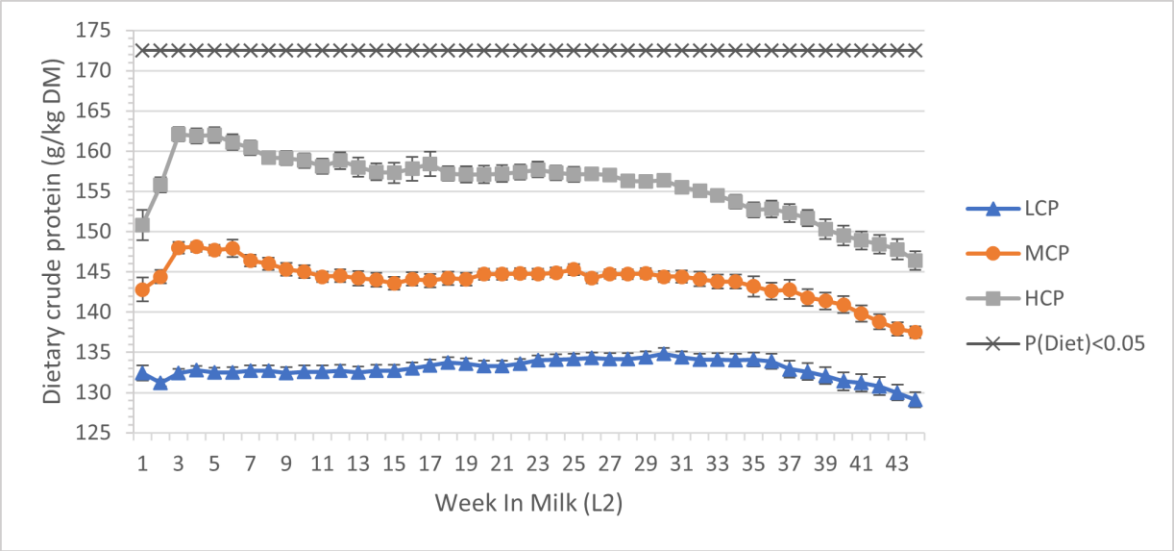


Figure 5.1. Realized crude protein level per week in milk during the second lactation (L2) for cows fed different dietary crude protein levels of 133, 144 and 156 g CP/kg DM (LCP, MCP, and HCP respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05).

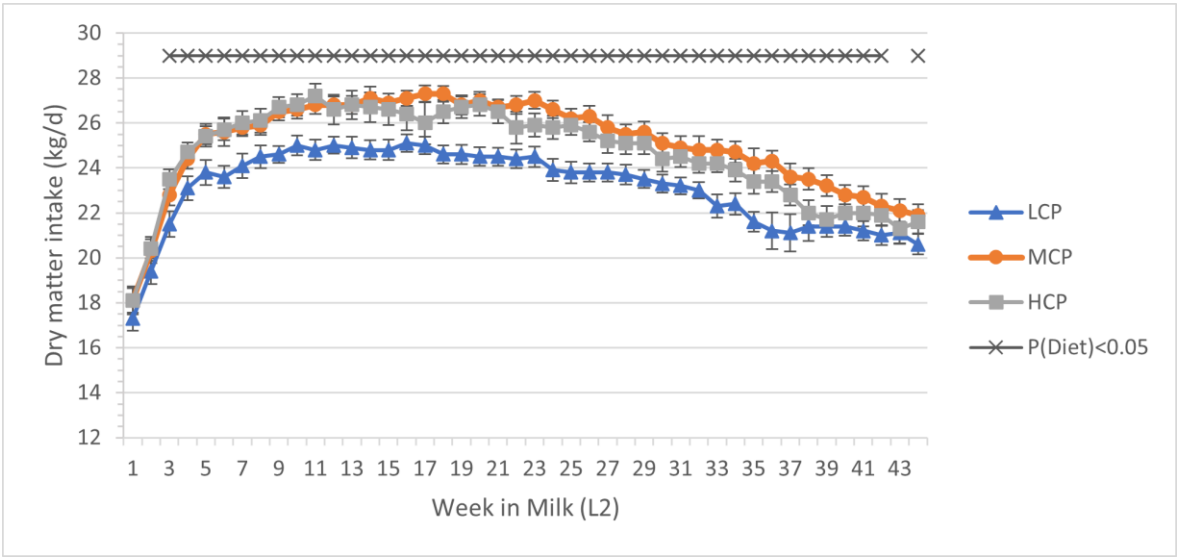


Figure 5.2. Dry matter intake per week in milk for cows fed different dietary crude protein (CP) levels of 133, 144, and 156 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the second lactation of the study (L2).

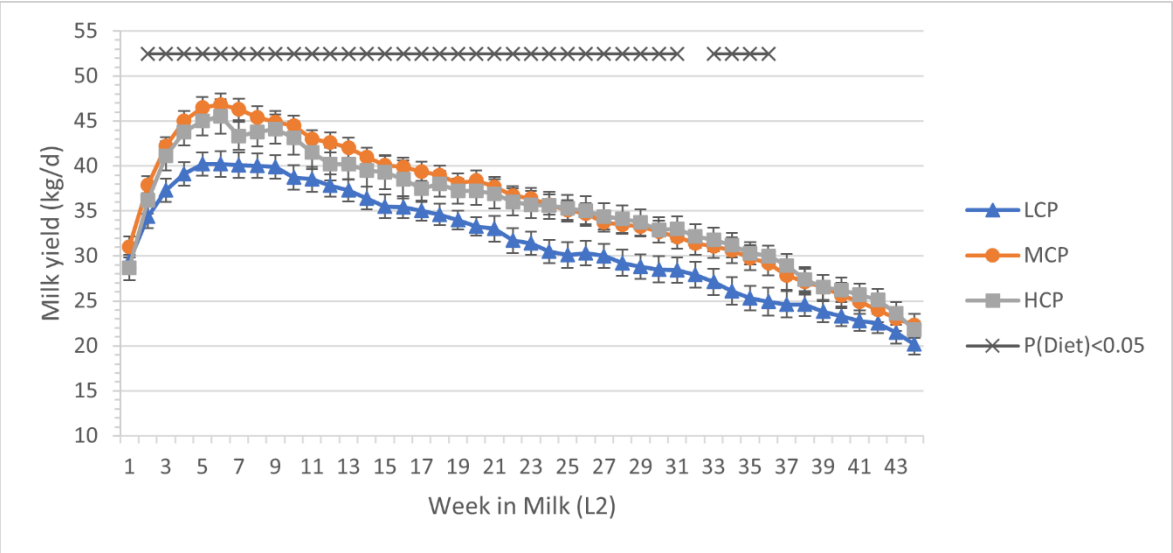


Figure 5.3. Milk yield per week in milk for cows fed different dietary crude protein (CP) levels of 133, 144, and 156 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the second lactation of the study (L2).

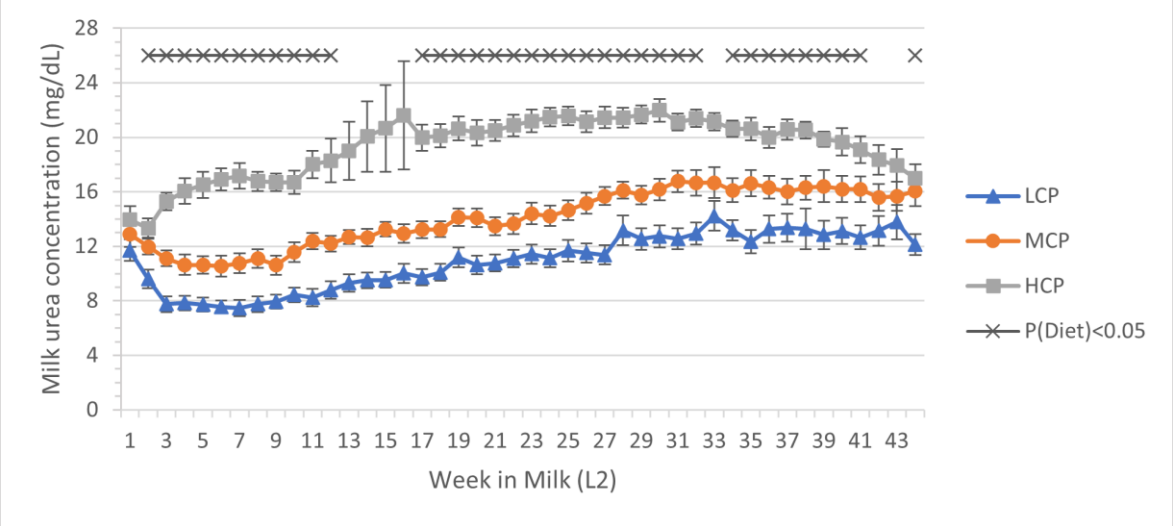


Figure 5.4. Milk urea concentration per week in milk for cows fed different dietary crude protein (CP) levels of 133, 144, and 156 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the second lactation of the study (L2).

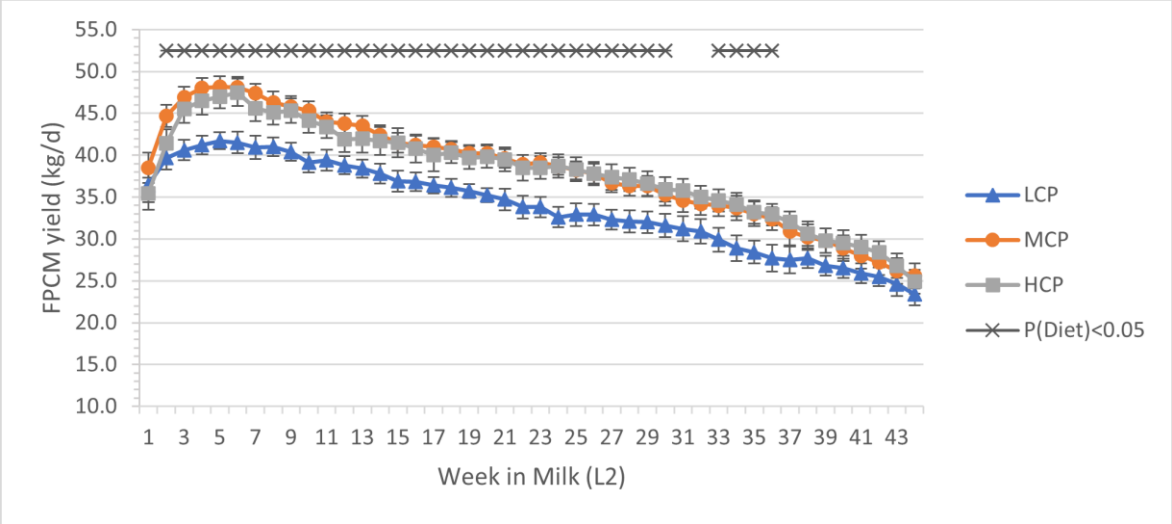


Figure 5.5. Fat and protein corrected milk (FPCM) yield per week in milk for cows fed different dietary crude protein (CP) levels of 133, 144, and 156 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) ≤ 0.05) during the second lactation of the study (L2).

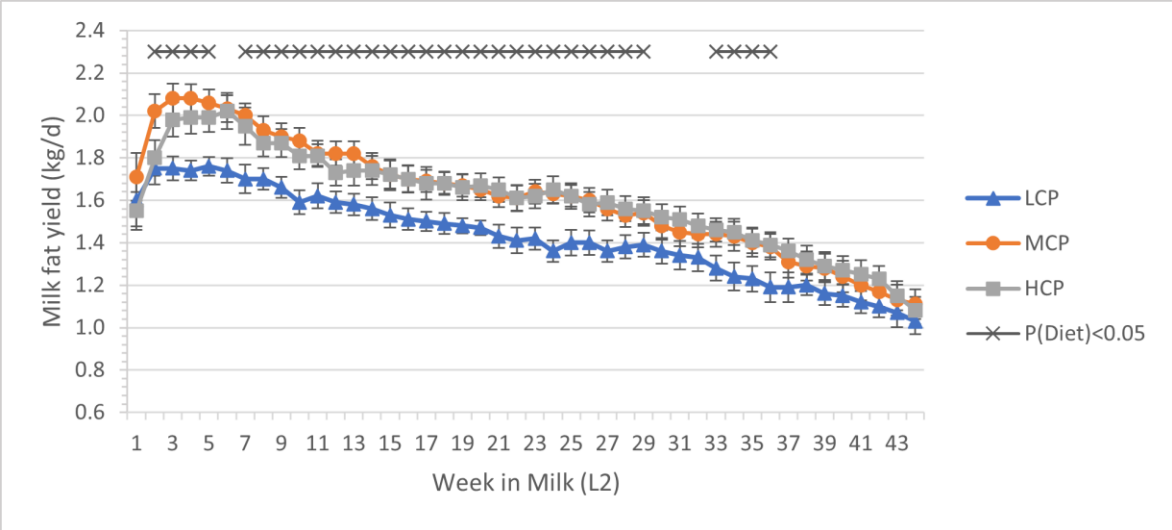


Figure 5.6. Fat yield per week in milk for cows fed different dietary crude protein (CP) levels of 133, 144, and 156 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the second lactation of the study (L2).

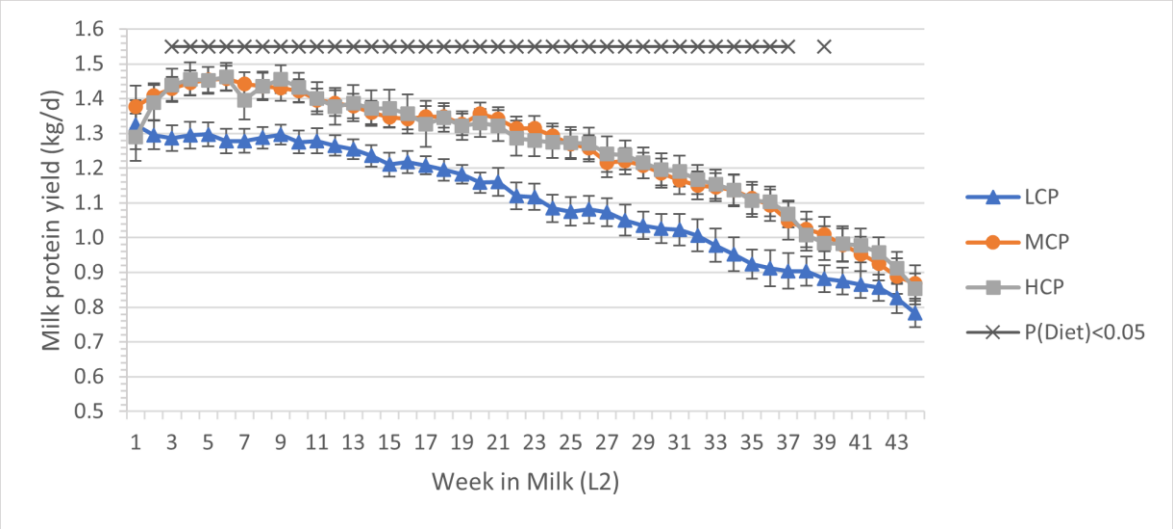


Figure 5.7. Protein yield per week in milk for cows fed different dietary crude protein (CP) levels of 133, 144, and 156 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the second lactation of the study (L2).

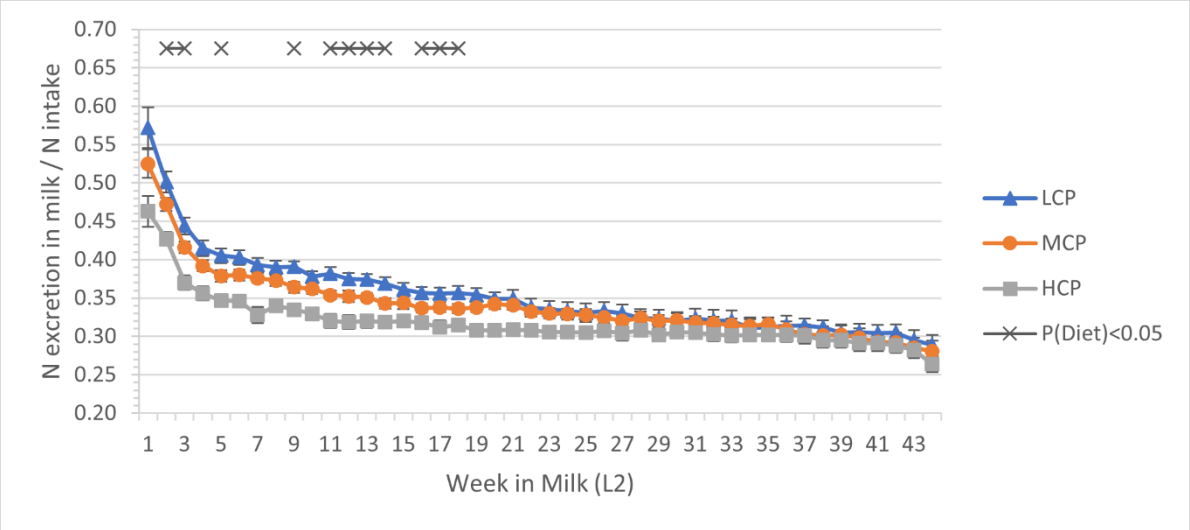


Figure 5.8. Nitrogen use efficiency for milk yield per week in milk for cows fed different dietary crude protein (CP) levels of 133, 144, and 156 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the second lactation of the study (L2).

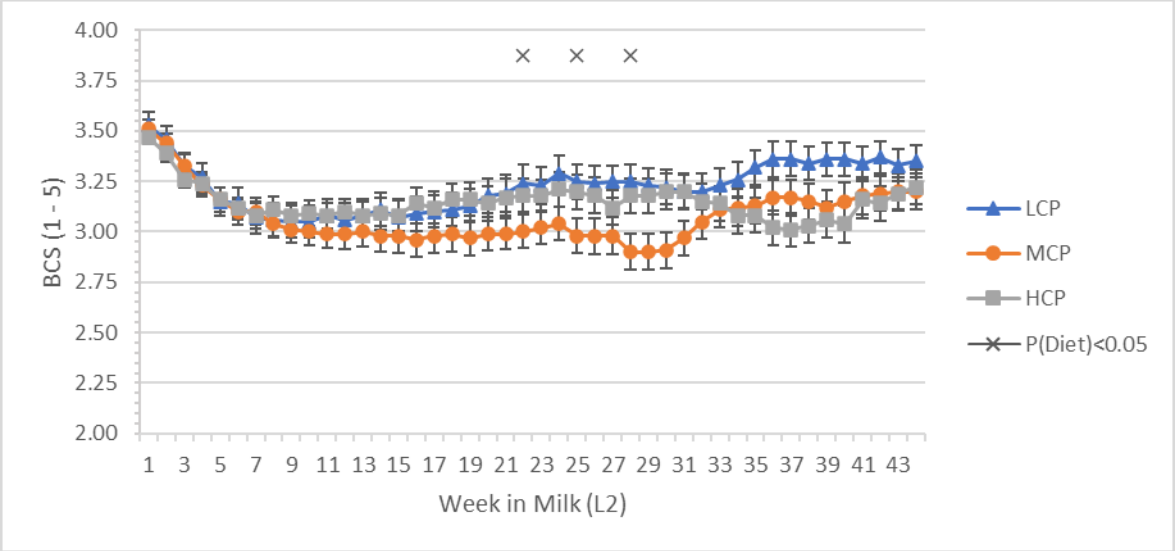


Figure 5.9. Body condition score (BCS) per week in milk for cows fed different dietary crude protein (CP) levels of 133, 144, and 156 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05) during the second lactation of the study (L2).

5.2 Interpretation of lactation 2 in relation to lactation 1

During the second lactation, realized CP levels were slightly different from the intended values (133, 144, and 156 g CP/kg DM vs. 130, 145, and 160 g CP/kg DM for LCP, MCP, and HCP, respectively). These deviations should be taken into account when interpreting the results, but the contrasts between treatments remained clearly defined and of a similar magnitude. When comparing the realized CP levels of the second lactation with those of the first lactation, the average dietary CP concentrations were similar (133, 143, 154 g CP/kg DM vs. 133, 144, 156 g CP/kg DM for LCP, MCP, and HCP, respectively). However, the temporal pattern of CP supply differed between lactations. During the first lactation, CP levels peaked at 4 weeks in milk and gradually declined thereafter, whereas in the second lactation this peak was less pronounced for LCP and MCP, and no clear (gradual) decline was observed between 15 and 30 weeks in milk for all three treatments. These differences likely resulted from changes in silage batches over time and discrepancies between the CP values used during diet formulation and the actual analyzed CP concentrations of the grass silages.

The feed intake and production results obtained during the second lactation were highly consistent with those from the first lactation, indicating a robust and repeatable response of dairy cows to dietary CP levels across production cycles. As observed previously, cows fed the LCP diet again had lower DMI and milk yield compared with those fed MCP and HCP. MCP supported the same level of production as HCP, indicating that increasing dietary CP beyond the medium level did not further enhance intake or milk yield. Milk fat content was not affected by dietary CP level, in L2 in contrast to L1 where fat was affected by an interaction between CP and week. Protein content showed a CP × week interaction in both lactations without a consistent overall effect. Milk urea concentration showed the same strong and consistent diet effect in both lactations (HCP > MCP > LCP). FPCM yield followed the same pattern as milk yield, with MCP and HCP outperforming LCP but not differing from each other.

Fat yield also showed a diet × week interaction, similar to the first lactation. Over extended periods, HCP and MCP remained higher than LCP in most of the lactation weeks, however differences between HCP and MCP were not significant in any week. The pattern of weekly differences was broadly similar to that observed in lactation 1. Protein yield displayed a consistent pattern across both lactations, with MCP and HCP having greater protein yield than LCP, without differences between HCP and MCP in both lactations. Feed efficiency also showed a diet × week interaction in both lactations, indicating diet dependent differences over time with a general pattern of LCP having lower feed efficiency than MCP or HCP.

Nitrogen efficiency followed a similar pattern across both study lactations. In both lactations, cows fed the LCP diet generally had higher nitrogen efficiency compared with those fed MCP and HCP diets, with nitrogen efficiency also being higher in MCP than in HCP. These differences were reflected in a CP × week interaction and most pronounced during the first half of lactation, whereas from approximately week 28 onwards no differences in nitrogen efficiency were observed among treatments.

These results emphasize that the trade-off between nitrogen efficiency and performance depends on the degree of protein restriction. Low CP diets (LCP) improved nitrogen efficiency, this occurred at the cost of reduced dry matter intake and milk yield. In contrast, across two consecutive lactations under the conditions of this study, no clear difference in milk yield or dry matter intake was observed between MCP and HCP. Moreover, MCP exhibited little or no reduction in milk N secretion compared with HCP despite a lower N intake, indicating a more efficient partitioning of dietary nitrogen. This improved N utilization in MCP relative to HCP is further supported by consistently lower milk urea concentrations for MCP across most weeks of lactation in both L1 and L2, which is indicative of lower excess rumen nitrogen and assumed reductions in urinary N losses.

5.3 Conclusion

The results of the second lactation confirm the outcomes observed in the first lactation. Cows fed LCP had lower intake and milk yield, but higher nitrogen efficiency compared with MCP and HCP. MCP supported the same performance as HCP, showing that additional protein did not provide production benefits but did increase N losses. These findings across two lactations demonstrate that, under the conditions of the present study, feeding MCP is sufficient to sustain performance while improving N efficiency compared with HCP, whereas a further reduction in CP level (LCP) enhanced N efficiency but at the cost of reduced milk yield.

6 Digestibility, nitrogen utilization, and metabolism in lactation 1

6.1 Results

The next chapter outlines the nutrient intake and total apparent total tract digestibility of key nutrients, the nitrogen utilization and blood serum metabolites as affected by the dietary CP level and lactation stage (peak (7-12 WIM) or mid-lactation (30-35 WIM)) during the first lactation of the study. The chemical compositions of the different diet components are presented in Table 6.1 for peak lactation and in Table 6.2 for mid lactation during the first lactation of the study. The realized dietary CP level at peak lactation was 139, 154 and 170 g/kg DM for LCP, MCP and HCP, respectively, and the dietary CP level at mid lactation was 138, 147 and 157 g/kg DM for LCP, MCP and HCP, respectively.

Table 6.1. Chemical composition of PMR and different types of concentrates offered during peak lactation (7-12 WIM) during the first lactation of the study.

	PMR[1]		Concentrates[2]					
			Low CP		High CP		GreenFeed	
Composition (g/kg DM)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM (g/kg product)	359	10.9	862	-	869	-	875	-
OM	901	2.2	936	-	937	-	925	-
CP	135	3.1	147	-	317	-	139	-
Ether extract	32	0.6	37	-	40	-	30	-
Gross energy (MJ/d)	18.6	0.05	18.0	-	19.1	-	17.9	-
NDF	434	6.1	259	-	239	-	378	-
ADF	262	4.2	149	-	133	-	228	-
ADL	18	0.5	12	-	37	-	16	-
Starch	123	7.1	272	-	191	-	106	-
Sugar	18	1.6	82	-	83	-	101	-

[1]PMR consisted of 58.8% grass silage, 34.7% corn silage, 5.7% rapeseed meal, and 0.8% mineral premix on a DM basis.
[2]Concentrates for different batches were pooled and analyzed as one sample, SD was not determined.

Table 6.2. Chemical composition of PMR and different types of concentrates offered during mid lactation (30-35 WIM) during the first lactation of the study.

	PMR[1]		Concentrates[2]					
			Low CP		High CP		GreenFeed	
Composition (g/kg DM)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM (g/kg product)	359	6.1	860	-	870	-	878	-
OM	904	3.0	945	-	938	-	921	-
CP	132	4.2	155	-	317	-	145	-
Ether extract	31	0.5	40	-	39	-	27	-
Gross energy (MJ/d)	18.5	0.02	18.2	-	19.2	-	17.8	-
NDF	430	3.8	251	-	245	-	382	-
ADF	258	2.7	82	-	130	-	225	-
ADL	19	0.3	14	-	34	-	18	-
Starch	125	7.3	272	-	197	-	122	-
Sugar	20	2.2	83	-	85	-	100	-

[1]PMR consisted of 57.7% grass silage, 35.8% corn silage, 5.7% rapeseed meal, and 0.8% mineral premix on a DM basis.
[2]Concentrates for different batches were pooled and analyzed as one sample, SD was not determined.

6.1.1 Nutrient intake and digestibility (L1)

Table 6.3 outlines the nutrient intake and total apparent total tract digestibility of key nutrients as affected by the dietary CP level and lactation stage (peak (7-12 WIM) or mid-lactation (30-35 WIM)) during the first lactation of the study.

- CP × lactation stage interactions were observed for CP intake, ADL intake, and sugar intake, but not for the other intake variables.
- DM intake was higher in MCP and HCP, compared with LCP, resulting in a proportional increase in intake of all other nutrients. Intake of OM, ether extract, GE, NDF, ADF, and starch was higher in

MCP and HCP compared with LCP ($P < 0.01$) with no differences between MCP and HCP. CP intake increased with increasing dietary CP level ($P < 0.01$).

- The period of measurement had an effect on all intake variables ($P < 0.01$).
- CP × lactation stage interactions were not observed for apparent total tract digestibility variables.
- In general, different dietary CP levels did not have a large effect on the apparent total tract digestibility of the nutrients, with exception of CP. Apparent total tract digestibility of CP was lower for LCP compared with MCP and HCP, and for MCP compared with HCP ($P < 0.01$). Apparent total tract digestibility of GE tended to differ between cows fed different dietary CP levels ($P = 0.06$). Apparent total tract digestibility of DM, OM, ether extract, NDF, and starch were not affected by the dietary CP level.
- The sampling period had an effect on the apparent total tract digestibility of DM, OM, ether extract, GE, and NDF ($P \leq 0.01$). However, the sampling period did not affect the apparent total tract digestibility of CP ($P = 0.15$) or starch ($P = 0.24$).

Table 6.3. Effect of dietary crude protein (CP) level (LCP, MCP, and HCP) and lactation stage (LS; peak or mid-lactation) on nutrient intake and apparent total tract digestibility of nutrients in lactating Holstein Friesian cows during the first lactation of the study.

	Diet (CP)				*P*-value		
	LCP	MCP	HCP	SEM	CP	LS	CP × LS
Nutrient intake (kg/d, unless otherwise stated)							
DM	21.5[b]	24.2[a]	24.1[a]	0.22	<0.01	<0.01	0.43
OM	19.7[b]	22.1[a]	22.0[a]	0.20	<0.01	<0.01	0.39
CP	2.98	3.65	3.93	0.048	<0.01	<0.01	<0.01
Ether extract	0.71[b]	0.80[a]	0.80[a]	0.008	<0.01	<0.01	0.17
Gross energy (MJ/d)	394[b]	447[a]	448[a]	4.1	<0.01	<0.01	0.40
NDF	8.11[b]	9.25[a]	9.16[a]	0.083	<0.01	<0.01	0.48
ADF	4.70[b]	5.42[a]	5.39[a]	0.053	<0.01	<0.01	0.49
ADL	0.36	0.45	0.49	0.006	<0.01	<0.01	<0.01
Starch	3.57[b]	3.77[a]	3.63[a]	0.042	<0.01	<0.01	0.21
Sugar	0.94	1.01	0.98	0.014	<0.01	<0.01	0.05
Apparent total tract digestibility (% of intake)							
DM	69.1	67.7	69.2	0.60	0.13	<0.01	0.75
OM	70.7	69.2	70.7	0.61	0.12	<0.01	0.76
CP	55.5[c]	58.3[b]	63.6[a]	0.63	<0.01	0.15	0.20
Ether extract	64.8	64.6	66.7	0.66	0.26	<0.01	0.78
Gross energy	68.0	66.9	68.6	0.59	0.06	0.01	0.95
NDF	62.1	60.1	61.3	0.67	0.29	<0.01	0.85
Starch	96.6	95.9	96.1	0.75	0.12	0.24	0.17

[a-c] Values with different superscript are significantly different ($P < 0.05$)

6.1.2 Nitrogen utilization (L1)

Table 6.4 shows the effect of dietary CP level and period of measurement (peak (7-12 WIM) or mid lactation (30-35 WIM)), on nitrogen intake and excretion during the first lactation of the study.

- There was an interaction between CP and lactation stage for total N intake and N intake per unit metabolic BW ($P < 0.01$), with N intake generally increasing with increasing dietary CP level, reflecting the gradient in dietary CP concentration.
- Milk yield and N secretion in milk were higher for MCP and HCP compared with LCP ($P < 0.01$), with no difference between MCP and HCP. There was a CP × lactation stage interaction for the ratio of milk N to N intake ($P < 0.01$), with the N ratio in milk generally being higher for LCP than MCP and HCP, and MCP being higher than HCP. This indicates that while total N secretion in milk decreased as dietary CP level decreased, a shift in nitrogen partitioning occurred, resulting in a increasing proportion of N intake directed to milk.
- Total fecal DM output was higher for HCP and MCP compared with LCP ($P < 0.01$), but not different from HCP compared with MCP. N excretion in feces was higher for MCP compared to LCP ($P <$

0.01), while HCP was intermediate and not different from either LCP and MCP. The effect of dietary CP level on fecal N per unit metabolic BW remained a trend (P = 0.06).

- The estimated urine volume was higher for HCP compared with LCP (P = 0.05), while MCP was not different from the other treatments. There was an CP × lactation stage interaction for urea N excretion, total urinary N excretion (Figure 6.1), urinary N per unit metabolic BW, and the proportion of urinary N relative to N intake (P < 0.01). This generally shows a decreasing N excretion in urine with a decreasing CP level.
- The N retention was affected by CP level (P < 0.01), with LCP being lower compared with HCP, while MCP did not differ from either LCP or HCP.
- The total N content in the rumen fluid was affected by CP level, with LCP and MCP being lower compared with HCP, while LCP and MCP did not differ from each other (P < 0.01). The NH_3 in the rumen fluid was lower for LCP compared with MCP and HCP, and lower for MCP compared with HCP (P < 0.01).

Table 6.4. Effect of dietary treatment (LCP, MCP, and HCP) and lactation stage (LS; early- and mid-lactation) on nitrogen intake and excretion of lactating dairy cows during the first lactation of the study.

		Diet (CP)				*P* - value		
		LCP	MCP	HCP	SEM	CP	LS	CP × LS
Metabolic BW	$BW^{0.75}$	132	137	137	0.86	0.01	0.36	0.67
CP	g/kg DM	139	150	163	0.98	<0.01	<0.01	<0.01
Intake								
DMI	kg/d	21.5[b]	24.2[a]	24.1[a]	0.22	<0.01	<0.01	0.41
Total N intake	g/d	477	583	629	7.7	<0.01	<0.01	<0.01
N intake	g/kg $BW^{0.75}$/d	3.63	4.26	4.61	0.056	<0.01	<0.01	<0.01
Milk								
Milk yield	kg/d	30.5[b]	35.2[a]	35.8[a]	0.71	<0.01	<0.01	0.43
Total Milk N secretion	g/d	158[b]	184[a]	191[a]	2.9	<0.01	<0.01	0.54
N secretion	g/kg $BW^{0.75}$/d	1.21[b]	1.35[a]	1.40[a]	0.023	<0.01	<0.01	0.75
N milk / N intake		0.331	0.314	0.303	0.0033	0.01	<0.01	<0.01
Feces								
Total output	kg DM/d	6.67[b]	7.83[a]	7.44[a]	0.104	<0.01	<0.01	0.70
Total fecal N excretion	g/d	213[b]	243[a]	229[ab]	3.2	<0.01	<0.01	0.51
N excretion	g/kg $BW^{0.75}$/d	1.62	1.78	1.68	0.024	0.06	<0.01	0.65
N feces / N intake		0.445[a]	0.417[b]	0.364[c]	0.0043	<0.01	0.15	0.20
Urine								
Urine volume	L	29.8[b]	31.7[ab]	33.9[a]	0.55	0.05	<0.01	0.32
Urea N excretion	g/d	34.2	70.6	110.4	3.22	<0.01	<0.01	<0.01
Total urinary N excretion	g/d	105	146	185	3.5	<0.01	<0.01	<0.01
N excretion	g/kg $BW^{0.75}$/d	0.80	1.07	1.35	0.026	<0.01	<0.01	<0.01
N urine / N intake		0.221	0.252	0.294	0.0041	<0.01	0.08	<0.01
N retention								
Total N retention	g/d	0.7[b]	9.4[ab]	24.0[a]	2.81	<0.01	<0.01	0.77
N retention	g/kg $BW^{0.75}$/d	0.006[b]	0.063[ab]	0.170[a]	0.0208	<0.01	<0.01	0.71
Rumen fluid								
N in content	g/kg	0.130[b]	0.147[b]	0.184[a]	0.0043	<0.01	<0.01	0.39
NH_3 in content	mg/L	13.5[c]	18.6[b]	25.8[a]	0.90	<0.01	<0.01	0.47

[a-c] Values with different superscript are significantly different (P < 0.05)

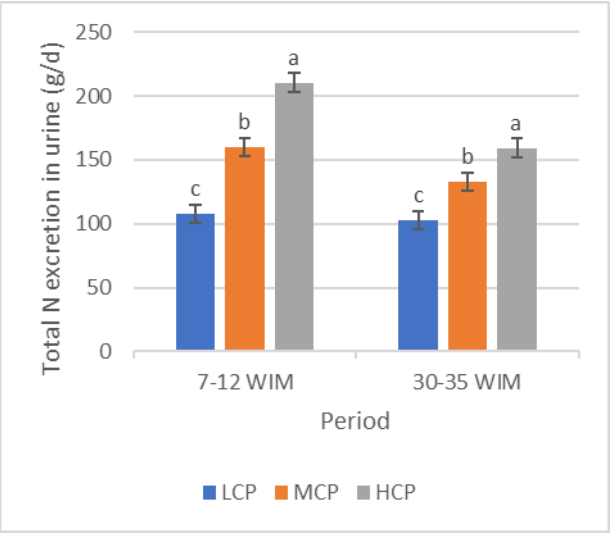


Figure 6.1. Total N excretion in urine for different dietary treatments (LCP, MCP, and HCP) during different lactation stages in the first lactation of the study (L1).

6.1.3 Blood analysis (L1)

Results of serum analysis from blood samples collected during peak (7-12 WIM) or mid (30-35 WIM) lactation in the first lactation of the study showed clear effects of dietary CP level, lactation stage, and their interaction (Table 6.5).

- A CP × lactation stage interaction was observed for blood serum concentrations of AST, Bilirubin, Phosphate, GGT, GLDH, Haptoglobin, total protein, and urea ($P \leq 0.03$), whereas magnesium tended to be affected by a CP × lactation stage interaction ($P = 0.08$). Average lever values of ALP, AST, Bilirubin, GGT, and GLDH were well within normal reference range. Figure 6.2 shows the GLDH, Haptoglobin total protein, and urea levels in blood serum for different dietary CP levels during both periods. During peak lactation GLDH levels were lower in MCP compared with HCP, with LCP not being different from both MCP and HCP, however there were no differences between CP levels in mid lactation cows. Haptoglobin levels were lowest in MCP during both periods. While HCP cows had higher haptoglobin levels than MCP in early lactation, LCP cows had higher haptoglobin levels than MCP and HCP in mid lactation. The blood serum levels of total protein and urea clearly depicture the effect of different dietary CP levels with increasing levels of total protein and urea with increasing dietary CP level.
- Blood serum concentrations of BHBA tended to be affected by diet ($P = 0.06$).
- In addition, an effect of lactation stage was observed for all blood parameters ($P \leq 0.01$) except BHBA and CPK ($P \geq 0.21$), indicating that physiological stage of lactation influenced most blood metabolites and indicators of liver function and protein metabolism.

Table 6.5. Effect of dietary treatment (LCP, MCP, and HCP), lactation stage (LS; early or mid-lactation), on blood serum metabolites of lactating dairy cows during the first lactation of the study.

		Diet (CP)				*P*-value		
		LCP	MCP	HCP	SEM	CP	LS	CP x LS
Albumin	g/L	34.8	36.2	36.3	0.14	0.16	<0.01	0.12
ALP	IU/L	36.4	34.9	33.9	0.50	0.77	<0.01	0.60
AST	IU/L	77.3	75.2	84.1	0.81	0.17	<0.01	<0.01
BHBA	mmol/L	0.79	0.82	0.89	0.010	0.06	0.21	0.19
Bilirubin	µmol/L	5.06	4.64	4.64	0.04	0.16	0.01	0.03
Calcium	mmol/L	2.44	2.47	2.48	0.006	0.31	<0.01	0.68
CPK	IU/L	129	152	120	5.1	0.28	0.88	0.10
Creatinine	µmol/L	68.6	67.9	63.7	0.45	0.10	<0.01	0.18
Inorganic phosphate	mmol/L	1.83	1.80	1.77	0.009	0.41	<0.01	0.03
GGT	IU/L	28.2	27.8	29.6	0.36	0.75	<0.01	<0.01
GLDH	IU/L	19.4	17.4	21.7	0.71	0.51	0.01	<0.01
Hp	g/L	0.22	0.13	0.18	0.010	0.25	<0.01	<0.01
Log Hp	Hp x 100	1.17	1.07	1.11	0.013	0.31	<0.01	0.23

Magnesium	mmol/L	0.97	0.97	0.96	0.004	0.99	<0.01	0.08
NEFA	mmol/L	0.15	0.15	0.16	0.004	0.50	<0.01	0.63
Total Protein	g/L	71.2	75.8	77.3	0.29	<0.01	<0.01	0.03
Urea	mmol/L	1.76	2.39	3.21	0.034	<0.01	<0.01	<0.01

[a-c] Values with different superscript are significantly different ($P < 0.05$)

ALP = alkaline phosphatase; AST = aspartate aminotransferase; BHBA = β-hydroxy butyric acid; CPK = creatinine phosphor kinase; GGT = gamma-glutamyl transferase; GLDH = glutamate dehydrogenase; Hp = Haptoglobin; NEFA = non Esterified Fatty Acid

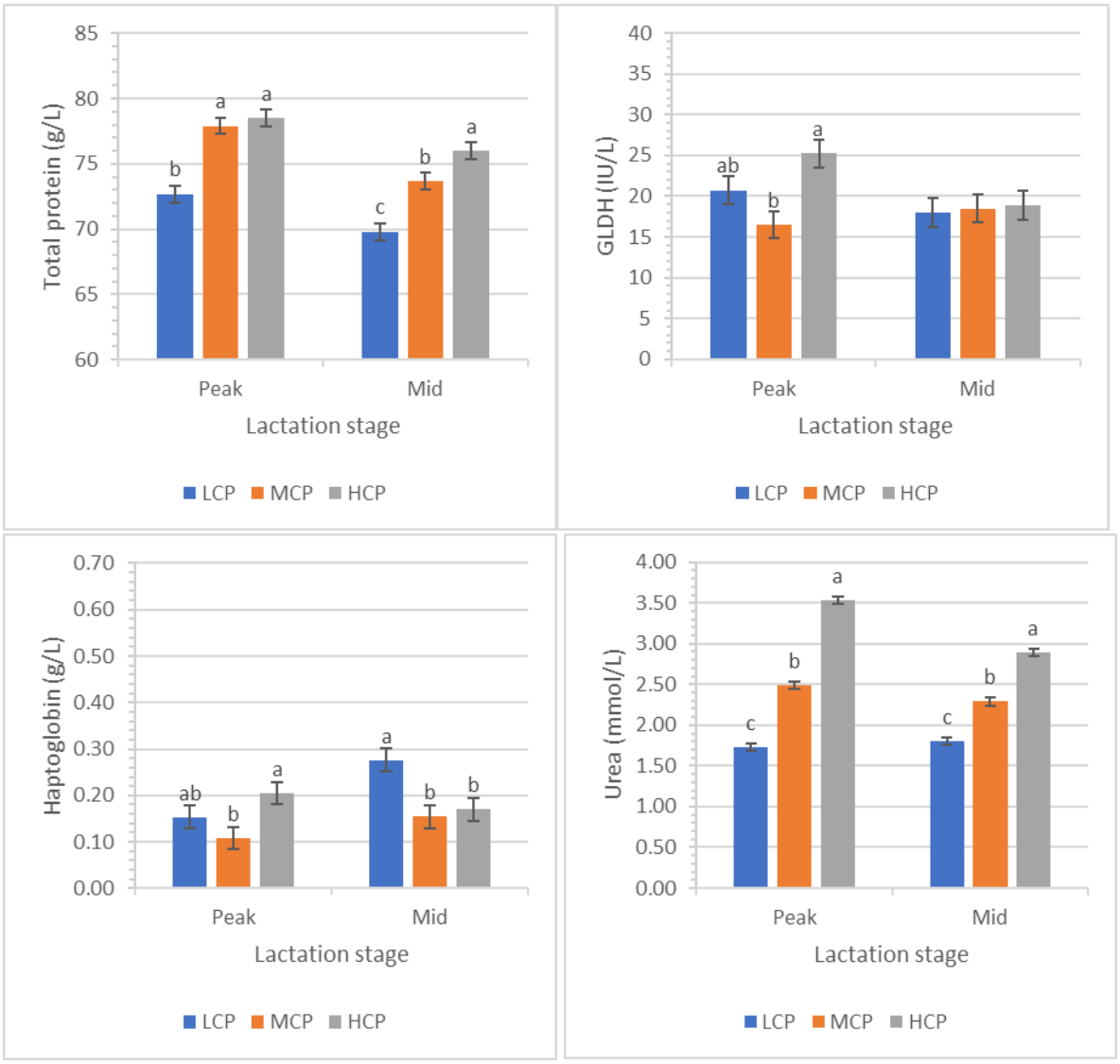


Figure 6.2. Blood serum levels of total protein, GLDH, Haptoglobin, and urea of dairy cows fed different dietary crude protein levels (LCP, MCP, and HCP) in different lactation stages (peak or mid-lactation) during the first lactation of the study.

6.2 Interpretation

Digestibility and nitrogen balance

The differences in dietary CP level led to clear differences in nutrient intake, especially between the low CP diet and the two higher CP diets. However, the apparent total tract digestibility of most nutrients did not differ among treatments. This indicates that the range of CP concentrations used in this study did not lead to major changes in overall digestibility.

The observed differences in apparent total tract digestibility of CP, particularly between the LCP and HCP diets, represent a key finding of the present study. These differences are most likely attributable to the lower dietary CP concentration rather than to changes in the true digestibility of feed protein. Endogenous protein losses are closely related to the flow of undigested organic matter through the lower gastrointestinal

tract. Assuming a similar flow of undigested organic matter across diets, a lower dietary CP concentration would result in a relatively greater contribution of endogenous nitrogen to fecal CP, thereby reducing apparent total tract digestibility of CP. Consequently, true CP digestibility may have been similar across all three diets. This distinction between apparent and true digestibility is a well-established principle in digestion studies.

The clear differences in total nitrogen intake among the treatments, with the lowest intake in the low CP diet and progressively higher intakes in the medium and high CP diets, were directly reflected in nitrogen excretion in urine and feces. Absolute nitrogen excretion in urine and feces differed among treatments. Lowering dietary CP reduced the proportion excreted in urine relative to nitrogen intake.

The reduction in urinary N excretion represents another key finding of this study. When expressed relative to metabolic body weight, urinary N excretion was approximately 21% lower in MCP cows compared with HCP cows, and as much as 41% lower in LCP cows compared with HCP cows. These results highlight the substantial potential for reducing NH_3 emissions to the environment through lowering dietary CP concentrations. As a general rule of thumb, a reduction of 1 g CP/kg DM is often associated with an approximate 1% reduction in NH_3 emissions; however, the reductions in urinary N excretion observed in the present study considerably exceed this expectation.

Increasing dietary CP increased total nitrogen intake, but most of the additional nitrogen in the medium and high CP diets was excreted in urine rather than secreted in milk. This kept milk nitrogen output similar between the medium and high CP treatments and reduced nitrogen use efficiency, indicating that the marginal efficiency of increasing CP above the medium CP level was low.

Blood analysis

Across both lactation stages, the absence of elevated AST activities indicates that none of the dietary CP treatments induced hepatocellular damage or overt liver overload. Although AST values were numerically slightly higher in HCP cows during both peak and mid lactation, these differences were biologically minor and remained well within the physiological range. In contrast, GLDH activity revealed a subtle increase in hepatic metabolic activity in HCP cows during peak lactation, suggesting a modest additional liver load associated with higher CP supply.

Serum total protein concentrations were within reference values for lactating dairy cows and differed only slightly among treatments. The somewhat higher total protein levels observed for MCP and HCP do not indicate improved protein utilization, but rather are consistent with a higher circulating nitrogen load at higher CP intakes.

Serum haptoglobin concentrations remained below approximately 0.30 g/L across all treatments and lactation stages, indicating the absence of a pronounced systemic inflammatory response. The slightly elevated haptoglobin concentrations observed for LCP cows during mid lactation may reflect a mild stress response, but without evidence of clinical relevance. Moreover, the lack of a CP × lactation stage interaction for log-transformed haptoglobin suggests that observed variation was more likely attributable to individual cow differences than to dietary treatment effects.

Although serum urea concentrations in LCP cows were notably low, total protein concentrations remained within the normal range for all CP treatments. Together, these findings indicate that reduced dietary CP levels, supported efficient N utilization without evidence of compromised liver function or increased inflammatory burden during either lactation stage.

6.3 Conclusion

Reducing dietary CP lowered N intake and substantially decreased urinary N excretion, thereby improving N use efficiency for milk without major effects on overall nutrient digestibility. Although apparent total tract CP digestibility declined with lower CP supply, milk N secretion was only reduced at the lowest CP level, and no additional benefit in milk N secretion was observed with the higher CP diet compared with the medium CP. Blood metabolites supported these findings, as lower serum urea concentrations in LCP cows reflected reduced excess N and more efficient N utilization.

Overall, a moderate reduction of dietary CP can effectively reduce N losses without substantially impairing productive performance, while maintaining the cow’s production and metabolic function.

7 Digestibility and nitrogen utilization in lactation 2

7.1 Results

The next chapter outlines the nutrient intake and total apparent total tract digestibility of key nutrients, the nitrogen utilization and blood serum metabolites as affected by the dietary CP level and lactation stage (peak (7-12 WIM) or mid-lactation (30-35 WIM)) during the second lactation of the study. The chemical compositions of the different diet components are presented in Table 7.1 for peak lactation and in Table 7.2 for mid lactation during the second lactation of the study. The realized dietary CP level at peak lactation was 140, 151 and 166 g/kg DM for LCP, MCP and HCP, respectively, and the dietary CP level at mid lactation was 150, 154 and 160 g/kg DM for LCP, MCP and HCP, respectively.

Table 7.1. Chemical composition of PMR and different types of concentrates offered during peak lactation (7-12 WIM) during the second lactation of the study.

	PMR[1]		Concentrates					
			Low CP		High CP		GreenFeed	
Composition (g/kg DM)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM (g/kg product)	395	19.6	868	5.7	883	3.5	873	4.5
OM	905	2.1	938	2.3	934	0.9	920	1.0
CP	137	9.2	150	2.3	315	4.3	142	2.6
Ether extract	31	3.2	38	0.8	41	1.3	33	1.0
Gross energy (MJ/d)	18.7	0.17	17.8	0.24	19.3	0.14	17.9	0.05
NDF	413	18.5	253	16.4	233	12.7	373	10.7
ADF	246	15.3	141	2.3	127	3.6	219	4.7
ADL	13	1.7	7	2.4	37	5.2	12	0.5
Starch	144	20.5	269	7.5	194	8.6	104	4.1
Sugar	39	21.5	80	6.4	78	5.5	110	10.8

[1]PMR consisted of 58.4% grass silage, 36.3% corn silage, 4.6% rapeseed meal, and 0.7% mineral premix on a DM basis.

Table 7.2. Chemical composition of PMR and different types of concentrates offered during mid lactation (30-35 WIM) during the second lactation of the study.

	PMR[1]		Concentrates					
			Low CP		High CP		GreenFeed	
Composition (g/kg DM)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM (g/kg product)	381	14.1	874	2.4	885	6.6	875	2.5
OM	906	4.3	940	2.0	935	0.4	920	1.6
CP	141	7.6	151	3.0	320	3.4	142	2.2
Ether extract	34	2.8	39	0.9	41	1.1	34	0.8
Gross energy (MJ/d)	18.8	0.16	18.0	0.15	19.2	0.14	17.9	0.05
NDF	404	9.4	241	6.9	222	8.6	370	7.1
ADF	229	8.3	142	3.9	124	1.4	217	2.1
ADL	12	1.1	4	1.0	32	1.4	12	0.8
Starch	144	12.2	263	5.2	187	4.6	106	6.0
Sugar	23	17.2	75	4.0	73	1.1	97	8.3

[1]PMR consisted of 58.8% grass silage, 34.7% corn silage, 5.7% rapeseed meal, and 0.8% mineral premix on a DM basis.

7.1.1 Nutrient intake and digestibility (L2)

Table 7.3 summarizes nutrient intake and apparent total tract digestibility of cows as influenced by dietary CP level and lactation stage (peak (7-12 WIM) or mid-lactation (30-35 WIM)) in the second lactation in the study.

- CP × lactation stage interactions were observed for CP intake and ADL intake, but not for the other intake variables. In general cows receiving the LCP diet had the lowest CP intake, with MCP and HCP having higher CP intake following the dietary CP gradient.
- Dietary CP level affected ether extract and gross energy intake ($P \leq 0.03$). Ether extract and GE intakes were higher in MCP compared with LCP, while HCP was not different from either treatment. There was a tendency for dietary CP level to affect the intake of DM, OM, NDF, and ADF.

- The lactation stage of cows affected the intake of all nutrients ($P < 0.01$).
- CP × lactation stage interactions were observed for apparent total tract digestibility of DM, OM, and NDF ($P \leq 0.03$), and tended to affect GE.
- Dietary CP level affected the apparent total tract digestibility of several nutrients. Apparent total tract digestibility of CP ($P < 0.01$) was lower for LCP and MCP compared with HCP, while LCP and MCP were not different. Apparent total tract digestibility for ether extract and GE ($P < 0.01$) were lower for LCP compared with HCP, while MCP did not differ from LCP and HCP. Starch digestibility was not affected by dietary CP level ($P = 0.13$).
- The lactation stage of cows affected the digestibility of CP and ether extract ($P \leq 0.03$).

Table 7.3. Effect of dietary treatment (LCP, MCP, and HCP) and lactation stage (LS; early or mid-lactation) on nutrient intake and apparent total tract digestibility of nutrients in lactating Holstein Friesian cows during the second lactation of the study.

	Diet (CP)				*P* - value		
	LCP	MCP	HCP	SEM	CP	LS	CP × LS
Nutrient intake (kg/d, unless otherwise stated)							
DM	24.9	26.7	26.1	0.32	0.06	<0.01	0.72
OM	22.8	24.4	23.9	0.30	0.06	<0.01	0.72
CP	3.61	4.06	4.26	0.055	<0.01	<0.01	0.02
Ether extract	0.85[b]	0.90[a]	0.88[ab]	0.009	0.03	<0.01	0.90
Gross energy (MJ/d)	460[b]	495[a]	488[ab]	5.9	0.02	<0.01	0.57
NDF	9.11	9.83	9.51	0.128	0.08	<0.01	0.52
ADF	5.28	5.71	5.55	0.079	0.07	<0.01	0.48
ADL	0.29	0.35	0.39	0.008	<0.01	<0.01	<0.01
Starch	4.31	4.40	4.29	0.066	0.55	<0.01	0.52
Sugar	1.26	1.37	1.36	0.052	0.40	<0.01	0.40
Apparent total tract digestibility (% of intake)							
DM	67.4	68.2	70.4	0.37	<0.01	0.34	0.03
OM	69.0	69.8	72.0	0.37	<0.01	0.28	0.03
CP	57.6[b]	59.3[b]	63.1[a]	0.50	<0.01	0.01	0.36
Ether extract	64.8[b]	65.2[ab]	68.1[a]	0.51	<0.01	0.03	0.37
Gross energy	67.0[b]	67.8[ab]	70.1[a]	0.39	<0.01	0.48	0.06
NDF	56.7	58.6	61.9	0.58	<0.01	0.37	0.02
Starch	95.6	95.8	95.9	0.19	0.13	0.46	0.28

[a-c] Values with different superscript are significantly different ($P < 0.05$)

7.1.2 Nitrogen utilization (L2)

Table 7.4 presents N intake and excretion for cows in their second lactation as affected by dietary CP level and period of measurement.

- There was an interaction between CP and lactation stage for total N intake and N intake per unit metabolic body weight ($P < 0.01$), which generally followed the gradient of dietary CP level.
- Milk yield and total milk N secretion did not differ among dietary CP treatments ($P \geq 0.20$), although values were numerically higher for MCP and HCP compared with LCP. Nitrogen secretion in milk per unit metabolic BW showed a similar pattern and was not affected by dietary CP level ($P = 0.51$). The ratio of milk N to N intake tended to decrease with increasing dietary CP level ($P = 0.06$). The lactation stage of cows had an effect on milk N secretion and the ratio of milk N to N intake ($P < 0.01$).
- Fecal N variables were not affected by a CP and lactation stage interaction or by dietary CP level. Total fecal N excretion and fecal N per unit metabolic BW did not differ between treatments ($P \geq 0.15$). However, the proportion of fecal N relative to N intake was affected by dietary CP level ($P < 0.01$), with LCP and MCP having a higher fecal N ratio than HCP, with no differences between LCP and MCP. The lactation stage of the cow had an effect on all fecal-related variables ($P \leq 0.01$).
- The estimated urine volume increased with dietary CP level ($P = 0.04$). There was an CP and lactation stage interaction for total urinary N excretion (Figure 7.1) and urinary N per unit

metabolic BW, which generally showed an increase as dietary CP increased ($P < 0.01$). There was an effect of dietary CP level on the proportion of urinary N relative to N intake ($P < 0.01$), with LCP cows having lower urinary N ratios compared with MCP and HCP, and MCP having lower urinary N ratios compared with HCP. The lactation stage of the cows affected all urinary N variables ($P \leq 0.03$).

- Total N retention and N retention per unit metabolic BW did not differ among dietary CP levels ($P \geq 0.87$) and were not affected by period ($P \geq 0.10$).
- Rumen fluid N and NH_3 concentrations were affected by dietary CP level ($P < 0.01$). HCP had higher rumen fluid N and NH_3 concentrations than LCP and MCP, while LCP and MCP did not differ from each other.

Table 7.4. Effect of dietary treatment (LCP, MCP, and HCP) and lactation stage (LS; early- and mid-lactation) on nitrogen intake and excretion of lactating dairy cows during the second lactation of the study.

		Diet (CP)				*P* - value		
		LCP	MCP	HCP	SEM	CP	LS	CP × LS
Metabolic BW	$BW^{0.75}$	133	135	135	1.0	0.68	0.23	0.21
CP	g/kg DM	145	152	163	1.0	<0.01	0.03	<0.01
Intake								
DMI	kg/d	25.0	26.7	26.1	0.32	0.10	<0.01	0.54
Total N intake	g/d	577	650	682	8.8	<0.01	<0.01	0.01
N intake	g/kg $BW^{0.75}$/d	4.36	4.83	5.07	0.072	<0.01	<0.01	0.04
Milk								
Milk yield	kg/d	34.5	37.4	36.5	0.83	0.31	<0.01	0.10
Total Milk N secretion	g/d	184	199	197	3.4	0.20	<0.01	0.50
N excretion	g/kg $BW^{0.75}$/d	1.40	1.49	1.47	0.029	0.51	<0.01	0.78
N milk / N intake		0.322	0.306	0.290	0.0046	0.06	<0.01	0.14
Feces								
Total output	kg DM/d	8.16	8.49	7.74	0.140	0.18	<0.01	0.33
Total fecal N excretion	g/d	243	264	252	4.1	0.15	<0.01	0.22
N excretion	g/kg $BW^{0.75}$/d	1.84	1.96	1.89	0.033	0.60	<0.01	0.41
N feces / N intake		0.424[a]	0.407[a]	0.369[b]	0.0050	<0.01	0.01	0.22
Urine								
Urine volume	L	26.7	30.1	34.6	1.01	0.04	<0.01	0.64
Urea N excretion	g/d	44.1	80.0	121.5	3.54	<0.01	<0.01	<0.01
Total urinary N excretion	g/d	116	155	194	3.9	<0.01	0.03	<0.01
N excretion	g/kg $BW^{0.75}$/d	0.87	1.16	1.45	0.030	<0.01	0.01	0.01
N urine / N intake		0.203[c]	0.240[b]	0.287[a]	0.0053	<0.01	0.02	0.33
N balance								
Total N retention	g/d	34.3	31.6	37.3	4.69	0.91	0.10	0.22
N retention	g/kg $BW^{0.75}$/d	0.252	0.229	0.245	0.0341	0.87	0.20	0.18
Rumen fluid								
N in content	g/kg	0.169[b]	0.181[b]	0.223[a]	0.0063	<0.01	<0.01	0.11
NH_3 in content	mg/L	21.8[b]	26.6[b]	38.5[a]	1.36	<0.01	<0.01	0.81

[a-c] Values with different superscript are significantly different ($P < 0.05$)

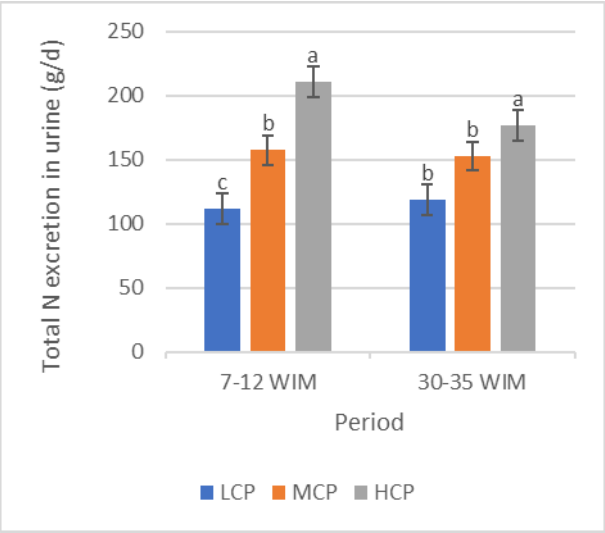


Figure 7.1. Total N excretion in urine for different dietary treatments (LCP, MCP, and HCP) during different lactation stages in the first lactation of the study (L2).

7.1.3 Blood analysis (L2)

The effect of dietary CP level and lactation stage on blood serum metabolites of lactating dairy cows during the second lactation of the study is presented in Table 7.5.

- A CP × period interaction was found for AST, calcium, CPK, NEFA, and urea ($P \leq 0.02$), and BHBA and GLDH tended to be affected by a CP × period interaction. Figure 7.2 shows the blood serum levels of calcium, CPK, NEFA and urea during both periods. The serum calcium levels were highest in LCP and MCP compared with HCP during peak lactation while during mid lactation LCP was showing lower calcium levels than HCP. During peak lactation CPK levels were higher for MCP compared with LCP, with HCP not being different from LCP and MCP, while in mid lactation there were no differences between treatments. During peak lactation NEFA was higher for MCP compared with LCP and HCP, with LCP and HCP not being different from each other, while during mid lactation there were no differences for dietary CP level. Urea increased with the increasing dietary CP level in both peak and mid lactation.
- There was a diet effect for bilirubin (P = 0.01), with LCP being higher than MCP and HCP, whereas MCP and HCP did not differ, and a diet effect for total protein, ($P < 0.01$), with LCP being lower than MCP and HCP, whereas MCP and HCP did not differ. Magnesium tended to be affected by diet ($P = 0.08$).
- BHBA, bilirubin, calcium, creatinine, phosphate, GGT, NEFA, total protein, and urea exhibited a period effect ($P \leq 0.02$). There was a tendency for CPK and haptoglobin (log10-transformed) to be affected by period.

Table 7.5. Effect of dietary treatment (LCP, MCP, and HCP), lactation stage (LS; early or mid-lactation), on blood serum metabolites of lactating dairy cows during the second lactation of the study.

		Diet (CP)				*P*-value		
		LCP	MCP	HCP	SEM	CP	LS	CP x LS
Albumin	g/L	36.4	35.6	35.2	0.18	0.64	0.90	0.47
ALP	IU/L	33.8	34.3	32.9	0.46	0.88	0.51	0.17
AST	IU/L	72.2	73.0	82.1	0.88	0.14	0.52	<0.01
BHBA	mmol/L	0.80	0.84	0.86	0.011	0.51	0.01	0.09
Bilirubin	µmol/L	4.90[a]	4.37[b]	4.27[b]	0.04	0.01	<0.01	0.25
Calcium	mmol/L	2.46	2.48	2.45	0.006	0.74	<0.01	<0.01
CPK	IU/L	117	136	131	5.7	0.59	0.08	0.02
Creatinine	µmol/L	70.2	66.2	71.4	0.73	0.68	<0.01	0.81
Inorganic phosphate	mmol/L	1.75	1.81	1.81	0.009	0.19	<0.01	0.44
GGT	IU/L	26.8	28.4	30.7	0.46	0.39	0.01	0.46
GLDH	IU/L	13.1	13.6	19.9	0.64	0.13	0.79	0.10
Hp	g/L	0.15	0.24	0.16	0.013	0.29	0.32	0.57

Log Hp	Hp x 100	1.10	1.18	1.06	0.013	0.20	0.09	0.53
Magnesium	mmol/L	1.01	0.96	0.99	0.004	0.08	0.73	0.25
NEFA	mmol/L	0.16	0.20	0.17	0.006	0.16	<0.01	0.01
Total Protein	g/L	73.7[b]	80.6[a]	78.6[a]	0.34	<0.01	0.02	0.34
Urea	mmol/L	2.10	2.70	3.80	0.047	<0.01	0.01	<0.01

[a-c] Values with different superscript are significantly different ($P < 0.05$)
ALP = alkaline phosphatase; AST = aspartate aminotransferase; BHBA = β-hydroxy butyric acid; CPK = creatinine phosphor kinase; GGT = gamma-glutamyl transferase; GLDH = glutamate dehydrogenase; Hp = Haptoglobin; NEFA = non Esterified Fatty Acid

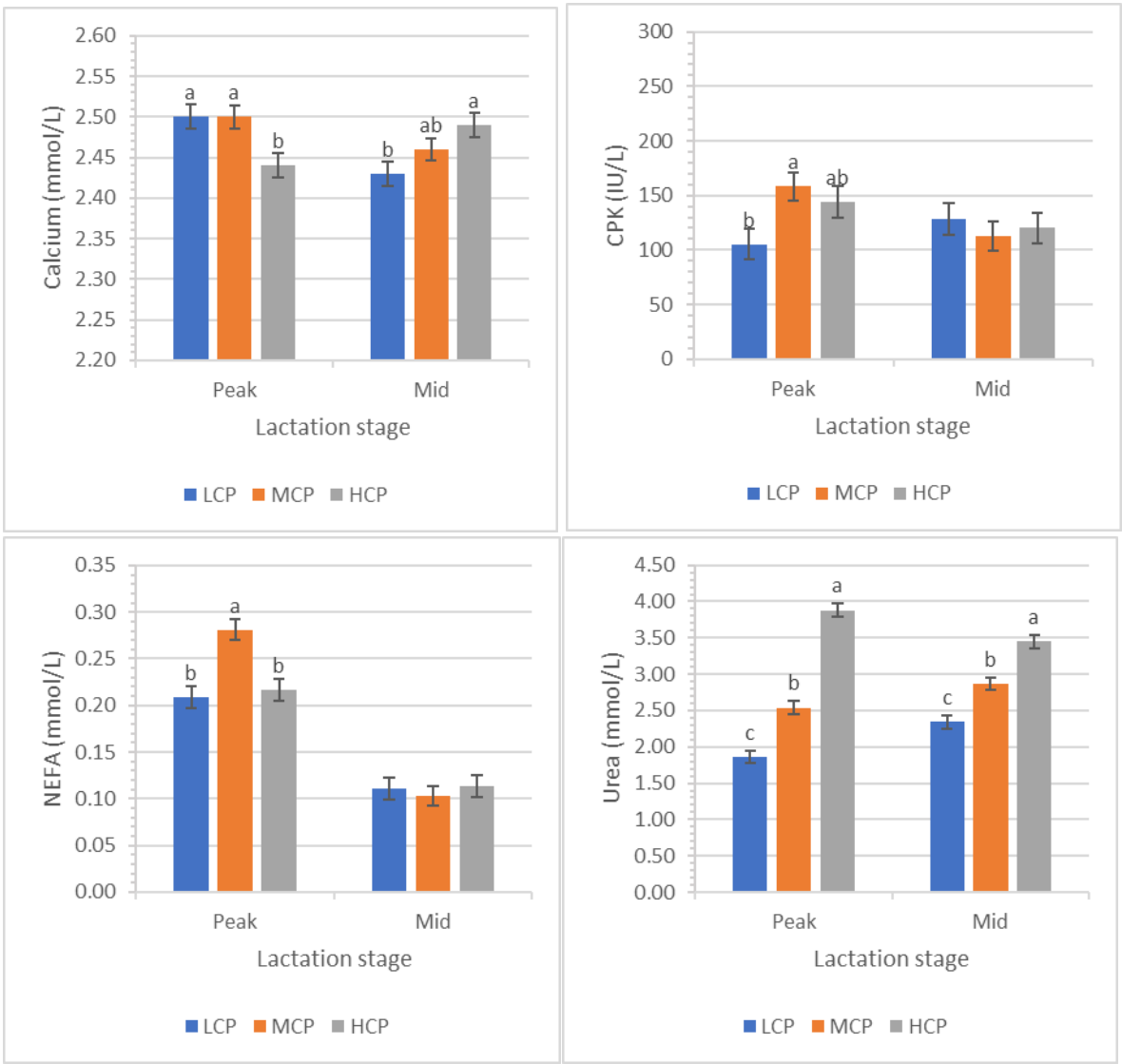


Figure 7.2. Blood serum levels of calcium, CPK, NEFA and urea of dairy cows fed different dietary crude protein levels (LCP, MCP, and HCP) in different lactation stages (peak or mid-lactation) during the second lactation of the study.

7.2 Interpretation of lactation 2 and relation to lactation 1

Digestibility and nitrogen balance

Significant CP × lactation stage interactions were observed for CP intake and ADL intake in both study lactations. In L1, DMI was higher for cows fed MCP and HCP compared with those fed LCP, resulting in a proportional increase in the intake of all other nutrients. In L2, this pattern was less pronounced, which may indicate either an attenuated biological response across successive lactations or reduced statistical power to detect treatment effects in the second lactation. In addition, the period of measurement

significantly affected all intake variables in both lactations, reflecting expected changes in intake across lactation stages.

In contrast to intake responses, CP × lactation stage interactions were not observed for apparent total tract digestibility variables in L1, however in L2, CP × lactation stage interactions were detected for the apparent total tract digestibility of multiple nutrients, suggesting that digestibility responses to dietary CP level were more dynamic in the second lactation. While dietary CP level influenced apparent total tract CP digestibility in both lactations, effects on the digestibility of other nutrients were evident only in L2. Overall, L1 was characterized by a stronger lactation stage effect, whereas L2 showed a more pronounced dietary CP effect on both intake and apparent total tract digestibility. These differences between lactations may be explained, at least in part, by variation in the composition and chemical characteristics of the PMR. Differences in PMR ingredient batches, harvest years, and growing and ensiling conditions of grass and corn silages are likely contributors. Notably, the PMR in L2 contained higher concentrations of CP, starch, and sugars, but lower NDF compared with L1, which may have enhanced fermentability and amplified the dietary CP effects observed in L2.

The results of the N intake and excretion reflect the intended gradient in dietary CP supply and was most evident in the L1. In L1, clear effects of dietary CP level and lactation stage were observed for most N intake and excretion variables, and urinary N excretion showed a pronounced CP × lactation stage interaction. In contrast, responses to dietary CP level were less pronounced in L2. Although differences in N intake and urinary N excretion among CP treatments were still evident in L2, treatment effects on fecal N excretion and milk N secretion were not detected, in contrast to L1. This suggests a reduced sensitivity of N partitioning to dietary CP level in the later lactation cycle or under the conditions prevailing in L2. Despite these differences between lactations, the consistently low N content of rumen fluid and the marked reductions in N excretion observed for the reduced-CP diets in both lactations support earlier conclusions. Specifically, reducing dietary CP to approximately 143–144 g/kg DM (MCP) did not compromise N secretion in milk, while consistently lowering total N excretion by approximately 20–25% relative to the HCP diet. These findings indicate that moderate CP reduction effectively improves N efficiency without impairing productive N output, across lactations and lactation stages.

Blood analysis

Reducing dietary CP consistently lowered systemic N status in both lactations, as reflected by reduced serum urea and total protein concentrations in cows fed the LCP diet compared with MCP and HCP. In L1, serum urea concentrations in both peak an mid lactation was low in the reference range in LCP cows, and in L2 this was only observed in peak lactation in the LCP group. These lower values likely reflect a reduced circulating N surplus rather than impaired protein metabolism per se. However, during these specific sampling periods in L1 the LCP diet was also associated with reduced DMI and milk yield, indicating that N supply may have become limiting for optimal performance at the lowest CP level. Importantly, no concurrent abnormalities in liver enzymes or other health indicators were detected, suggesting that the response was nutritional rather than pathological.

The results obtained in L1 and L2 were not fully consistent. Although CP × lactation stage interactions were observed in both lactations, only AST activity and serum urea concentration were consistently affected. Like in L1, also in L2 most serum metabolites remained within established reference ranges for lactating dairy cows, generally suggesting a stable metabolic profile for all dietary CP levels in both lactation stages. In L1, lactation stage exerted a more pronounced effect on several response variables than in L2. This difference may partly be explained by the slower decline in dietary CP levels in L2 relative to L1, resulting in more similar CP concentrations between peak and mid lactation in L2. Consequently, metabolic responses associated with changes in lactation stage may have been attenuated in L2 compared with L1.

From a practical perspective, reducing dietary CP lowers circulating total protein and serum urea without compromising metabolic health. This supports the feasibility of moderate CP reduction as a strategy to improve N efficiency without impairing performance.

7.3 Conclusion

Reducing dietary CP effectively improves N use efficiency by lowering urinary N excretion and shifting N partitioning toward milk, thereby reducing potential environmental N losses. While the HCP diet increased apparent total tract digestibility of CP, this did not translate into greater N retention in milk compared with MCP. The MCP diet maintained milk yield while enhancing N secretion in milk, whereas LCP diets reduced intake and milk production.

Blood analyses confirmed that lowering CP decreases systemic N status, as reflected by reduced serum urea and total protein, without inducing metabolic stress. Overall, these results demonstrate that moderate

reductions in dietary CP can improve N efficiency and reduce environmental impact without compromising cow health or performance.

8 Vaccination

8.1 Results

8.1.1 Acute phase response

Performance and blood variables in the first days following vaccination (Table 8.1) were affected by dietary CP level, day relative to vaccination, and their interaction, with effects differing by study lactation (L1 or L2) and lactation stage (mid = 14–19 WIM; late = 35–40 WIM).

- In L1 during mid lactation, a CP × day interaction was detected for DMI and serum BHBA (P = 0.01). Crude protein level affected creatinine (P = 0.05), with LCP being higher than MCP, while HCP did not differ from LCP and MCP. Crude protein level affected urea concentrations (P < 0.01), with LCP being lower than MCP and HCP, and MCP being lower than HCP. Whereas day relative to vaccination affected DMI, milk yield, albumin, BHBA, creatinine, NEFA, total protein, and urea (P ≤ 0.02).
- In L1 during late lactation, no CP × day interactions were observed, although a tendency was detected for BHBA. Crude protein level affected DMI (P = 0.04) with LCP being lower than HCP and MCP not being different from either LCP and HCP. Crude protein level affected bilirubin (P = 0.05). Crude protein level affected log-transformed haptoglobin (P = 0.03), with LCP being lower than MCP while HCP did not differ from LCP and MCP. Crude protein level affected urea (P < 0.01), with LCP and MCP being lower compared with HCP and with LCP and MCP not being different. Crude protein level tended to affect BHBA and haptoglobin. Day affected DMI, log-transformed haptoglobin, NEFA, and urea (P ≤ 0.04), with a tendency for albumin.
- In L2 during mid lactation, a CP × day interaction was observed for log-transformed haptoglobin (P = 0.03), whereas no main effects of CP were detected. Day relative to vaccination affected DMI, milk yield, and creatinine concentrations (P ≤ 0.04).
- In L2 during late lactation, no CP × day interactions were detected, although a tendency was observed for albumin. Crude protein level affected bilirubin (P < 0.01) with LCP being lower than MCP and HCP, while MCP and HCP did not differ. Crude protein level affected NEFA (P = 0.02), with MCP being lower than HCP, and LCP not being different from MCP and HCP. Crude protein level affected total protein (P = 0.04), with LCP being lower than MCP, and HCP not being different from LCP and MCP. Crude protein level affected urea (P = 0.04) and there was a tendency for creatinine (P = 0.09). Day effects were observed for DMI, milk yield, bilirubin, creatinine, and log-transformed haptoglobin (P ≤ 0.04), with tendencies for haptoglobin and NEFA.

Table 8.1. Effect of dietary crude protein level (LCP, MCP and HCP) and day after vaccination (1, 2, 3, and 5), with day 0 included as covariate, for different study lactations (L1 and L2) and lactation stages (mid = 14-19 and late = 35-40 WIM), on animal performance (DMI and milk yield) and blood serum metabolites in dairy cows.

		Treatment (CP)				*P*-value		
L1 mid		LCP	MCP	HCP	SEM	CP	Day	CP x Day
Dietary CP	g/kg DM							
DMI	kg/d	23.0	25.0	25.1	0.18	<0.01	<0.01	0.01
Milk yield	kg/d	33.3	33.6	34.1	0.34	0.45	<0.01	0.66
Albumin	g/L	36.0	35.4	35.8	0.21	0.22	<0.01	0.78
BHBA	mmol/L	0.78	0.81	0.84	0.012	0.41	<0.01	0.01
Bilirubin	µmol/L	5.1	5.1	5.1	0.06	0.99	0.93	0.98
Creatinine	µmol/L	64.4[a]	61.7[b]	63.6[ab]	0.56	0.05	0.01	0.37
GLDH	IU/L	22.9	24.8	24.7	1.29	0.36	0.14	0.20
Haptoglobin	g/L	0.22	0.22	0.22	0.026	0.99	0.56	0.71
Log Hp		1.09	1.12	1.11	0.021	0.91	0.19	0.83
NEFA	mmol/L	0.12	0.11	0.12	0.003	0.39	0.02	0.17
Total protein	g/L	76.5	75.6	77.1	0.41	0.14	0.01	0.89

Urea	mmol/L	2.18[c]	2.51[b]	2.94[a]	0.046	<0.01	<0.01	0.27
L1 late		LCP	MCP	HCP	SEM	CP	Day	CP x Day
Dietary CP	g/kg DM							
DMI	kg/d	21.0[b]	21.5[ab]	22.2[a]	0.16	0.04	<0.01	0.87
Milk yield	kg/d	27.8	27.5	27.9	0.47	0.56	0.22	0.53
Albumin	g/L	35.9	35.7	35.6	0.18	0.49	0.08	0.85
BHBA	mmol/L	0.83	0.79	0.87	0.014	0.08	0.27	0.10
Bilirubin	µmol/L	5.1	5.0	4.9	0.05	0.05	0.22	0.58
Creatinine	µmol/L	73.9	74.3	72.1	0.72	0.13	0.60	0.78
GLDH	IU/L	18.0	17.3	16.6	0.68	0.54	0.99	0.55
Haptoglobin	g/L	0.08	0.15	0.10	0.008	0.06	0.35	0.30
Log Hp		0.88[b]	1.02[a]	0.95[ab]	0.014	0.03	<0.01	0.26
NEFA	mmol/L	0.09	0.10	0.10	0.002	0.68	<0.01	0.29
Total protein	g/L	75.6	75.7	75.5	0.39	0.99	0.21	0.26
Urea	mmol/L	1.99[b]	2.06[b]	2.28[a]	0.032	<0.01	0.04	0.69
L2 mid		LCP	MCP	HCP	SEM	CP	Day	CP x Day
Dietary CP	g/kg DM							
DMI	kg/d	25.3	26.3	26.7	0.22	0.13	0.01	0.23
Milk yield	kg/d	38.2	37.4	38.0	0.40	0.50	0.04	0.14
Albumin	g/L	36.4	36.8	36.7	0.18	0.46	0.19	0.72
BHBA	mmol/L	0.72	0.74	0.73	0.013	0.92	0.86	0.46
Bilirubin	µmol/L	4.7	4.8	4.8	0.07	0.47	0.61	0.41
Creatinine	µmol/L	64.6	64.8	65.2	0.57	0.92	<0.01	0.20
GLDH	IU/L	15.4	16.3	17.9	0.72	0.45	0.86	0.36
Haptoglobin	g/L	0.15	0.22	0.14	0.017	0.42	0.50	0.18
Log Hp		1.15	1.18	1.12	0.013	0.54	0.66	0.03
NEFA	mmol/L	0.12	0.14	0.11	0.007	0.43	0.18	0.19
Total protein	g/L	79.8	81.0	81.1	0.45	0.23	0.18	0.59
Urea	mmol/L	2.52	2.65	2.54	0.054	0.24	0.31	0.28
L2 late		LCP	MCP	HCP	SEM	CP	Day	CP x Day
Dietary CP	g/kg DM							
DMI	kg/d	22.0	22.4	22.0	0.21	0.66	0.01	0.54
Milk yield	kg/d	26.0	26.3	26.0	0.43	0.80	0.01	0.77
Albumin	g/L	35.4	36.0	35.5	0.22	0.12	0.28	0.07
BHBA	mmol/L	0.72	0.76	0.74	0.016	0.68	0.48	0.45
Bilirubin	µmol/L	4.9[b]	5.2[a]	5.2[a]	0.06	<0.01	0.04	0.75
Creatinine	µmol/L	71.4	71.2	69.3	0.67	0.09	0.03	0.16
GLDH	IU/L	14.2	14.2	14.0	0.67	0.97	0.63	0.69
Haptoglobin	g/L	0.20	0.23	0.24	0.026	0.91	0.06	0.26
Log Hp		1.17	1.20	1.20	0.018	0.71	<0.01	0.23
NEFA	mmol/L	0.103[ab]	0.099[b]	0.120[a]	0.0035	0.02	0.07	0.94
Total protein	g/L	78.2[b]	79.8[a]	79.3[ab]	0.44	0.04	0.61	0.19
Urea	mmol/L	2.54	2.77	2.88	0.053	0.04	0.71	0.55

[a-c] Values with different superscript are significantly different

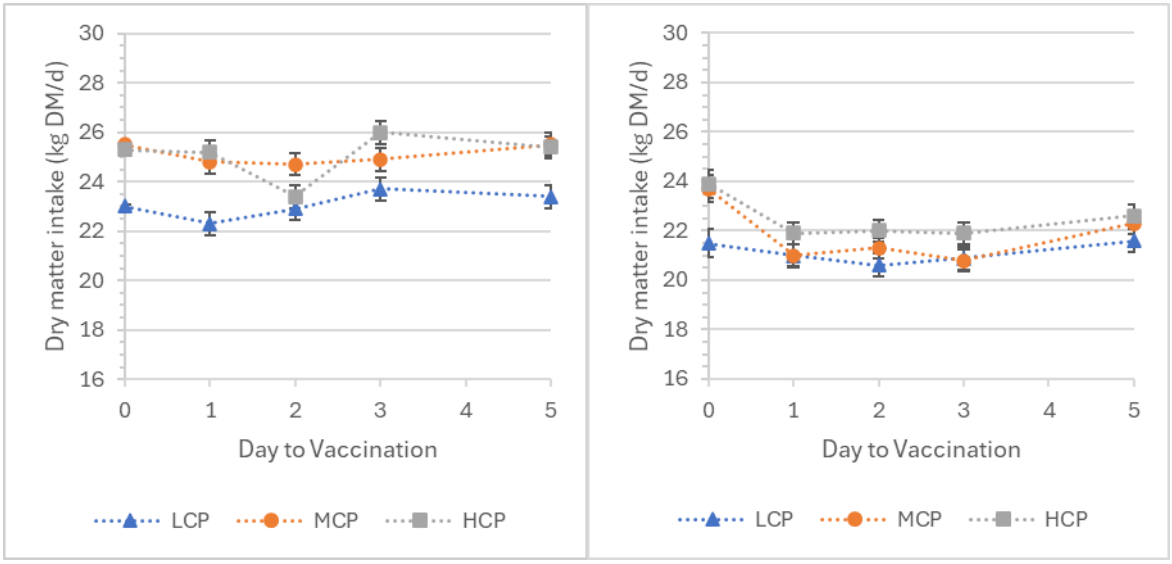


Figure 8.2. Dry matter intake response to vaccination of dairy cows fed different dietary crude protein levels (LCP, MCP, and HCP) during mid (left) and late (right) lactation in the first lactation of the study.

8.1.2 Antibody titer response

Antibody titer responses to vaccination were primarily driven by time after vaccination, with limited effects of dietary CP level (Table 8.2).

- In L1 during mid lactation, no CP × day interactions or main effects of CP were detected for BHV or BVDV titers or their log-transformed values. However, an effect of day after vaccination was observed for BHV and BVDV titers as well as for log-transformed BHV and BVDV titers ($P \leq 0.01$).
- In L1 during late lactation, no main effects of CP were detected, whereas a CP × day interaction was observed for the log-transformed BHV titer ($P = 0.05$). Day after vaccination significantly affected all four response variables in this group ($P < 0.01$).
- In L2 during mid lactation, no CP × day interactions were detected. There was no main effect of CP, although a tendency was observed for BHV titer ($P = 0.10$). Day after vaccination significantly affected BHV and BVDV titers and their log-transformed values ($P \leq 0.03$).
- In L2 during late lactation, CP × day interactions were detected for BHV titer and its log-transformed value ($P \leq 0.05$), whereas no main effects of CP were observed. As observed for the other vaccination moments, day after vaccination affected all four antibody response variables ($P \leq 0.04$).

Table 8.2. Effect of dietary crude protein level (LCP, MCP and HCP) and day after vaccination (14, 28, and 42), with day 0 included as covariate, for different study lactations (L1 and L2) and lactation stages (mid = 14-19 and late = 35-40 WIM), on blood serum antibody titer response (BHV and BVDV) in dairy cows.

	Treatment (CP)				*P*-value		
	LCP	MCP	HCP	SEM	CP	Day	CP x Day
L1 mid							
Dietary CP (g/kg DM)							
BHV titer	743	825	808	54.1	0.43	<0.01	0.17
BHV log	2.74	2.79	2.79	0.024	0.26	<0.01	0.16
BVDV titer	757	605	764	165.3	0.63	0.01	0.48
BVDV log	2.50	2.48	2.54	0.030	0.48	<0.01	0.95
L1 late							
Dietary CP (g/kg DM)							
BHV titer	1,288	1,235	1,355	115.9	0.78	<0.01	0.42
BHV log	3.00	2.96	2.95	0.024	0.46	<0.01	0.05
BVDV titer	1,552	1,261	1,203	226.9	0.55	<0.01	0.67
BVDV log	2.78	2.82	2.76	0.035	0.56	<0.01	0.93

L2 mid							
Dietary CP (g/kg DM)							
BHV titer	1,223	1,040	1,060	72.6	0.10	<0.01	0.20
BHV log	2.98	2.93	2.92	0.236	0.43	<0.01	0.20
BVDV titer	585	737	609	127.7	0.61	0.03	0.46
BVDV log	2.52	2.49	2.50	0.338	0.85	<0.01	0.50
L2 late							
Dietary CP (g/kg DM)							
BHV titer	1,080	1,094	1,055	73.3	0.96	<0.01	0.05
BHV log	2.91	2.93	2.90	0.025	0.85	0.04	<0.01
BVDV titer	1,509	1,178	1,384	256.8	0.22	<0.01	0.37
BVDV log	2.77	2.72	2.73	0.040	0.71	<0.01	0.77

BHV= Bovine Herpes Virus; BVDV = Bovine Viral Diarrhea Virus

The effect of dietary CP level on the area under the curve (AUC) of blood serum antibody titers was also evaluated in L1 and L2 during mid and late lactation. AUC responses were mainly affected by lactation period, with limited effects of dietary CP level (Table 8.3).

- Across both lactations, no interaction between diet and period was observed. In L1, there was an effect of diet for log-transformed BHV antibody titers ($P = 0.04$), with MCP being higher than LCP, whereas HCP did not differ from either MCP or LCP.
- A period effect was found for most antibody titer variables ($P \leq 0.01$), with the exception of BHV titers in lactation 2, which showed a tendency ($P = 0.09$).

Table 8.3. Effect of dietary crude protein level (LCP, MCP and HCP), during two lactation stages (LS; 14-19 and 35-40 WIM), on the area under the curve (AUC) of blood antibody titers in dairy cows.

		Diet (CP)				*P*-value		
	unit	LCP	MCP	HCP	SEM	CP	LS	CP × LS
L1								
BHV titer		30,690	54,512	38,310	4,019	0.17	0.01	0.49
BHV log		115[b]	124[a]	118[ab]	1.1	0.04	<0.01	0.69
BVDV titer		45,736	41,240	22,653	7,318	0.59	<0.01	0.25
BVDV log		107	111	105	1.5	0.49	<0.01	0.78
L2								
BHV titer		40,355	52,674	40,221	3,373	0.41	0.09	0.20
BHV log		120	125	121	1.2	0.35	0.01	0.43
BVDV titer		60,656	35,390	22,821	9,909	0.51	<0.01	0.74
BVDV log		109	110	106	1.9	0.81	<0.01	0.13

BHV= Bovine Herpes Virus; BVDV = Bovine Viral Diarrhea Virus

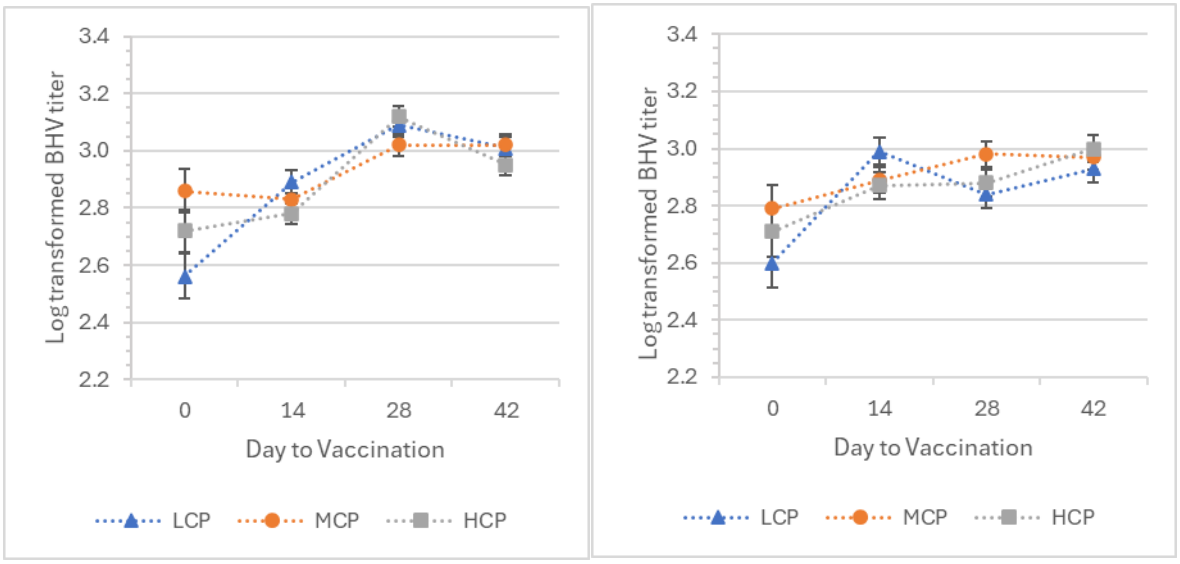


Figure 8.2. Blood serum levels of log transformed BHV titers of dairy cows fed different dietary crude protein levels (LCP, MCP, and HCP) in during late-lactation in the first (left) en second (right) lactation of the study.

8.2 Interpretation

Acute phase response

The vaccination induced clear, time-dependent changes in intake, production, and metabolic and inflammatory indicators, consistent with activation of an acute-phase immune response. Across both lactations and lactation stages, day relative to vaccination was the most consistent driver of variation, particularly for DMI, milk yield, and several blood metabolites associated with energy metabolism and inflammation. This indicates that the physiological response to immune activation largely overrode dietary effects in the immediate post-vaccination period.

Dry matter intake was the variable most consistently affected in L1, showing modest day effects. In contrast, DMI responses in cows during the L2 appeared to be driven primarily by the immune challenge itself rather than by dietary CP level, as indicated by the absence of CP × day interactions.

Inflammatory markers, particularly haptoglobin, showed indications of an acute-phase response following vaccination. The magnitude of the acute-phase response observed after vaccination was relatively modest, particularly when compared with responses reported following experimental inflammatory challenges such as lipopolysaccharide administration. Although temporal changes were detected in inflammatory markers, including haptoglobin, the increases were limited in amplitude and not consistently present across both lactations and lactation stages. This subdued response is not unexpected, as commercial vaccines are designed to stimulate adaptive immunity while minimizing systemic inflammation and clinical disturbance.

Antibody response

Across lactation number and stage, the adaptive antibody response to vaccination was largely driven by time after vaccination, with only limited effects by dietary CP level. The consistent and significant day effects observed for BHV and BVDV titers and their log-transformed values in all groups indicate a robust adaptive immune response, reflecting normal kinetics of antigen recognition, clonal expansion, and antibody production. This overall coherence suggests that, under the dietary conditions of this study, protein supply was generally sufficient to support the humoral immune response.

Mid-lactation cows, in both lactations, showed a highly consistent pattern: no CP × day interactions and no main effects of CP on antibody titers, combined with clear time effects. This indicates that antibody development followed a similar trajectory regardless of dietary CP level, reinforcing the concept that mid-lactation cows had adequate metabolic capacity and nutrient availability to buffer potential dietary variation in CP when mounting an adaptive immune response.

In late lactation, subtle differences emerged. In L1, a CP × day interaction was detected for the log-transformed BHV titer, whereas in L2 similar interactions were observed for both absolute and log-transformed BHV titers. These findings suggest that, particularly in late lactation, dietary CP may influence the temporal dynamics of the BHV-specific antibody response, rather than its overall magnitude. The absence of main CP effects in these groups indicates that CP level did not constrain the total antibody

response but may have modulated response kinetics. Among the measured variables, BHV titers appeared more sensitive to these interactions than BVDV titers, pointing to antigen-specific differences in regulation of adaptive immunity.

Analysis of the area under the curve (AUC) further supports this interpretation. In the first lactation of the study, dietary effects were detected for log-transformed BHV antibody titers. However, it should be noted that AUC calculations inherently reflect cumulative concentrations over time and do not account for pre-existing differences. Consequently, variation in initial antibody titers before vaccination may have contributed to differences in AUC values.

8.3 Conclusions

Overall, the results indicate that dietary crude protein level had little influence on immune responsiveness in lactating dairy cows. The acute phase response following vaccination was characterized by time-dependent changes in performance and metabolic and inflammatory markers, but these responses were largely independent of dietary CP level. Similarly, the adaptive immune response, as reflected by BHV and BVDV antibody titers, was driven primarily by time after vaccination and lactation stage, with only minor and inconsistent CP-related effects on response kinetics or cumulative antibody exposure. Collectively, these findings suggest that, within the range of CP levels evaluated, there was no clear evidence that reduced dietary CP impaired the measured acute phase or adaptive immune responses, and that immune activation and regulation after vaccination were governed predominantly by physiological stage and immune signaling rather than by dietary CP level.

9 Methane emissions

9.1 Results

Gas exchange measurements obtained using the GreenFeed system during both lactations (L1 and L2) are presented in Table 9.1. In L1, dietary CP level had limited effects on GreenFeed (GF) visit behavior. The number of visits per day tended to differ among treatments ($P = 0.09$), while time spent in the GF per visit did not differ among CP treatments ($P = 0.32$).

- Dietary CP level affected methane (CH_4) excretion in L1 ($P < 0.01$). Cows fed LCP emitted less CH_4 than cows fed MCP and HCP (442 vs. 484 and 488 g/d, respectively), whereas MCP and HCP did not differ. However, CH_4 yield expressed per unit of DMI or milk did not differ among CP treatments ($P \geq 0.30$). Methane intensity per unit of FPCM showed a CP × WIM interaction ($P = 0.01$). Week in milk affected all CH_4-related variables ($P \leq 0.01$).
- In L1, CP × WIM interactions were observed for total carbon dioxide (CO_2) excretion and CO_2 per unit of FPCM ($P \leq 0.03$), with a tendency for CO_2 per unit of milk ($P = 0.06$). CO_2 yield expressed per unit of DMI, milk, or FPCM was not affected by dietary CP ($P \geq 0.25$). Week in milk affected all CO_2 variables ($P < 0.01$).
- Significant CP × WIM interactions were detected for all Hydrogen (H_2) excretion parameters ($P \leq 0.02$) in L1.

In L2, cows visited the GreenFeed unit less frequently in the HCP treatment compared with LCP and MCP ($P = 0.04$), while time spent per visit did not differ among treatments ($P = 0.59$).

- Methane excretion in L2 was lower in LCP compared with MCP and HCP (438 vs. 476 and 473 g/d, respectively; $P = 0.01$). However, CH_4 yield expressed per unit of DMI or FPCM was not affected by dietary CP level ($P \geq 0.51$). A CP × WIM interaction was observed for CH_4 per unit of milk ($P = 0.02$) and week in milk affected all CH_4-related variables ($P < 0.01$).
- A CP × WIM interaction was observed for CO_2 excretion ($P = 0.01$), with CO_2 excretion in L2 generally increased with increasing dietary CP level, whereas CO_2 expressed relative to DMI, milk, or FPCM was not affected by treatment ($P \geq 0.51$). In contrast, WIM affected all CO_2 parameters ($P < 0.01$).
- Hydrogen excretion in L2 was not affected by dietary CP × WIM interactions or by CP level, regardless of expression basis ($P \geq 0.07$). However, WIM influenced all H_2 variables ($P < 0.01$).

Table 9.1. Effect of dietary crude protein (CP) level (LCP, MCP, and HCP) on enteric methane (CH_4), carbon dioxide (CO_2), and hydrogen (H_2) excretion of dairy cows across weeks in milk (WIM), presented separately for two consecutive 44-wk study lactations, with CP levels of 133, 143, and 154 g CP/kg DM in lactation 1 and 133, 144, and 156 g CP/kg DM in lactation 2, respectively.

		Treat (CP)				*P*-value		
Lactation 1		LCP	MCP	HCP	SEM	CP	WIM	CP × WIM
Visits GF	per day	2.69	2.66	2.48	0.011	0.09	<0.01	0.07
Time in GF	sec/visit	295	289	288	0.7	0.32	<0.01	0.13
CH_4								
CH_4 excretion	g/d	442[b]	484[a]	488[a]	1.1	<0.01	<0.01	0.08
CH_4/DMI	g/kg	20.5	20.3	20.4	0.04	0.96	<0.01	0.78
CH_4/Milk	g/kg	15.8	15.5	15.1	0.09	0.70	0.01	0.15
CH_4/FPCM	g/kg	14.8	14.3	13.7	0.08	0.30	<0.01	0.01
CO_2								
CO_2 excretion	g/d	14,248	15,233	15,309	24.6	<0.01	<0.01	0.03
CO_2/DMI	g/kg	661	642	644	1.3	0.33	<0.01	0.82
CO_2/Milk	g/kg	510	492	475	3.3	0.56	<0.01	0.06
CO_2/FPCM	g/kg	479	456	431	2.8	0.25	<0.01	<0.01
H_2								
H_2 excretion	g/d	1.81	1.71	1.65	0.009	0.07	<0.01	<0.01
H_2/DMI	g/kg	0.084	0.072	0.069	0.0004	<0.01	<0.01	<0.01
H_2/Milk	g/kg	0.063	0.053	0.051	0.0004	<0.01	<0.01	0.02
H_2/FPCM	g/kg	0.060	0.049	0.046	0.0003	<0.01	<0.01	<0.01
Lactation 2								
Visits GF	per day	2.62	2.71	2.41	0.009	0.04	<0.01	<0.01

Time in GF	sec/visit	325	327	329	0.3	0.59	0.02	0.55
CH_4								
CH_4 excretion	g/d	438[b]	476[a]	473[a]	1.3	0.01	<0.01	0.08
CH_4/DMI	g/kg	19.0	19.1	19.3	0.05	0.51	<0.01	0.47
CH_4/Milk	g/kg	14.9	14.4	14.2	0.10	0.80	<0.01	0.02
CH_4/FPCM	g/kg	13.7	13.3	13.0	0.09	0.72	<0.01	0.25
CO_2								
CO_2 excretion	g/d	14,393	15,530	15,602	28.9	<0.01	<0.01	0.01
CO_2/DMI	g/kg	630	628	643	1.8	0.58	<0.01	0.96
CO_2/Milk	g/kg	497	474	478	3.8	0.80	<0.01	0.68
CO_2/FPCM	g/kg	455	437	436	3.2	0.51	<0.01	0.41
H_2								
H_2 excretion	g/d	1.63	1.58	1.59	0.011	0.83	<0.01	0.11
H_2/DMI	g/kg	0.071	0.064	0.065	0.0005	0.13	<0.01	0.55
H_2/Milk	g/kg	0.055	0.046	0.047	0.0004	0.10	<0.01	0.14
H_2/FPCM	g/kg	0.050	0.042	0.043	0.0004	0.07	<0.01	0.39

GF = GreenFeed unit

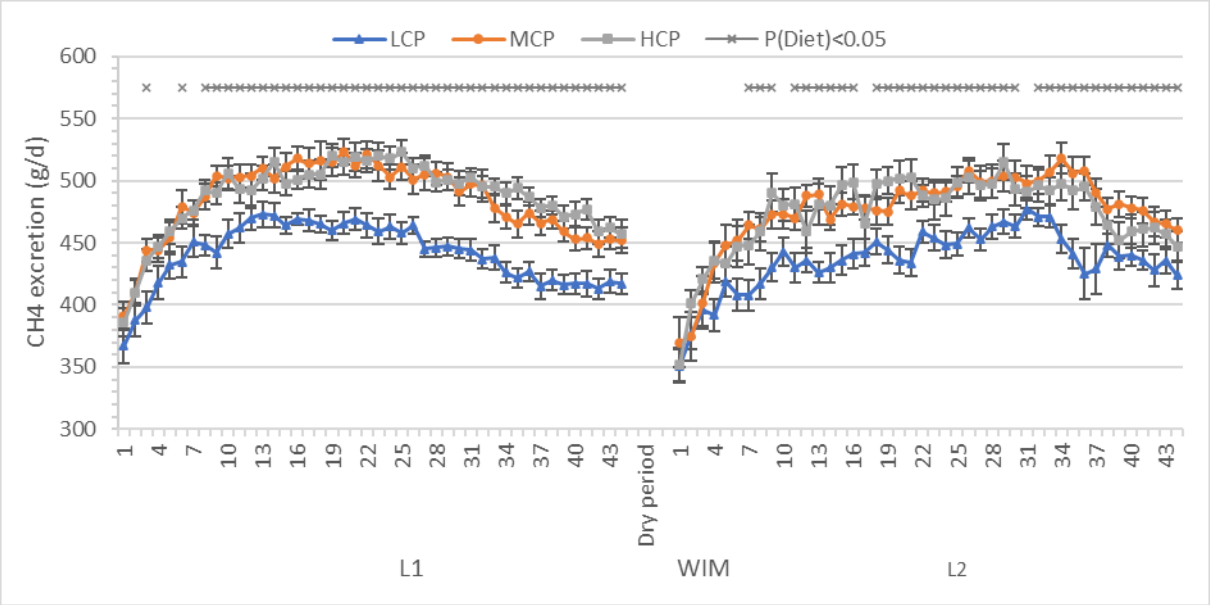


Figure 9.1. Methane (CH_4) excretion per week in lactation 1 and 2 (L1 & L2) for dairy cows fed different dietary crude protein (CP) levels in L1 of 133, 143, and 154 g CP / kg DM (for LCP, MCP, and HCP, respectively) and in L2 of 133, 144, and 156 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05).

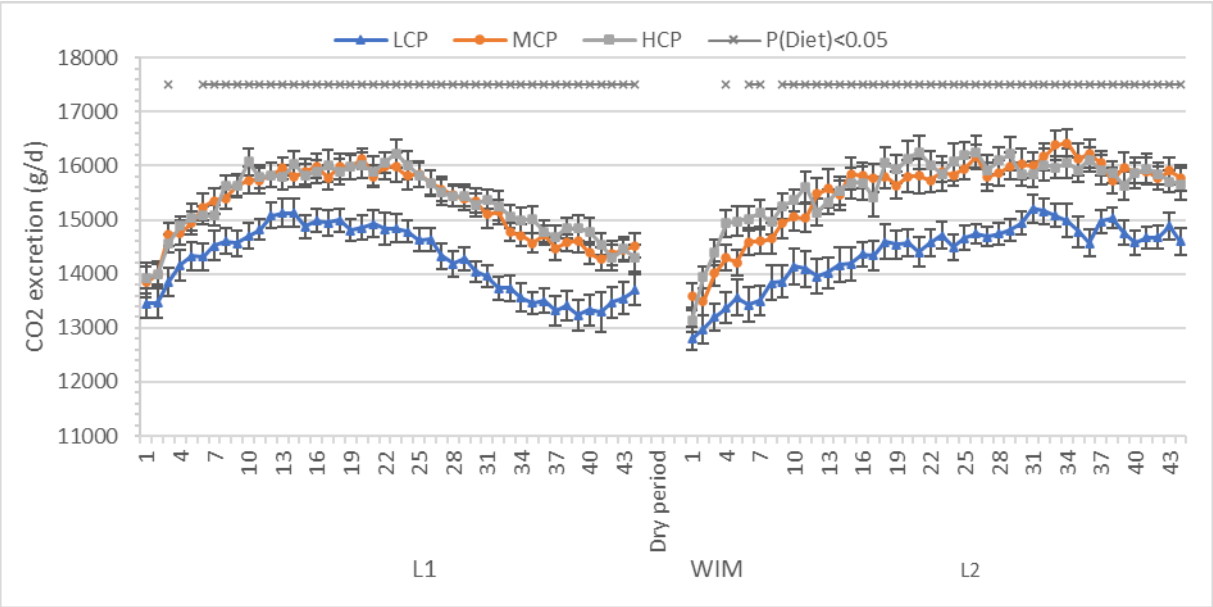


Figure 9.2. Carbon dioxide (CO_2) excretion per week in lactation 1 and 2 (L1 & L2) for dairy cows fed different dietary crude protein (CP) levels in L1 of 133, 143, and 154 g CP / kg DM (for LCP, MCP, and HCP, respectively) and in L2 of 133, 144, and 156 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05).

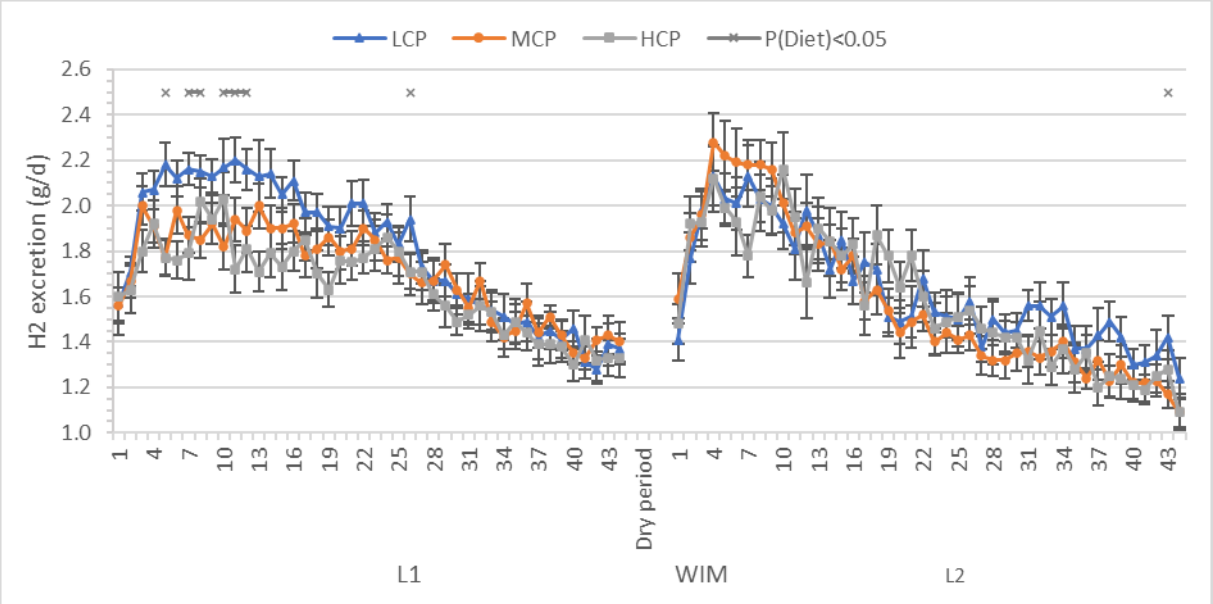


Figure 9.3. Hydrogen (H_2) excretion per week in lactation 1 and 2 (L1 & L2) for dairy cows fed different dietary crude protein (CP) levels in L1 of 133, 143, and 154 g CP / kg DM (for LCP, MCP, and HCP, respectively) and in L2 of 133, 144, and 156 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05).

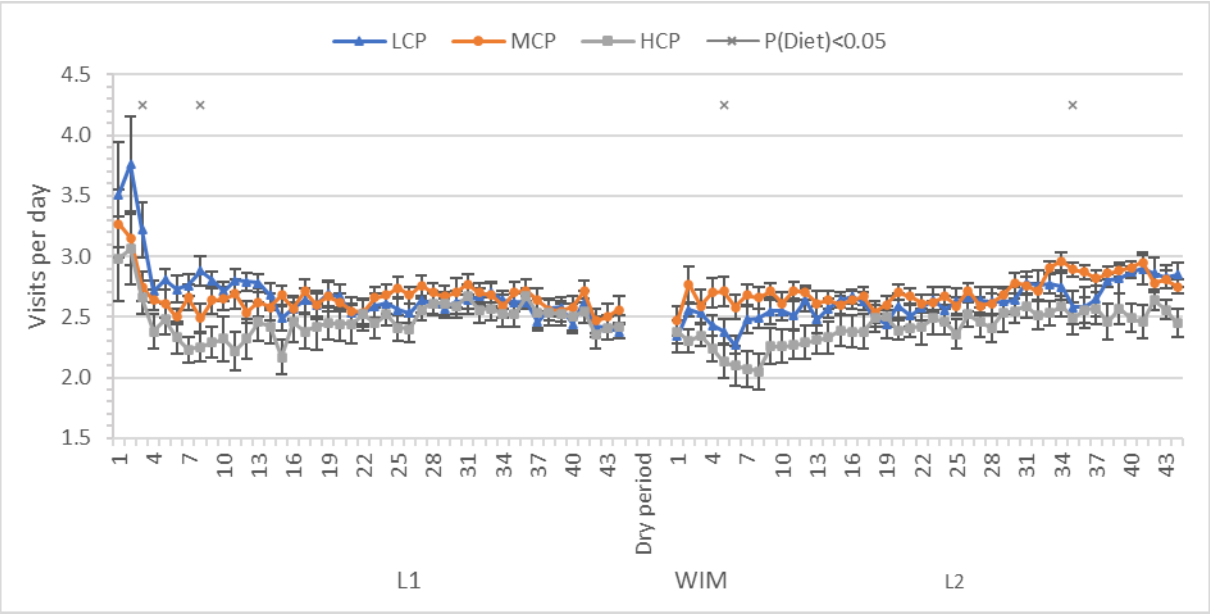


Figure 9.4. GreenFeed visits per day during different weeks in lactation 1 and 2 (L1 & L2) for dairy cows fed different dietary crude protein (CP) levels in L1 of 133, 143, and 154 g CP / kg DM (for LCP, MCP, and HCP, respectively) and in L2 of 133, 144, and 156 g CP / kg DM (for LCP, MCP, and HCP, respectively) and the significant differences of dietary treatment per week (P(Diet) < 0.05).

9.2 Interpretation

GreenFeed visit frequency averaged approximately 2.4 to 2.7 visits per cow per day, with around 290 sec in L1 and 325 sec in L2 spent in the unit per successful visit. These variables were not substantially affected by dietary CP level, except for a lower visit frequency in the HCP treatment during L2. This level of visitation and occupation time, above 180 sec per visit, is within the range generally considered adequate for estimating daily CH_4 emissions using GreenFeed systems, provided visits are distributed across the day. Therefore, it is unlikely that differences in CH_4 output among treatments were confounded by unequal sampling intensity.

Dietary CP level affected absolute CH_4 excretion, with lower daily CH_4 emissions in LCP compared with MCP and HCP in both lactations. However, when CH_4 was expressed relative to DMI, milk yield, or FPCM, no consistent differences among CP treatments were detected. This indicates that the lower absolute CH_4 emissions in LCP were primarily driven by lower intake and lower production level rather than by improved enteric methane efficiency per unit of feed or milk. Therefore, the lower CH_4 excretion observed in LCP should not be interpreted as a direct methane-mitigation effect of reducing dietary CP. Instead, it reflects the lower feed intake and productive output of cows receiving the lowest CP diet.

From a practical perspective, reducing dietary CP may contribute to modest reductions in total CH_4 emissions through effects on intake and overall production level, but it does not appear to intrinsically reduce CH_4 yield or intensity. This distinction is important because a reduction in total daily CH_4 output is only beneficial if it is not accompanied by a disproportionate reduction in milk production. In the present study, LCP reduced absolute CH_4 emissions but also reduced DMI and milk performance, whereas MCP maintained performance comparable to HCP without increasing CH_4 yield or intensity.

Carbon dioxide excretion was also lower in LCP compared with MCP and HCP, particularly when expressed as total daily CO_2 output. This pattern is consistent with lower feed intake and lower overall metabolic activity in cows receiving the lowest CP diet. However, CO_2 expressed relative to DMI, milk, or FPCM was generally not affected by dietary CP level, indicating that the observed differences mainly reflected changes in intake and production level rather than differences in metabolic efficiency.

Hydrogen emissions showed some CP-related responses, particularly in L1, where H_2 expressed relative to DMI, milk, and FPCM was lower in MCP and HCP compared with LCP. However, these responses were not consistent across both lactations. Therefore, the H_2 results should be interpreted cautiously and mainly as supporting information on fermentation dynamics rather than as a primary outcome of dietary CP level.

9.3 Conclusions

Reducing dietary crude protein decreased absolute methane emissions; however, methane yield and intensity were unaffected when emissions were expressed relative to DMI, milk yield, or FPCM. The lower absolute CH_4 excretion observed in LCP was therefore mainly associated with lower feed intake and milk production, rather than improved enteric methane efficiency. Overall, these results indicate that reducing CP to the LCP level does not provide a direct methane-mitigation benefit when animal performance is considered.

10 Survival, treatments for disease or preventive, and fertility

10.1 Results

10.1.1 Survival (L1 and L2)

The survival rate of the cows in the study over two lactations of 308 DIM, with one dry period between lactations, is presented in Figure 10.1. Reasons for culling during the complete study period are presented below.

- During lactation 1, from calving to the next calving, four cows were culled or removed from the study. Reasons for removal included teat trampling followed by mastitis (HCP; cow replaced), chronic pneumonia (LCP), chronic indigestion (LCP), and failure to become pregnant after 44 weeks in milk (HCP). No cows were culled during the subsequent dry period.
- During lactation 2, from calving to the next calving, eleven cows were culled or removed. In the LCP group, removals were due to acute pneumonia with cardiovascular complications, trauma resulting in severe lameness, and bluetongue followed by lameness. In the MCP group, cows were removed because of trauma with a dislocated hip, severe pneumonia, and failure to become pregnant after 44 weeks in milk. In the HCP group, reasons for removal included hypocalcemia followed by trauma, chronic udder cleft dermatitis, claw necrosis and chronic lameness after claw amputation, severe lameness, and abortion or miscarriage.
- In early lactation 3, during the first 4 weeks of lactation, three additional cows were removed from the study: one HCP cow due to incorrect allocation of treatment concentrates, one MCP cow due to digital dermatitis resulting in missing data, and one LCP cow due to stillbirth and absence of milk production.
- No differences in the risk of removal from the study were detected among treatments (Table 10.1; $P \geq 0.09$). Nevertheless, the risk of removal tended to be higher for HCP than for MCP (HR = 3.32; $P = 0.09$). The wide 95% confidence intervals indicate considerable uncertainty around the estimated hazard ratios.

Table 10.1. Pairwise comparisons of the risk of removal from the study among dietary treatments (LCP, MCP, and HCP).

	HR	95%-CI	*P*-value
MCP vs. LCP	0.46	0.12-1.76	0.26
HCP vs. LCP	1.54	0.41-5.74	0.52
HCP vs. MCP	3.32	0.82-13.55	0.09

HR = hazard ratio; CI = confidence interval

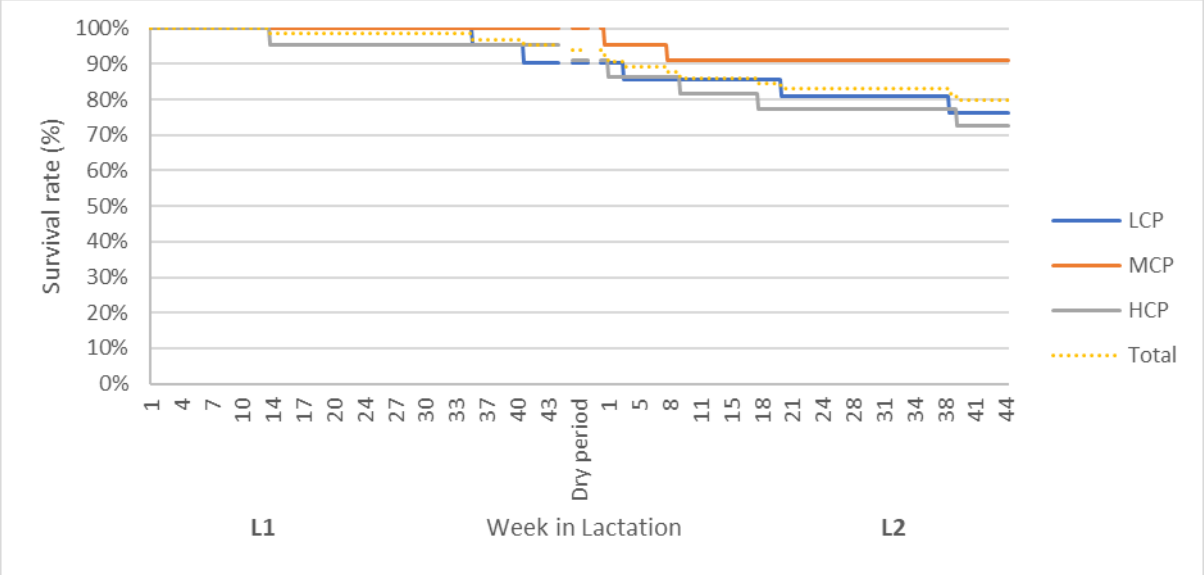


Figure 10.1. Survival rate of lactating Holstein Friesian dairy cows fed different levels of crude protein (133 (LCP), 143/144 (MCP), and 154/156 (HCP)) during two consecutive 44-wk lactations (L1 and L2).

10.1.2 Treatments for disease or preventive (L1, L2, and L3)

Across the study lactations, dietary CP level did not affect the number of disease or preventive treatments per cow (Table 10.2).

- In L1, the number of treatments per cow tended to decrease with increasing CP level (P = 0.09). There was no effect of CP level on the proportion of cows treated for disease or preventive (P = 0.36).
- In L2, the number of treatments per cow was similar among treatments (P = 0.45), despite numerically lower values for HCP compared with LCP and MCP. The proportion of cows treated for disease or preventive was affected by CP level (P =0.04) and was high across all treatments, particularly in MCP and HCP (95.5 and 90.0%, respectively), compared with LCP (68.4%). The percentage cows treated for disease or preventive was lower in LCP cows than in MCP cows, while HCP did not differ from both LCP and MCP.
- In L3 (0–28 DIM), neither the number of treatments per cow or the proportion of cows treated for disease or preventive differed among dietary CP levels ($P \geq 0.57$), with similar values observed across treatments.

Table 10.2 Animals treated for disease or preventive for cows fed different CP levels (LCP, MCP and HCP) during complete study lactations in lactation 1 and 2 (L1 and L2) and the first 28 days into lactation 3 (L3).

L1	LCP	MCP	HCP	SEM	*P*-value
Number of cows, n	21	22	22		
Treatments for disease per cow, n	2.15	1.45	1.14	0.227	0.09
Cows treated for disease, %	66.7	59.1	45.5	10.47	0.36
L2					
Number of cows, n	19	22	20		
Treatments for disease per cow, n	3.02	3.00	2.24	0.344	0.45
Cows treated for disease, %	68.4[b]	95.5[a]	90.0[ab]	27.41	0.04
L3					
Number of cows, n	13	16	13		
Treatments for disease per cow, n	1.50	1.37	1.34	0.160	0.91
Cows treated for disease, %	92.3	81.3	92.3	8.18	0.57

Across lactations and dry periods, the incidence and type of treatments for disease or preventive varied, but no consistent pattern related to dietary CP level was observed (Table 10.3).

- In lactation and dry period 1, the total number of treatments decreased numerically with increasing CP level (45, 32, and 25 for LCP, MCP, and HCP, respectively). The most frequently observed treatments were for milk fever (n = 24), clinical mastitis (n = 23), and fever (n = 17). Notably, treatments for stomach and intestine problems and pneumonia were only observed in LCP and MCP cows, while absent in HCP cows.
- In lactation and dry period 2, the total number of treatments was highest in MCP (66), followed by LCP (55) and HCP (45). Milk fever (n = 44) and clinical mastitis (n = 33) remained the most prevalent treated conditions. Although some treatments for conditions such as pneumonia and stomach and intestine problems were less frequent or absent in HCP cows, other conditions (e.g., ketosis) were numerically higher in this group, indicating no consistent effect of CP level.
- During the first 4 weeks of lactation 3, treatments were mainly for milk fever, with similar numbers across dietary treatment groups (13 cases in each group). The total number of treatments was comparable among CP levels (19, 22, and 17 for LCP, MCP, and HCP, respectively), and the occurrence of treatments for other diseases was generally low and evenly distributed.

Overall, treatments for milk fever and clinical mastitis were the most common conditions throughout the study, and although some numerical differences between dietary treatment groups were observed, there was no clear or consistent relationship between dietary CP level and the number of treatments for disease or preventive.

Table 10.3. Overview on the number of cows treated for certain disease or preventive for complete study lactations (L1, L2, and L3 (first 28 DIM)) and different dietary CP level (LCP, MCP, or HCP)

Lactation and dry period 1	**LCP**	**MCP**	**HCP**	**Total**
Number of cows, n	21	22	22	65
Milk fever	8	10	6	24
Ketosis	1	2	1	4
Clinical mastitis	8	10	5	23

Vaginal discharge	0	2	1	3
Endometritis	0	1	0	1
Cystic ovaries	2	1	3	6
Claw and leg problems	0	0	0	0
Stomach and intestine problems[1]	6	3	0	9
Pneumonia	4	0	0	4
Fever	11	2	4	17
Other[2]	5	1	5	11
Total	**45**	**32**	**25**	**102**
0-5 DIM	10	10	5	25
6-28 DIM	3	1	2	6
≥ 29 DIM	32	21	18	71
Lactation and dry period 2	**LCP**	**MCP**	**HCP**	**Total**
Number of cows	19	22	20	61
Milk fever	11	17	16	44
Ketosis	1	1	3	5
Clinical mastitis	13	12	8	33
Vaginal discharge	3	4	2	9
Endometritis	4	7	5	16
Cystic ovaries	4	3	2	9
Claw and leg problems	2	4	3	9
Stomach and intestine problems[1]	1	2	0	3
Pneumonia	3	2	0	5
Fever	10	10	5	25
Other[2]	3	4	1	8
Total	**55**	**66**	**45**	**166**
0-5 DIM	15	23	22	60
6-28 DIM	9	9	5	23
≥ 29 DIM	31	34	18	83
Lactation 3 (first 28 DIM)	**LCP**	**MCP**	**HCP**	**Total**
Number of cows	13	16	13	42
Milk fever	13	13	13	39
Ketosis	1	2	0	3
Clinical mastitis	1	2	0	3
Vaginal discharge	0	0	0	0
Endometritis	0	0	1	1
Cystic ovaries	0	0	0	0
Claw and leg problems	0	2	1	3
Stomach and intestine problems[1]	1	0	0	1
Pneumonia	0	0	0	0
Fever	2	3	1	6
Other[2]	1	0	1	2
Total	**19**	**22**	**17**	**58**
0-5 DIM	14	16	15	45
6-28 DIM	5	6	2	13

[1]Main stomach and intestine problems: diarrhea, peritonitis, rumen acidosis.
[2]Main diagnoses in 'other': abscess, 3 teats.

Across the three complete study lactations (L1, L2, and L3), dietary CP level did not affect somatic cell count (SCC) or the distribution of SCC categories (Table 10.4).

- In L1, average lactation length, number of days with SCC above 150,000 cells/mL, mean SCC, and log-transformed SCC were not different among treatments ($P \geq 0.30$). Similarly, the proportion of days within the different SCC classes (<150; 150–500; 500–1,500; and >1,500 × 1,000 cells/mL) did not differ among CP levels ($P \geq 0.20$).
- In L2, no significant effects of dietary CP were observed on average lactation days, days with SCC above 150,000 cells/mL, mean SCC, or log SCC ($P \geq 0.26$). Although numerically fewer days with SCC above 150,000 cells/mL and lower mean SCC values were observed for HCP compared with LCP, these differences were not statistically significant. The distribution of SCC across categories was also unaffected by dietary treatment ($P \geq 0.34$).
- In L3, which comprised a shorter observation period, dietary CP level again did not influence lactation length, days with SCC above 150,000 cells/mL, mean SCC, or log SCC ($P \geq 0.24$). While cows fed HCP showed numerically lower mean SCC and a greater proportion of days with SCC above 150,000 cells/mL compared with LCP and MCP, these differences were not statistically significant ($P \geq 0.16$). Overall, varying dietary CP concentration from 133 to 154 g/kg DM did not significantly affect SCC levels or the proportion of days within SCC categories during complete lactations.

Table 10.4. Somatic cell count (SCC × 1,000 cells/ml) and percentage of days increased during study lactations (L1, L2 and L3) of dairy cows with different dietary CP levels (LCP, MCP, or HCP).

L1	LCP	MCP	HCP	SEM	*P*-value
Cows, n	21	22	22		
Average lactation days	348	357	357	8.4	0.87
Days >150 SCC	140	153	122	15.4	0.67
SCC mean	269	379	314	46.2	0.59
SCC (log)	2.07	2.09	1.92	0.056	0.30
SCC (% of days)					
<150	58.6	57.7	68.0	4.11	0.48
150-500	29.4	26.1	18.3	2.66	0.20
500-1500	10.2	9.9	8.4	1.73	0.88
1500<	2.1	6.1	5.3	1.29	0.42
L2	LCP	MCP	HCP	SEM	*P*-value
Cows, n	19	21	19		
Average lactation days	338	347	314	12.2	0.53
Days >150 SCC	148	155	101	15.8	0.26
SCC	496	378	311	63.0	0.48
SCC (log)	2.15	2.10	1.98	0.058	0.47
SCC (% of days)					
<150	59.3	58.5	67.9	1.17	0.56
150-500	19.6	23.1	19.8	2.51	0.79
500-1500	13.8	13.4	7.5	2.14	0.34
1500<	7.4	5.0	5.0	1.41	0.73
L3	LCP	MCP	HCP	SEM	*P*-value
Cows, n	13	15	13		
Average lactation days	24	25	23	0.4	0.34
Days >150 SCC	8	8	3	1.4	0.24
SCC	502	427	108	126.6	0.43
SCC (log)	2.07	1.95	1.76	0.080	0.31
SCC (% of days)					
<150	63.9	68.8	85.7	0.06	0.27
150-500	19.9	24.6	10.3	0.05	0.29
500-1500	12.6	3.5	2.7	0.02	0.16
1500<	3.2	5.3	0.0	0.02	0.42

10.1.3 Fertility (L1 and L2)

During the first lactation, dietary CP (133, 143, and 154 g/kg DM for LCP, MCP, and HCP, respectively) did not affect fertility parameters (Table 10.5). The number of inseminated and pregnant cows was similar among treatments, and only one cow (HCP) was not pregnant before 305 DIM. Days to first insemination, days open, calving interval, and number of inseminations to pregnancy were not different among CP levels ($P \geq 0.54$). In addition, inclusion of the corresponding response variables of the previous lactation as a covariate did not influence days open, calving interval or inseminations needed for pregnancy ($P \geq 0.64$). Pregnancy rate after first insemination was comparable among treatments (61.9, 55.6, and 57.1% for LCP, MCP, and HCP, respectively). Overall, varying dietary CP concentration during the first lactation did not affect reproductive performance.

Table 10.5. Effect of dietary crude protein (133, 143, and 154 for LCP, MCP, and HCP, respectively) on fertility during the first lactation of the study.

	CP-level				*P*-value	
	LCP	MCP	HCP	SEM	CP-level	CP_cov[1]
Cows (all), n	21	22	21			
Inseminated cows, n	21	22	21			
Pregnant cows, n	21	22	20			
Not pregnant before 44 WIM, n	0	0	1			
Days to first insemination	96.4	103.5	99.9	2.66	0.54	
Days open	125	132	131	7.20	0.88	0.90
Calving interval, days	405	409	413	7.5	0.91	0.97
Inseminations to pregnancy	2.1	1.9	1.9	0.17	0.70	0.64
Pregnancy after first insemination, %	61.9	55.6	57.1	2.15	0.88	

[1]Initial lactation fertility performance was used as a covariate.

In the second lactation, some fertility parameters tended to be affected by dietary CP level (Table 10.6). Days to first insemination tended to decrease with increasing CP concentration (112.5, 99.3, and 90.5 days for LCP, MCP, and HCP, respectively; $P = 0.08$). Similarly, days open tended to be shorter in HCP cows compared with LCP and MCP cows ($P = 0.09$), and this effect became significant when the corresponding response variables of the previous lactation was included as a covariate ($P = 0.04$). Calving interval was affected by dietary treatment ($P = 0.02$), with HCP having a shorter calving interval than LCP, while MCP was not different from LCP and HCP. This effect was also visible when the calving interval of the lactation before the start of the study was included as a covariate ($P = 0.01$). The number of inseminations to pregnancy was not influenced by dietary CP level ($P \geq 0.70$). Pregnancy rate after first insemination was comparable among treatments (44.4, 50.0, and 35.3% for LCP, MCP, and HCP, respectively). The pregnancy rate after different number of inseminations during L1 and L2 was presented in Figure 10.2. Overall, decreasing dietary CP concentration during L2 was associated with a longer calving interval and tended to deteriorate some reproductive parameters.

Table 10.6. Effect of dietary crude protein (133, 144, and 156 for LCP, MCP, and HCP, respectively) on fertility during the second lactation of the study.

	CP-level				*P*-value	
	LCP	MCP	HCP	SEM	CP-level	CP_cov[1]
Cows (all), n	19	22	20			
Inseminated cows, n	18	20	17			
Pregnant cows, n	15	18	15			
Not pregnant before 44 WIM, n	1	2	1			
Days to first insemination	112.5	99.3	90.5	4.03	0.08	
Days open	146	136	107	7.3	0.09	0.04
Calving interval, days	426[a]	416[ab]	377[b]	7.6	0.02	0.01
Inseminations to pregnancy	2.1	1.9	1.7	0.15	0.70	0.78
Pregnancy after first insemination, %	44.4	50.0	35.3	4.29	0.67	

[ab] Values with different superscript are significantly different ($P \leq 0.05$)
[1]Previous lactation fertility performance was used as a covariate.

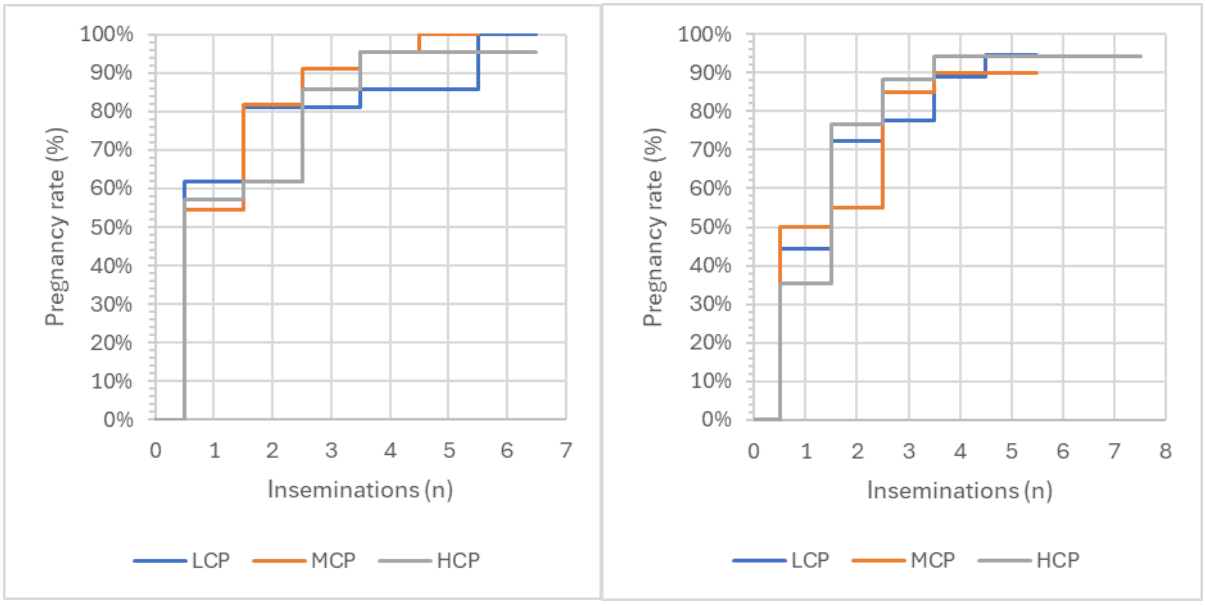


Figure 10.2. Cumulative pregnancy rate by number of inseminations in lactation 1 (left) and 2 (right) for lactating dairy cows fed different dietary crude protein (CP) levels in L1 of 133, 143, and 154 g CP / kg DM (for LCP, MCP, and HCP, respectively) and in L2 of 133, 144, and 156 g CP / kg DM (for LCP, MCP, and HCP, respectively).

10.2 Interpretation

Across the two consecutive lactations, overall survival remained relatively high, with 95% of cows completing L1 and 80% also completing L2. The low culling rate in L1 likely reflects the initial selection of clinically healthy, multiparous cows, which reduced the likelihood of early removals. In contrast, the more pronounced culling during lactation 2 is consistent with the advancing age and cumulative health challenges in this population, as well as exposure to external factors such as the bluetongue epidemic in the winter of 2023/24. Differences among treatments were modest, although numerically higher survival was observed in MCP (91%) compared with LCP (76%) and HCP (73%) after two lactations. Given the relatively small number of events and the diverse, largely incidental causes of culling (e.g., trauma, infectious disease, reproductive failure), these differences are not readily attributable to dietary CP level per se. Notably, no cows were culled during the dry period, and relatively few removals were directly associated with metabolic diseases. Compared with commercial practice, the overall survival rate of 80% over two lactations can be considered high. This is likely partly explained by the study design, in which cows were not culled for production-related reasons, whereas such criteria commonly contribute to culling decisions on commercial farms. Together, these findings suggest that variation in dietary CP level had limited impact on cow survival under the conditions of this study.

Across the three study lactations, dietary CP level did not affect disease incidence or the number of disease treatments per cow. This absence of treatment effects is not unexpected, as epidemiological studies on animal health outcomes typically require large datasets to detect robust differences, particularly when disease incidence is multifactorial and relatively infrequent. Under the controlled conditions of the present study and given the limited number of animals per treatment, only large effects of dietary CP on health status would be likely to be detected.

Although no effects were observed, a consistent numerical pattern was present across lactations, with the number of treatments for disease generally decreasing as dietary CP level increased. This trend was most apparent during L1, where the number of treatments per cow and the proportion of cows treated for disease were numerically higher in LCP compared with MCP and HCP. However, the clinical relevance of these differences should be interpreted with caution. In L1, a relatively large number of treatments in the LCP group were associated with a diagnosis of fever without clear accompanying clinical signs. These cases typically involved cows identified via the herd attention list and treated preventively with anti-inflammatory drugs, after which they often did not reappear on the attention list. Such management-driven treatments inflate treatment counts but do not necessarily reflect more severe or persistent disease, thereby complicating biological interpretation of treatment numbers alone.

Differences in overall treatment incidence between lactations further underline the importance of management context. The number of cows treated for disease was lower in L1 than in L2 and L3, which is largely explained by herd management practices at the experimental facility. Older cows received preventive milk fever treatments during later lactations, contributing to a higher number of recorded milk fever treatments in L2 and L3 compared with L1. Milk fever was the dominant disorder in all lactations, particularly during the early postpartum period, and therefore strongly influenced overall treatment numbers. Moreover, diseases occurring during the first days after calving, such as milk fever, cannot be readily attributed to dietary CP treatments, as experimental diets were applied from calving onwards. Indeed, in L1 approximately 25% of all treatments occurred within the first five days postpartum, increasing to 38% in L2. Because all cows received the same dry-period diet, carry-over effects of dietary CP from one lactation to the next are unlikely. Importantly, even when early-lactation treatments were excluded from consideration, cows fed the HCP diet still received fewer disease treatments than those fed LCP or MCP, although these differences remained numerical rather than statistically significant.

Interpretation of health outcomes during the third study lactation (L3) is further constrained by study design. Only a subset of cows entered L3 because some animals were excluded earlier due to disease or reproductive failure, and the observation period was limited to the first four weeks of lactation. Consequently, both the absolute number of disease cases and treatments per cow were lower in L3 than in L1 and L2.

Somatic cell count (SCC) was not affected by dietary CP level across the complete study, either in terms of average SCC, log-transformed SCC, days above 150,000 cells/mL, or the distribution among SCC categories. This lack of response was consistent across all three lactations. Nonetheless, HCP cows numerically exhibited fewer days with SCC above 150,000 cells/mL and lower mean SCC values compared with LCP and MCP, particularly in L2. Although these differences were not statistically significant, they may reflect the numerically lower incidence of clinical mastitis observed in HCP cows or, alternatively, a modest improvement in udder health that could not be confirmed within the statistical power of the present study.

Overall, the health and SCC results indicate that reducing dietary CP from HCP to MCP and LCP did not adversely affect disease incidence or udder health. The numerical trends toward lower disease treatments and SCC in HCP should be interpreted cautiously and primarily as hypothesis-generating, emphasizing the need for larger-scale studies to disentangle dietary protein effects from management- and parity-related influences on dairy cow health.

Overall, dietary crude protein level had limited effects on fertility, with no differences observed during the L1 and only modest effects during the L2, where HCP cows exhibited a shorter calving interval than LCP cows. It should be noted that robust assessments of fertility generally require large numbers of animals to obtain sufficient statistical power or more detailed measurements of reproductive physiology than were available in the present study. The shorter calving interval observed in HCP cows during L2 appears to result from a combination of fewer days to first insemination, fewer days open, and a shorter gestation length.

The results in the current study suggests that reproductive performance was largely robust to variation in CP supply within the range studied. These findings are consistent with Law et al. (2009b), who reported that fertility was more strongly associated with energy balance and endocrine status than with dietary CP concentration per se. While high CP levels have been linked to impaired fertility in other studies (e.g. Barton et al., 1996; Lean et al., 2012), the CP levels applied here fall within a moderate range and therefore do not reflect the conditions under which negative reproductive effects are typically observed. Conversely, studies such as Reynolds et al. (2022) indicate that insufficient protein supply can compromise fertility, supporting the notion that both under- and oversupply lie outside an optimal range. Taken together, the literature and the present results suggest that moderate variation in dietary CP has inconsistent and generally small effects on fertility, making it difficult to attribute clear reproductive responses to CP level alone.

10.3 Conclusions

Under the conditions of this study, variation in dietary crude protein concentration had limited effects on cow survival, health, somatic cell count, and fertility. Reducing dietary CP from HCP to MCP or LCP did not adversely affect disease incidence or udder health, although numerical trends toward lower disease treatments and SCC at higher CP levels warrant attention. Overall, the present results indicate that moderate variation in dietary CP concentration has small and inconsistent effects on fertility.

11 Dry-periods, calvings and early lactation performance

11.1 Results

11.1.1 Dry period (L1 and L2)

All treatments received the same diet during the dry period. The dry period diet consisted of a mixture of grass silage, corn silage, straw, soybean meal, and premix (Table 11.1). On average, the diet contained 32.5% grass silage, 27.8% corn silage, 29.1% straw, 9.7% soybean meal, 0.9% premix, and 0.5% water on a DM basis. Additionally, cows received concentrates during the close-up period, starting at 0.5 kg (product) per day at 21 days before expected calving and gradually increasing to 2.0 kg per day on the expected day of calving (Table 11.2).

Table 11.1. Chemical composition (g/kg DM or otherwise determined) of grass silages, corn silages, straw and soybean meal offered during the dry period (Jul '23 to Apr '25).

	Grass silage		Corn silage		Straw		Soybean meal	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM (g/kg)	400	30.5	423	42.7	859	17.3	863	7.4
Ash	113	8.4	38	3.8	63	10.0	70	1.2
Crude protein	150	12.1	71	7.4	30	4.3	506	7.0
Ether extract	37	5.2	31	1.2	13	2.3	17	3.3
Sugar	59	33.2	n.d.	n.d.	n.d.	n.d.	124	6.0
Starch	n.d.	n.d.	422	46.9	n.d.	n.d.	16	3.0
NDF	466	74.3	334	42.3	798	23.3	118	7.1
ADF	300	22.3	181	29.8	503	29.6	68	5.8
ADL	18	2.9	14	2.2	52	7.4	5	0.6

n.d. = Not determined

Table 11.2. Chemical composition and ingredients of concentrate fed during the last 3 weeks of the dry period (Aug '23 to Apr '25).

Composition (g/kg DM)	Mean	SD
DM (g/kg)	883	1.7
Ash	72	3.1
CP	199	3.8
Ether extract	47	6.0
Sugar	89	4.0
Starch	249	23.0
NDF	293	4.2
ADF	168	5.2
ADL	41	3.7
Ingredients (%)		
Wheat	39.0	
Palm kernel expeller	20.0	
Lupines	9.8	
Rape seed meal	6.6	
Beet pulp	6.5	
Sugar beet molasses	5.6	
Rapeseed meal, rumen bypass (AminoGo)	4.9	
Premix	7.6	

NE_L = Net energy for lactation, calculated with the VEM system (Van Es, 1978)
n.d. = Not determined

The chemical composition of the diets consumed by the different treatment groups is presented in Table 11.3. Cows were offered the same diets regardless of treatment; therefore, only minor differences in the composition of consumed diets were observed between treatments. Some small differences were observed between D1 and D2, most notably in CP content (132 vs. 127 for D1 and D2, respectively).

Table 11.3. Chemical composition (g/kg DM) of diets consumed by the low, medium and high CP treatment groups (LCP, MCP and HCP, respectively) calculated based on total daily dry matter intake and averaged for the last 6 weeks of dry periods (D1 and D2).

	LCP		MCP		HCP	
D1	Mean	SD	Mean	SD	Mean	SD
Ash	85	1.3	86	0.9	87	1.1
CP	132	1.5	133	1.3	132	1.3
Ether extract	27	0.4	28	0.3	28	0.4
Sugar	41	2.0	40	1.9	39	2.5
Starch	113	4.5	110	3.7	113	4.2
NDF	486	4.4	486	3.3	489	4.8
ADF	297	2.7	297	2.0	299	2.8
ADL	27	0.3	26	0.4	27	0.4
D2						
Ash	77	0.6	77	0.4	77	0.7
CP	127	1.0	127	1.1	127	1.7
Ether extract	26	0.4	26	0.4	27	0.4
Sugar	32	3.2	30	2.9	26	2.8
Starch	121	4.2	123	4.3	129	4.1
NDF	473	3.8	473	2.2	477	3.6
ADF	300	4.0	297	4.1	294	3.7
ADL	26	0.4	26	0.3	26	0.5

n.d. = Not determined

Animal performance during the last six weeks of the dry period 1 (Table 11.4) was not affected by CP level, as expected given that all cows received the same diet during this period. No differences were observed for dietary CP content, total DMI, PMR intake, or concentrate intake ($P \geq 0.31$). Similarly, BCS and BW did not differ among treatments ($P \geq 0.46$). In contrast, week of the dry period affected all measured variables ($P < 0.01$), reflecting the expected changes over time as cows approached calving. No CP × week interactions were observed for any of the parameters ($P \geq 0.39$), indicating that temporal patterns were consistent across treatments.

Table 11.4. Performance of non-lactating dairy cows during the last 6 weeks of the first dry period of the study receiving the same dietary CP level during after being fed different levels of CP during the previous lactation stage (133, 143 and 154, for LCP, MCP and HCP, respectively).

	CP-level				*P*-value		
	LCP	MCP	HCP	SEM	CP	Week	CP x Week
Number of animals	19	22	20				
Dry period length (d)	45.3	47.2	44.6	1.02	0.57		
DMI total (kg/d)	15.1	15.6	14.9	0.14	0.59	<0.01	0.54
DMI PMR	14.6	15.1	14.4	0.14	0.57	<0.01	0.54
DMI concentrates	0.5	0.5	0.5	0.03	0.86	<0.01	0.86
BCS[1]	3.5	3.5	3.5	0.02	0.67	<0.01	0.70
BW[2] (kg)	756	778	758	4.6	0.46	<0.01	0.88

[1]Body Condition Score, from 1 = very skinny to 5 = very fat

[2]BW = Body Weight

Animal performance during the last six weeks of the D2 is presented in Table 11.5. Dry period length tended to differ among CP treatments, with a shorter duration observed in HCP compared with LCP and MCP (P = 0.05). However, no effects of CP level were observed for total DMI, PMR intake, concentrate intake, BCS, or BW ($P \geq 0.10$). In contrast, week of the dry period affected all performance variables ($P <$ 0.01), reflecting expected changes as cows approached calving. No significant CP × week interactions were observed ($P \geq 0.10$), indicating that patterns over time were consistent across treatments.

Table 11.5. Performance of non-lactating dairy cows during the last 6 weeks of the second dry period of the study receiving the same dietary CP level during after being fed different levels of CP during the previous lactation stage (133, 143 and 154, for LCP, MCP and HCP, respectively).

	CP-level				*P*-value		
	LCP	MCP	HCP	SEM	CP	Week	CP x Week
Number of animals	13	16	14				
Dry period length (d)	49.1[a]	47.2[a]	39.2[b]	1.07	0.05		
DMI total (kg/d)	15.9	16.2	15.5	0.19	0.66	<0.01	0.10
DMI PMR	15.3	15.6	15.2	0.19	0.81	<0.01	0.11
DMI concentrates	0.6	0.6	0.4	0.04	0.10	<0.01	0.91
BCS[1]	3.7	3.5	3.5	0.02	0.22	<0.01	0.91
BW[2] (kg)	787	783	757	6.6	0.61	<0.01	0.22

[1]Body Condition Score, from 1 = very skinny to 5 = very fat
[2]BW = Body Weight

Blood samples dry period 1

Blood parameters measured during the first dry period are presented in Table 11.3.

- No CP × week interactions were detected for any of the measured parameters ($P \geq 0.21$), indicating that temporal patterns were consistent across treatments.
- No effects of dietary CP level were observed for any of the measured metabolites and biomarkers, including albumin, BHBA, calcium, creatinine, phosphorus, GLDH, haptoglobin (Hp), magnesium, NEFA, total protein, and urea ($P \geq 0.14$).
- In contrast, week of the dry period affected several variables. Concentrations of phosphorus, GLDH, NEFA, and total protein changed over time ($P \leq 0.03$), whereas albumin, calcium, haptoglobin, magnesium, and urea were not influenced by week ($P \geq 0.30$).

Table 11.6. Blood serum metabolites on during dry period (4 and 2 weeks before expected calving) of cows receiving the same dietary CP level during dry period after being fed different levels of CP during the previous lactation stage (133, 143 and 154, for LCP, MCP and HCP, respectively).

		CP-level				*P*-value		
		LCP	MCP	HCP	SEM	CP	Week	CP × Week
Albumin	g/L	34.2	35.0	33.4	0.35	0.39	0.99	0.95
BHBA	mmol/L	0.51	0.55	0.59	0.014	0.14	0.07	0.49
Calcium	mmol/L	2.48	2.51	2.45	0.011	0.16	0.47	0.42
Creatinine	µmol/L	78	83	78	1.2	0.28	0.08	0.73
Phosphorus	mmol/L	1.94	2.00	1.94	0.022	0.51	0.03	0.48
GLDH	IU/L	11.6	10.6	13.2	0.66	0.46	0.03	0.61
Haptoglobin (Hp)	g/L	0.48	0.20	0.21	0.053	0.14	0.73	0.35
Hp $\log_{10}$	Hp × 100	1.32	1.17	1.20	0.032	0.24	0.63	0.21
Magnesium	mmol/L	0.91	0.92	0.92	0.007	0.86	0.66	0.73
NEFA	mmol/L	0.11	0.11	0.10	0.005	0.78	0.02	0.24
Total protein	g/L	79	81	80	0.6	0.20	<0.01	0.43
Urea	mmol/L	4.0	3.9	4.3	0.08	0.25	0.30	0.31

11.1.2 Calvings (L0, L1, and L2)

Dietary crude protein CP level did not affect most calving variables across successive calvings (Table 11.7).

- At the start of the study (Calving 0), when cows had not yet received the experimental diets, gestation length, calf birth weight, and birth ease were similar among groups ($P \geq 0.16$), with all calves born alive.
- During Calving 1, after the first lactation, no differences were observed in gestation length, birth weight, or birth ease among CP treatments ($P \geq 0.21$). The number of calves born alive was numerically lower in LCP compared with MCP and HCP, but this was not statistically evaluated.
- In Calving 2, gestation length was affected by dietary CP level ($P = 0.04$), with cows fed HCP having a shorter gestation length (272 days) compared with LCP and MCP (both 280 days). However, birth weight and birth ease were not influenced by dietary treatment ($P \geq 0.20$), and the number of calves born alive was comparable across treatments.

Overall, dietary CP concentration had minimal impact on calving performance, with the exception of a shorter gestation length observed in HCP cows during the second calving.

Table 11.7. Effect of dietary crude protein level on calving performance across successive calvings.

	CP-level				***P*-value**
Calving 0[1]	LCP	MCP	HCP	SEM	CP-level
Cows total, n	21	22	21		
Calves total, n	21	22	21		
Born alive, n	21	22	21		
Gestation length, days	281	281	279	0.6	0.16
Birth weight, kg	40.8	42.6	41.9	0.61	0.41
Birth ease[1]	2.10	2.00	1.95	0.035	0.24
Calving 1	LCP	MCP	HCP	SEM	CP-level
Cows total, n	19	21	20		
Calves total, n	21	23	21		
Born alive, n	16	19	19		
Gestation length, days	278	278	278	0.7	0.96
Birth weight, kg	37.6	39.9	39.1	0.68	0.21
Birth ease[2]	1.94	2.07	2.34	0.099	0.27
Calving 2	LCP	MCP	HCP	SEM	CP-level
Cows total, n	13	17	14		
Calves total, n	14	19	14		
Born alive, n	12	14	11		
Gestation length, days	280	280	272	1.5	0.04
Birth weight, kg	38.2	38.8	38.7	1.03	0.97
Birth ease[2]	2.03	2.30	2.63	0.138	0.20

[1]Calving at the start of the study before the dietary treatment
[2]Birth ease (1 = easy, 2 = normal, 3 = heavy, 4 = obstetric)

11.1.3 Early lactation (L1, L2, and L3)

Early lactation (1-4 WIM) performance of dairy cows fed different levels of crude protein (LCP, MCP and HCP) during 3 consecutive study lactations were presented in Table 11.6.

- In L1 a CP × WIM interaction was observed for dietary CP level, milk yield, protein content in milk, urea concentration in milk, FPCM yield, and fat yield ($P < 0.01$).
- Effects of dietary CP level were detected for total DMI, DMI of PMR, and DMI intake of GreenFeed concentrates ($P \leq 0.05$). Total DMI, and DMI of PMR was lower for LCP compared with MCP and HCP, with no differences between MCP and LCP. DMI of GreenFeed concentrates was higher for LCP compared with HCP, with MCP not being different from LCP and HCP.
- WIM affected most variables ($P < 0.01$) with the exception of urea concentration in milk and protein yield.
- In L2 a CP × WIM interaction was observed for dietary CP level, DMI of PMR, milk yield, urea concentration in milk, FPCM yield, fat yield, and protein yield ($P \leq 0.03$).
- Effects of dietary CP level was detected for DMI intake of GreenFeed concentrates ($P = 0.04$) and WIM affected all variables ($P \leq 0.01$).
- In L3 a CP × WIM interaction was observed for dietary CP level, DMI of PMR, milk yield, fat content in milk, urea concentration in milk, FPCM yield, Fat yield, and protein yield ($P \leq 0.03$).
- WIM affected all variables ($P \leq 0.05$).

Table 11.6. Early lactation (1-4 WIM) performance of dairy cows fed different levels of crude protein (LCP, MCP and HCP) during 3 consecutive study lactations.

		Diet (CP)				P-value		
L1		LCP	MCP	HCP	SEM	CP	WIM	CP × WIM
Cows	n	21	22	22				
Parity		3.5	3.6	3.5				
Dietary CP	g/kg DM	135	148	159	0.8	<0.01	<0.01	<0.01
DMI total	kg/d	19.9[b]	21.3[a]	21.6[a]	0.18	<0.01	<0.01	0.15
PMR	kg/d	11.7[b]	13.3[a]	13.8[a]	0.11	<0.01	<0.01	0.08
Conc CP	kg/d	6.1	6.0	6.0	0.15	0.64	<0.01	0.76
Conc GF	kg/d	2.0[a]	1.9[ab]	1.7[b]	0.03	0.05	<0.01	0.65
Milk yield	kg/d	35.0	38.1	37.6	0.41	0.08	<0.01	<0.01
Fat	g/100 g milk	4.91	5.02	4.82	0.042	0.40	<0.01	0.07
Protein	g/100 g milk	3.76	3.63	3.86	0.038	0.02	<0.01	<0.01
Lactose	g/100 g milk	4.42	4.43	4.43	0.013	0.98	<0.01	0.39
Urea	mg/dL	9.6	11.7	14.9	0.27	<0.01	0.34	<0.01
FPCM	kg/d	39.4	43.1	42.2	0.42	0.09	<0.01	<0.01
Fat yield	kg/d	1.707	1.900	1.810	22.4	0.11	<0.01	<0.01
Protein yield	kg/d	1.301	1.366	1.413	11.7	0.06	0.16	0.11
L2		LCP	MCP	HCP	SEM	CP	WIM	CP × WIM
Cows	n	19	22	20				
Parity		4.5	4.5	4.3				
Dietary CP	g/kg DM	132	146	158	0.8	<0.01	<0.01	<0.01
DMI total	kg/d	20.3	21.4	21.7	0.22	0.08	<0.01	0.24
PMR	kg/d	12.2	13.2	13.7	0.16	0.06	<0.01	0.03
Conc CP	kg/d	6.2	6.2	6.2	0.14	0.77	<0.01	0.55
Conc GF	kg/d	1.8	1.8	1.6	0.03	0.04	<0.01	0.84
Milk yield	kg/d	35.1	39.0	37.5	0.50	0.09	<0.01	<0.01
Fat	g/100 g milk	4.93	5.09	4.94	0.050	0.59	<0.01	0.73
Protein	g/100 g milk	3.77	3.69	3.80	0.037	0.32	<0.01	1.00
Lactose	g/100 g milk	4.50	4.51	4.48	0.013	0.70	<0.01	0.99
Urea	mg/dL	9.2	11.6	14.7	0.27	<0.01	<0.01	<0.01
FPCM	kg/d	39.5	44.5	42.2	0.51	0.05	<0.01	<0.01
Fat yield	kg/d	1.711	1.970	1.830	26.0	0.05	<0.01	<0.01
Protein yield	kg/d	1.301	1.414	1.393	14.2	0.16	0.01	0.01
L3		LCP	MCP	HCP	SEM	CP	WIM	CP × WIM
Cows	n	13	15	13				
Parity		5.2	5.3	5.2				
Dietary CP	g/kg DM	132	146	157	0.9	<0.01	<0.01	<0.01
DMI total	kg/d	19.9	21.6	22.3	0.29	0.11	<0.01	0.14
PMR	kg/d	11.1	13.0	13.9	0.23	0.04	<0.01	0.03
Conc CP	kg/d	6.1	6.1	6.0	0.19	0.39	<0.01	0.19
Conc GF	kg/d	2.2	2.1	2.0	0.02	0.11	0.02	0.06
Milk yield	kg/d	37.8	38.8	36.9	0.70	0.81	<0.01	0.01
Fat	g/100 g milk	5.43	5.31	5.13	0.073	0.65	<0.01	0.01
Protein	g/100 g milk	3.79	3.85	3.90	0.047	0.56	<0.01	0.87
Lactose	g/100 g milk	4.43	4.44	4.44	0.017	0.98	<0.01	0.62
Urea	mg/dL	11.4	13.8	16.9	0.41	<0.01	<0.01	<0.01
FPCM	kg/d	44.6	45.5	42.8	0.73	0.70	<0.01	<0.01
Fat yield	kg/d	2.014	2.041	1.890	36.6	0.62	<0.01	<0.01
Protein yield	kg/d	1.410	1.461	1.400	21.8	0.81	0.05	0.01

[ab] Values with different superscript are significantly different ($P \leq 0.05$)

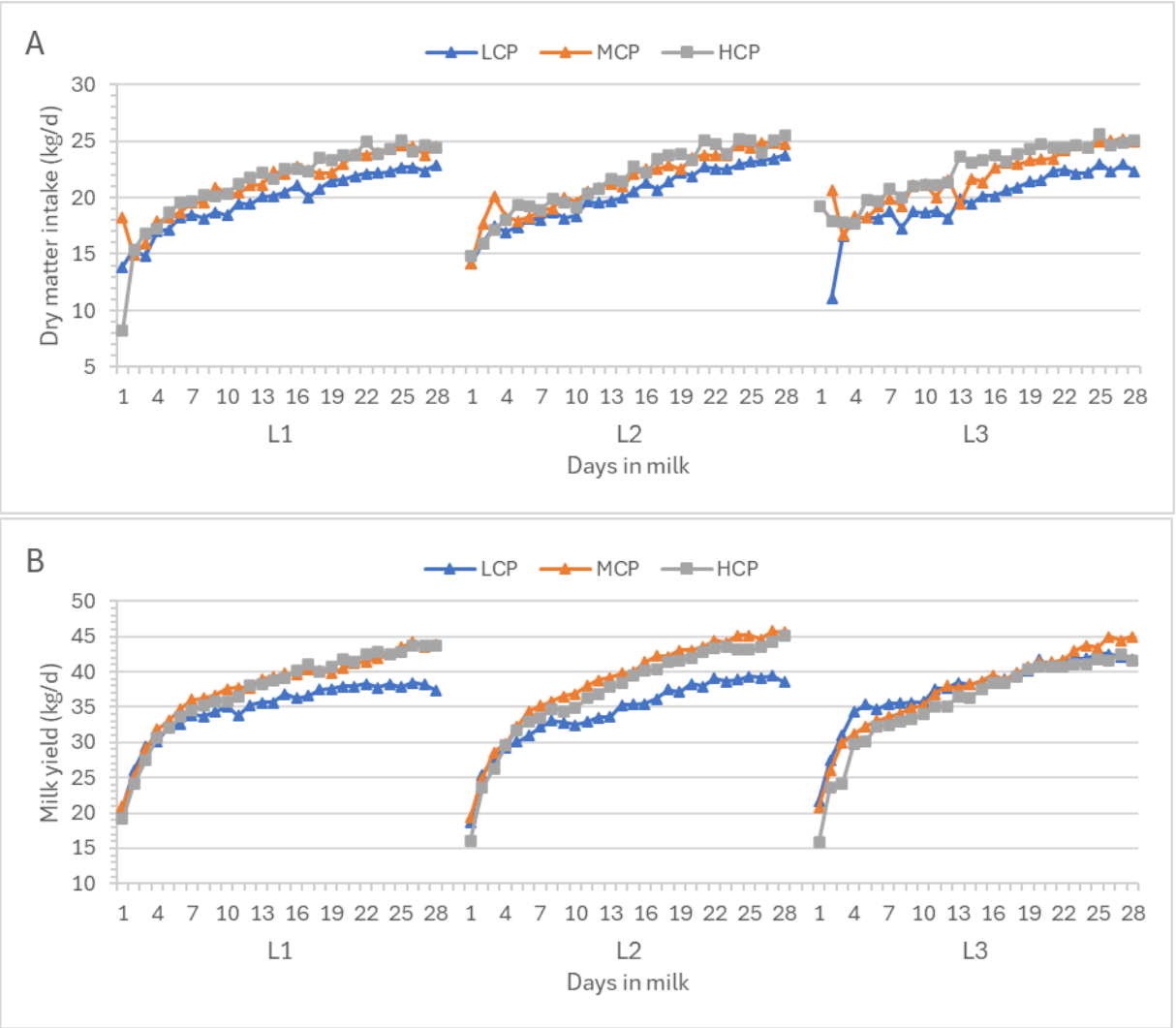


Figure 11.2. Early lactation (1-28 DIM) dry matter intake (A) and milk yield (B) of Holstein Friesian dairy cows fed different levels of CP during three consecutive lactations (L1 135/148/160 g CP/kg DM; L2 and L3, 132/146/158 g CP/kg DM for LCP/MCP/HCP respectively).

Analysis of serum blood samples taken once a week during week 1 to 4 after parturition are presented in table 11.7, with only minor effects visible between dietary treatments.

- A CP x WIM interaction was found for albumin, creatinine, and total protein ($P \leq 0.03$).
- A tendency for a dietary CP effect was found for CPK ($P = 0.07$) and WIM effects were found for all variables ($P \leq 0.04$) with the exception of CPK. BHBA, calcium, phosphorus, GLDH, haptoglobin, log-transformed haptoglobin,

Table 11.7. Early lactation (1, 2, 3, and 4 WIM) serum blood analysis of dairy cows fed different levels of CP (135, 148, and 160 g/kg DM for LCP, MCP, and HCP, respectively) during the first lactation of the study.

		CP level				*P*-value		
		LCP	MCP	HCP	SEM	CP	WIM	CP × WIM
Albumin	g/L	35.2	34.9	34.9	0.15	0.81	0.03	0.03
BHBA	mmol/L	0.85	0.83	0.89	0.020	0.76	<0.01	0.81
Calcium	mmol/L	2.47	2.44	2.43	0.012	0.43	<0.01	0.80
CPK	IU/L	224	162	151	14.0	0.07	0.37	0.37
Creatinine	µmol/L	74	75	70	0.7	0.03	<0.01	0.01
Phosphorus	mmol/L	1.77	1.80	1.76	0.017	0.71	0.04	0.13
GLDH	IU/L	20.1	15.6	21.6	1.14	0.31	0.01	0.41
Haptoglobin (Hp)	g/L	0.17	0.24	0.21	0.016	0.39	<0.01	0.62
Hp $\log_{10}$	Hp × 100	1.11	1.21	1.16	0.019	0.14	<0.01	0.41

Insulin	µIU/ml	7.4	7.9	10.4	0.48	0.83	<0.01	0.35
Magnesium	mmol/L	0.90	0.91	0.91	0.008	0.37	<0.01	0.28
NEFA	mmol/L	0.37	0.41	0.35	0.014	0.11	<0.01	0.26
Total protein	g/L	72	74	75	0.4	<0.01	<0.01	<0.01
Urea	mmol/L	1.5	2.1	2.7	0.05	0.25	<0.01	0.52

Table 11.7. Early lactation blood results (L2)

		CP-level				P-value		
		LCP	MCP	HCP	SEM	CP	Week	CP × Week
Albumin	g/L	34.0	34.2	34.1	0.30	0.99	<0.01	0.70
BHBA	mmol/L	0.87	0.99	0.88	0.021	0.27	0.03	0.42
Calcium	mmol/L	2.43	2.42	2.43	0.015	0.99	<0.01	0.53
CPK	IU/L	165	157	578	37.9	0.38	0.04	0.50
Creatinine	µmol/L	78	76	73	0.9	0.37	<0.01	0.67
Phosphorus	mmol/L	1.71	1.72	1.72	0.019	0.96	0.03	0.91
GLDH	IU/L	14.1	15.9	17.5	0.70	0.44	0.19	0.69
Haptoglobin (Hp)	g/L	0.50	0.52	0.29	0.039	0.07	<0.01	0.50
Hp $\log_{10}$	Hp × 100	1.43	1.44	1.30	0.027	0.10	<0.01	0.78
Insulin	µIU/ml	11.7	11.1	12.3	0.42	0.61	<0.01	0.31
Magnesium	mmol/L	0.95	0.93	0.94	0.009	0.62	<0.01	0.64
NEFA	mmol/L	0.38	0.46	0.38	0.015	0.13	<0.01	0.63
Total protein	g/L	73	76	77	0.5	0.20	<0.01	0.11
Urea	mmol/L	1.8	2.1	2.7	0.05	<0.01	<0.01	<0.01

11.2 Interpretation

The absence of performance differences among cows fed different dietary CP levels during lactation indicates that, under the conditions of this study, CP supply during previous lactation did not limit intake when the same dietary treatment was applied during the dry period. The shorter dry period length observed for HCP cows in D2 was associated with a shorter gestation length rather than an effect of dietary CP during the dry period itself. Because dry matter intake during both dry periods was similar among treatments, carryover effects of dietary CP from one lactation to the next are unlikely. Furthermore, the lack of differences in dry-period intake behavior and blood serum metabolite concentrations indicates that cows did not exhibit compensatory responses during the dry period, which was consistent with the experimental design and confirms that dry-period nutritional status was comparable across treatments.

Calving performance variables, including gestation length, calf birth weight, and calving ease, were largely unaffected by dietary CP level across successive calvings, indicating that calving outcomes are relatively resilient to dietary CP levels fed in the current study. Subtle differences observed, like the gestation length, may reflect normal biological variability rather than true dietary effects and may require larger datasets or more targeted physiological measurements. The observed reduction in gestation length for HCP cows at calving 2 occurred without concurrent differences in calf birth weight or calving ease, suggesting that the biological and practical significance of this finding is limited, although it cannot be excluded that calf birth weights might have been higher had full gestation length been maintained.

The uniform dietary CP levels applied during the dry period may have masked potential carryover effects of lactational CP treatments on calving characteristics or may support the concept that nutritional management in late gestation is more critical for determining calving performance than protein supply earlier in lactation.

Interpretation of calving ease should be made with caution, as calving ease was not assessed using a standardized or protocolized scoring system. As such, this variable is inherently subjective and highly susceptible to observer variation, which reduces its reliability for detecting subtle treatment effects.

The proportion of calves born alive was lower at calving 1 and calving 2 compared with calving 0, largely due to the occurrence of twin births, as neither calf was born alive in any twin calving event. In contrast to calving 1 and 2, no twin births were recorded at calving 0. In addition, external factors such as

bluetongue virus cases may have contributed to adverse outcomes, including a small number of early calvings, shortened gestation lengths in HCP cows during L2, and an increased number of calves born not alive. These events are unlikely to be directly related to dietary CP level.

Overall, the absence of adverse effects on calving performance across lactational CP levels, in combination with uniform CP supply during the dry period, supports the feasibility of reducing dietary CP within the evaluated range without compromising calving outcomes.

One noteworthy observation was the milk yield of HCP cows in L3, which was numerically lower compared with LCP cows. This contrasts with the overall pattern observed throughout the study, where LCP cows generally produced less milk than MCP and HCP cows. Furthermore, milk production would generally be expected to increase with advancing parity, making the lower milk yield of HCP cows in L3 compared with L2 particularly unexpected. The numerical differences observed in L3 may have been partly influenced by changes in the composition of the experimental population resulting from culling and exclusion of cows over time. Milk yield variation appeared greater in L3 than in previous lactations, suggesting that the performance of a limited number of cows may have had a stronger influence on treatment means. In addition, HCP cows experienced a shorter dry period before L3, which may have contributed to their lower subsequent milk production. In contrast, milk yield patterns during the first four weeks in milk were highly similar across L1, L2, and L3, indicating a consistent early-lactation response across study lactations despite changes in animal numbers and composition, thereby supporting overall robustness of the treatment effects observed throughout the study.

11.3 Conclusions

In conclusion, feeding different dietary CP levels during lactation had no detrimental effects on intake, performance, or calving outcomes when cows received a uniform diet during the dry period. Calving performance was largely unaffected by the applied CP ranges. Milk yield patterns during early lactation were consistent across study lactations, supporting the reliability of the results. Overall, these findings indicate that dietary CP can be reduced within the evaluated range without compromising cow performance during dry period or calving outcomes, but do affect early lactation performance.

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13 Appendices

13.1 Appendix 1: Feed values

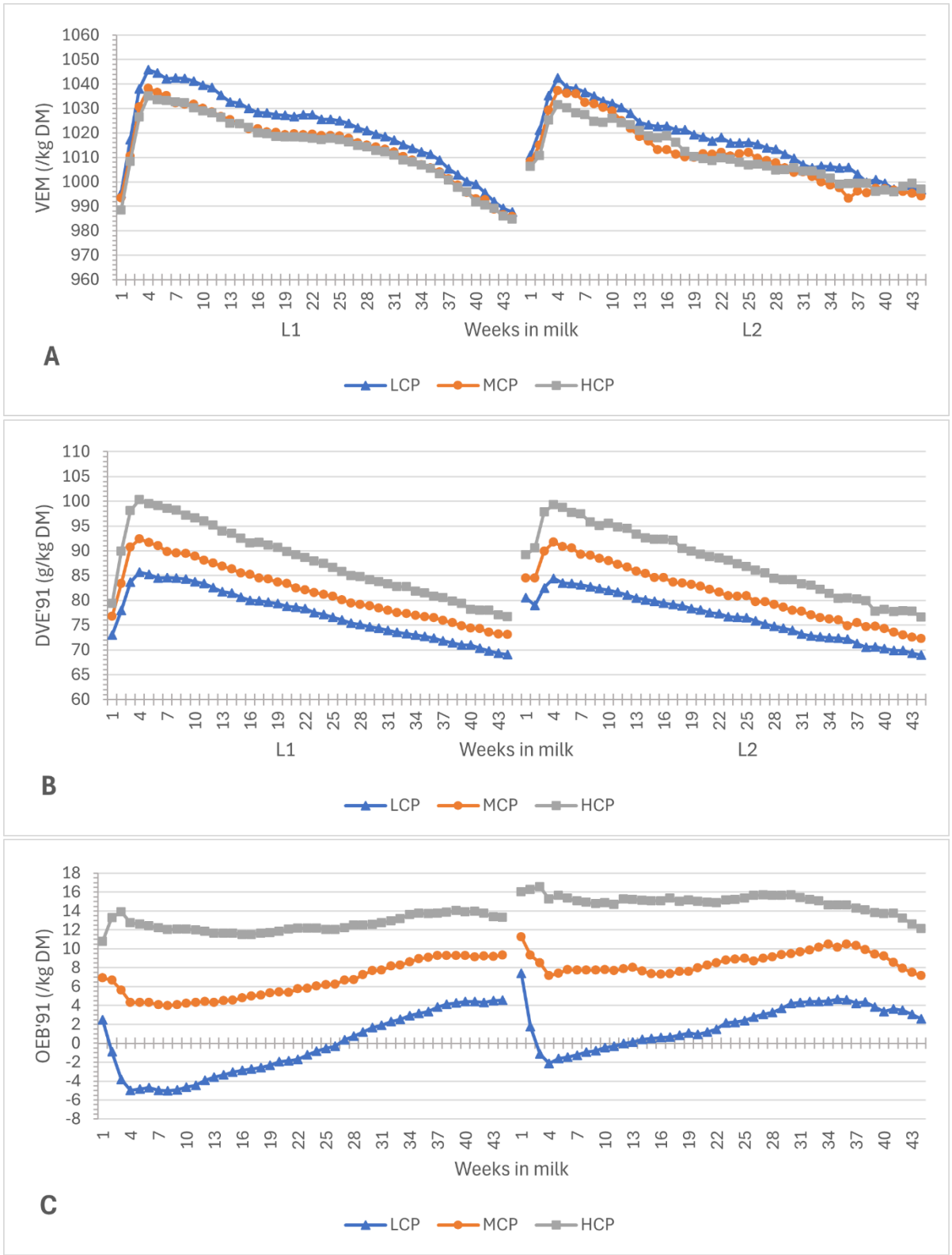


Figure 13.1.1. VEM (A), DVE (B), and OEB (C) levels in the diet per week in lactation for cows fed different dietary crude protein levels 133, 143 and 154 g CP / kg DM for LCP, MCP, and HCP respectively.

13.2 Appendix 2: Data overview per 60 d period

	L1				L2			
DIM	**LCP**	**MCP**	**HCP**	**SD**	**LCP**	**MCP**	**HCP**	**SD**
N (number of cows)								
1-60	21	22	22		18	22	19	
61-120	21	22	21		18	20	18	
121-180	21	22	21		17	20	17	
181-240	21	22	21		17	20	17	
241-300	19	22	21		16	20	16	
Parity								
1-60	3.52	3.59	3.55		4.56	4.52	4.32	
61-120	3.52	3.59	3.38		4.56	4.50	4.39	
121-180	3.52	3.59	3.38		4.59	4.50	4.24	
181-240	3.52	3.59	3.38		4.59	4.50	4.24	
241-300	3.48	3.59	3.38		4.57	4.50	4.21	

	L1				L2			
DIM	**LCP**	**MCP**	**HCP**	**SD**	**LCP**	**MCP**	**HCP**	**SD**
DMI total (kg/d)								
1-60	21.6	23.4	23.6	1.61	22.5	24.0	24.1	2.61
61-120	23.1	25.6	25.4	1.93	24.8	27.1	26.4	2.17
121-180	22.6	25.2	25.2	2.11	24.2	26.9	26.3	2.04
181-240	21.6	23.9	24.0	2.04	23.0	25.1	24.7	2.02
241-300	20.1	21.9	22.4	1.81	21.3	23.5	22.6	2.36
DMI PMR (kg/d)								
1-60	12.0	13.9	14.2	1.63	12.8	14.3	14.8	2.41
61-120	13.2	15.8	15.9	1.88	14.8	17.2	16.7	2.02
121-180	14.3	16.9	17.1	2.03	15.8	18.3	18.0	1.90
181-240	15.1	17.3	17.6	1.95	16.3	18.4	18.2	1.92
241-300	15.1	16.8	17.4	1.78	15.9	18.0	17.4	2.06
DMI Concentrate (kg/d)								
1-60	9.5	9.4	9.2	0.38	9.4	9.4	9.0	0.96
61-120	9.9	9.8	9.5	0.47	10.0	10.0	9.6	0.57
121-180	8.3	8.3	8.1	0.48	8.4	8.5	8.3	0.44
181-240	6.5	6.5	6.4	0.49	6.8	6.8	6.6	0.44
241-300	5.1	5.1	5.0	0.46	5.3	5.4	5.2	0.64

	L1				L2			
DIM	**LCP**	**MCP**	**HCP**	**SD**	**LCP**	**MCP**	**HCP**	**SD**
Milk yield (kg/d)								
1-60	37.0	41.0	41.2	5.43	37.5	42.8	41.2	6.68
61-120	34.9	39.4	38.9	5.26	36.9	41.9	39.7	6.09
121-180	30.5	34.7	34.8	5.11	32.2	37.1	36.6	5.64
181-240	26.9	30.5	31.5	4.92	28.4	32.5	33.2	5.91
241-300	22.0	25.0	26.8	4.60	24.1	26.6	27.7	5.77
Fat content (g/100 g milk)								
1-60	4.49	4.52	4.48	0.402	4.57	4.67	4.66	0.403
61-120	4.05	4.17	4.31	0.404	4.25	4.29	4.46	0.422
121-180	4.25	4.35	4.50	0.425	4.47	4.47	4.61	0.438
181-240	4.51	4.60	4.68	0.437	4.74	4.63	4.70	0.472
241-300	4.69	4.68	4.83	0.460	4.87	4.84	4.89	0.522
Protein content (g/100 g milk)								
1-60	3.44	3.31	3.50	0.227	3.47	3.39	3.49	0.204
61-120	3.27	3.28	3.37	0.231	3.40	3.31	3.48	0.218
121-180	3.43	3.49	3.55	0.219	3.55	3.58	3.61	0.239
181-240	3.54	3.60	3.63	0.218	3.63	3.68	3.65	0.252
241-300	3.63	3.71	3.75	0.232	3.74	3.83	3.75	0.289
Lactose content (g/100 g milk)								
1-60	4.48	4.49	4.49	0.139	4.58	4.59	4.57	0.130
61-120	4.47	4.51	4.51	0.149	4.63	4.65	4.63	0.145
121-180	4.44	4.45	4.47	0.179	4.57	4.59	4.60	0.134
181-240	4.49	4.48	4.49	0.172	4.52	4.54	4.56	0.148
241-300	4.50	4.49	4.53	0.171	4.46	4.46	4.53	0.185
Urea content in milk (mg/dL)								
1-60	8.5	11.4	15.9	3.90	8.4	11.1	15.9	3.97
61-120	7.1	10.9	16.5	4.67	9.1	12.5	18.6	6.36
121-180	8.9	11.8	16.0	3.87	11.1	14.1	19.7	4.42
181-240	10.1	13.3	16.8	3.70	12.8	16.2	20.1	4.09
241-300	11.1	13.2	16.0	3.19	13.1	16.1	18.5	4.25
SCC in milk (x 1.000 cels/ml)								
1-60	155	270	232	334	250	254	402	404
61-120	314	238	304	422	271	430	322	784
121-180	254	274	270	414	545	366	146	573
181-240	242	348	245	389	605	310	136	621
241-300	323	376	268	421	791	349	254	926
SCC.log10								
1-60	1.89	1.97	1.84	0.479	1.84	1.89	1.98	0.448
61-120	1.97	1.86	1.81	0.563	1.90	2.03	1.92	0.559
121-180	2.05	1.96	1.83	0.546	2.15	2.04	1.87	0.565
181-240	2.12	2.07	1.84	0.530	2.25	2.10	1.84	0.530
241-300	2.16	2.16	1.96	0.524	2.38	2.18	2.06	0.525

	L1				L2			
DIM	**LCP**	**MCP**	**HCP**	**SD**	**LCP**	**MCP**	**HCP**	**SD**
FPCM yield (kg/d)								
1-60	39.5	43.9	43.7	5.65	40.1	46.3	44.7	7.04
61-120	34.9	40.1	40.2	4.79	38.0	43.2	41.8	5.78
121-180	31.5	36.3	37.1	5.13	34.2	39.6	39.7	5.47
181-240	28.8	33.0	34.5	5.22	31.1	35.5	36.4	5.98
241-300	24.1	27.5	30.0	5.04	27.0	30.0	31.1	6.25
Fat yield (kg/d)								
1-60	1.661	1.867	1.839	0.2740	1.701	1.997	1.907	0.3292
61-120	1.407	1.639	1.662	0.2140	1.560	1.791	1.739	0.2573
121-180	1.289	1.498	1.554	0.2280	1.427	1.651	1.676	0.2408
181-240	1.209	1.396	1.468	0.2380	1.328	1.498	1.550	0.2625
241-300	1.028	1.165	1.288	0.2270	1.162	1.284	1.347	0.2814
Protein yield (kg/d)								
1-60	1.268	1.366	1.409	0.1530	1.284	1.436	1.425	0.2036
61-120	1.135	1.288	1.302	0.1420	1.244	1.380	1.368	0.1716
121-180	1.041	1.204	1.228	0.1660	1.134	1.323	1.313	0.1754
181-240	0.947	1.091	1.138	0.1660	1.019	1.188	1.203	0.1919
241-300	0.795	0.926	1.003	0.1680	0.895	1.013	1.033	0.2043

	L1				L2			
DIM	**LCP**	**MCP**	**HCP**	**SD**	**LCP**	**MCP**	**HCP**	**SD**
Feed efficiency of milk								
1-60	1.71	1.75	1.74		1.67	1.78	1.71	
61-120	1.51	1.54	1.53		1.49	1.55	1.51	
121-180	1.35	1.37	1.38		1.33	1.38	1.39	
181-240	1.24	1.28	1.31		1.23	1.30	1.34	
241-300	1.09	1.14	1.20		1.13	1.13	1.22	
Feed efficiency of FPCM								
1-60	1.82	1.87	1.85		1.78	1.93	1.85	
61-120	1.51	1.56	1.58		1.53	1.59	1.58	
121-180	1.39	1.44	1.47		1.41	1.47	1.51	
181-240	1.33	1.38	1.44		1.35	1.41	1.47	
241-300	1.20	1.26	1.34		1.27	1.28	1.38	

	L1				L2			
DIM	**LCP**	**MCP**	**HCP**	**SD**	**LCP**	**MCP**	**HCP**	**SD**
CP (g/kg DM)								
1-60	136	149	162	10.9	132	146	159	11.0
61-120	135	146	159	10.1	132	143	157	11.0
121-180	133	143	154	8.5	133	144	156	9.7
181-240	130	139	148	7.5	136	146	156	8.7
241-300	127	136	144	6.7	136	144	156	8.2
N intake (g/d)								
1-60	470	558	614	65.3	474	557	612	78.3
61-120	498	599	646	71.2	522	619	669	74.5
121-180	481	575	618	67.1	524	621	649	70.4
181-240	450	529	568	59.8	510	607	608	64.1
241-300	410	475	515	52.0	485	611	558	63.9
N output in milk (g/d)								
1-60	199	214	221	24.1	201	225	223	31.9
61-120	178	202	204	22.3	195	216	214	26.9
121-180	163	189	192	26.0	178	207	206	27.5
181-240	148	171	178	26.1	160	186	189	30.1
241-300	125	145	157	26.4	140	159	162	32.0
N efficiency								
1-60	0.432	0.386	0.368	0.0461	0.435	0.410	0.372	0.0452
61-120	0.359	0.338	0.316	0.0314	0.372	0.347	0.324	0.0367
121-180	0.340	0.328	0.312	0.0338	0.344	0.336	0.312	0.0348
181-240	0.331	0.322	0.315	0.0345	0.317	0.314	0.297	0.0400
241-300	0.305	0.304	0.305	0.0393	0.315	0.318	0.272	0.0448

	L1				L2			
DIM	LCP	MCP	HCP	SD	LCP	MCP	HCP	SD
CH4 (g/d)								
1-60	423	454	454	42.2	402	435	437	65.0
61-120	467	508	503	41.7	433	478	485	47.5
121-180	464	513	518	45.8	448	492	504	44.7
181-240	443	494	500	43.4	464	509	506	49.3
241-300	421	461	477	38.3	440	492	482	53.8
CH4 (g/kg DMI)								
1-60	19.6	19.5	19.2	1.42	18.1	18.2	18.3	1.90
61-120	20.3	19.9	19.8	1.64	17.6	17.8	18.3	1.31
121-180	20.7	20.5	20.6	1.84	18.8	18.3	19.1	1.48
181-240	20.6	20.8	20.9	1.72	20.5	20.4	20.4	1.62
241-300	21.2	21.2	21.3	1.58	20.6	21.1	21.4	1.84
CH4 (g/kg Milk)								
1-60	11.5	11.2	11.2	1.91	10.8	10.2	10.5	1.84
61-120	13.6	13.1	13.1	1.90	12.0	11.6	12.3	1.76
121-180	15.5	15.2	15.1	2.72	14.3	13.5	13.8	2.26
181-240	16.7	16.9	16.1	3.46	17.0	16.0	15.5	3.56
241-300	20.0	19.9	18.2	5.51	18.5	20.0	18.0	5.59
CO2 (g/d)								
1-60	14,167	14,861	14,884	966.0	13,394	13,874	14,286	1,281.1
61-120	14,943	15,827	15,865	996.0	13,672	15,373	14,940	1,443.2
121-180	14,841	15,923	15,904	1,067.0	13,886	14,451	15,535	1,742.2
181-240	13,996	15,194	15,239	1,130.0	14,892	16,262	15,701	1,272.2
241-300	13,381	14,486	14,650	1,030.0	14,738	15,998	15,825	1,001.6
H2 (g/d)								
1-60	2.03	1.84	1.80	0.297	2.04	2.14	2.03	0.369
61-120	2.11	1.90	1.81	0.350	1.93	1.87	2.05	0.356
121-180	1.93	1.81	1.75	0.312	1.76	1.77	1.86	0.335
181-240	1.62	1.61	1.54	0.311	1.55	1.36	1.55	0.313
241-300	1.42	1.44	1.37	0.245	1.40	1.27	1.32	0.282

13.3 Appendix 3: Overview animals in experiment

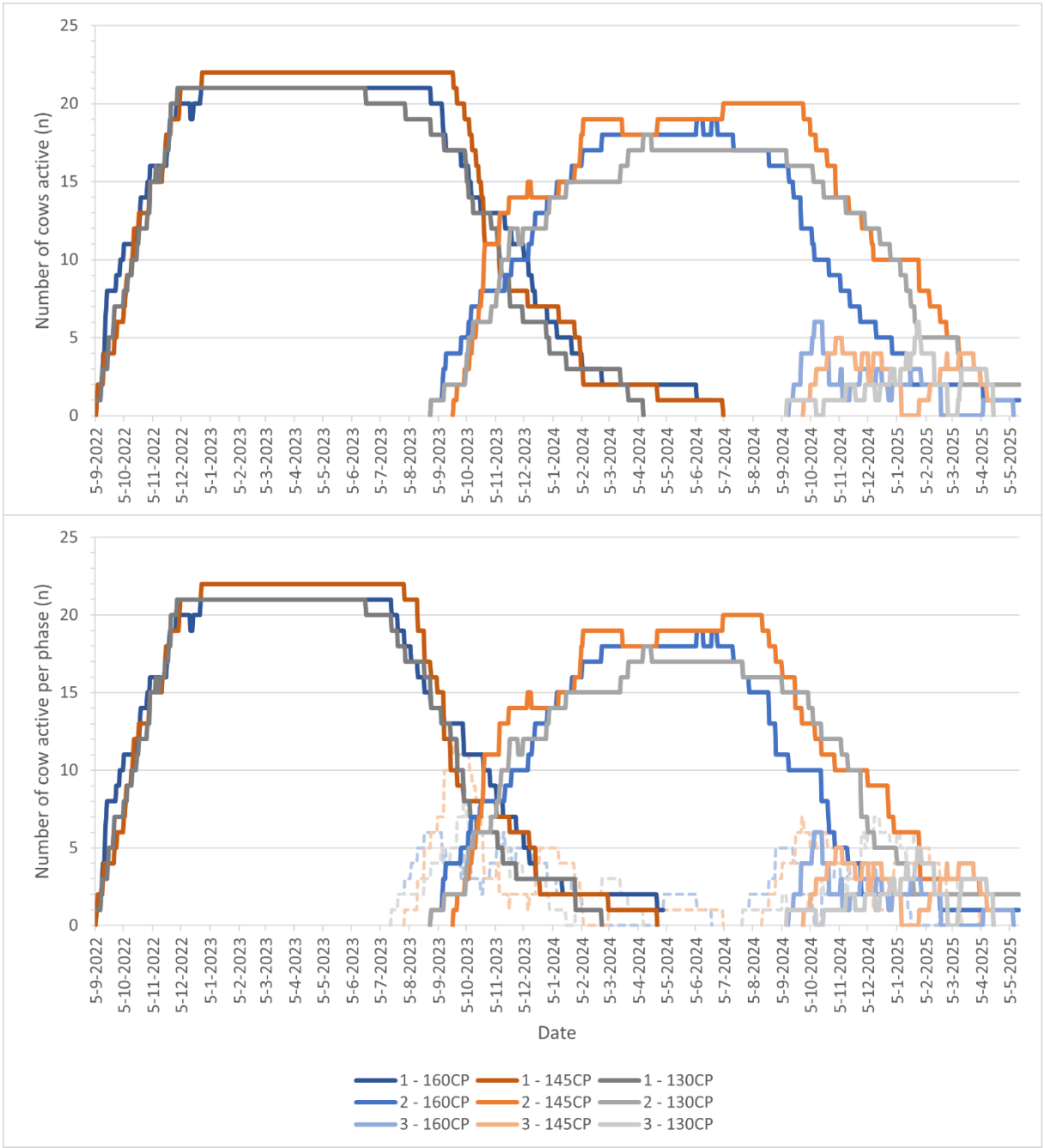


Figure 13.3.1. Overview of animals per treatment group (160CP, 145CP, 130CP) active in trial (A) and active in trial per phase (B: — lactating or --- dry).

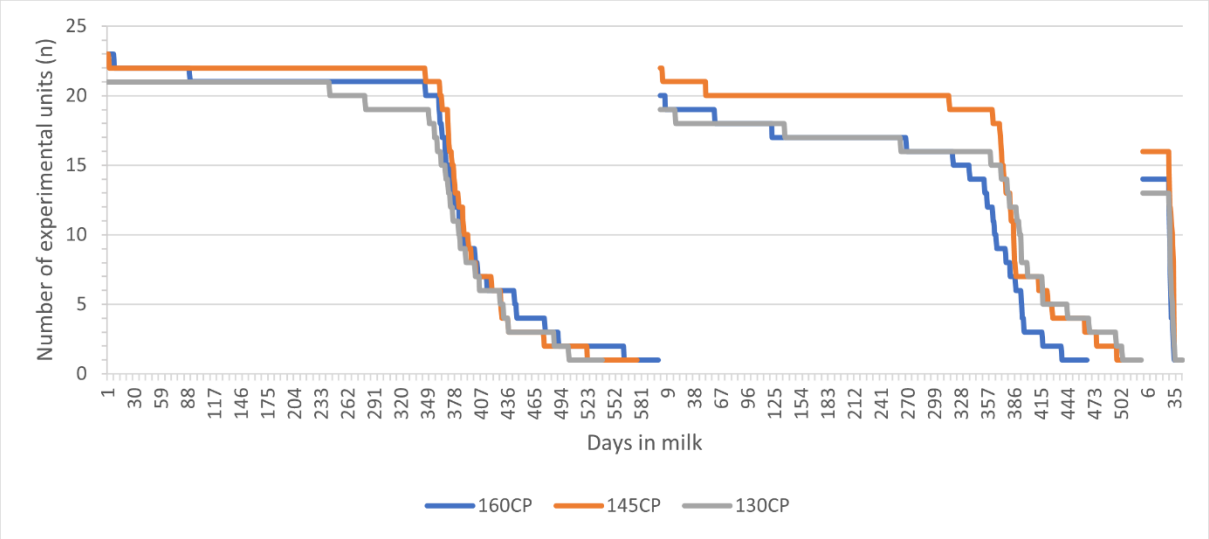


Figure 13.3.2. Total experimental units for different dietary treatment groups (LCP (133 g RE/kg DM) MCP (143/144 g RE/kg DM), and HCP (154/156 g RE/kg DM)) per days in milk.

Table 13.3.1. Details of excluded animals, dates, and reasons.

Date	Cow	Treat	Lactation	DIM	Reason	Note
14-12-2022	8127	160CP	L1	90	Trauma/disease	Teat trampling (65 DIM), mastitis (75, 83 DIM) severe fever (89 DIM) no recovery
19-6-2023	2715	130CP	L1	281	Chronic disease	Chronic and recurrent indigestion (34, 60, 146, 250 and 278 DIM) no prospect of recovery
31-7-2023	907	130CP	L1	242	Chronic disease	Chronic and recurrent pneumonia (153, 209, 238 DIM), no prospect of recovery
28-11-2023	2160	130CP	L2	17	Acute disease	Pneumonia (6 DIM), severe heart disease (13 DIM)
4-12-2023	1597	160CP	L1	443	Practical	Not pregnant after 305 DIM, producing less than 12 kg milk/d
12-12-2023	1192	145CP	L2	3	Trauma	Hypocalcemia and dislocated hip (1 DIM), no prospect of recovery
18-3-2024	1868	145CP	L2	50	Acute disease	Mastitis (37 DIM), severe pneumonia (44 DIM)
18-4-2024	2435	130CP	L2	136	Trauma	Jumped on during heat (132 DIM), aggravating lameness
12-6-2024	1681	160CP	L2	6	Trauma	Hypocalcemia (0 DIM), slipped and severe muscle damage (3 DIM)
27-6-2024	98	160CP	L2	122	Chronic disease	Uddercleft dermatitis (98, 110 DIM) with severe fever and anorexia (119 DIM)
14-7-2024	1740	160CP	L2	268	Chronic disease	Claw necrosis (146 DIM), lameness (227 DIM), severe lameness (255 DIM)
21-8-2024	2845	160CP	L2	60	Acute disease	Fever (45 DIM), lameness and nerve damage (51 DIM), no prospect of recovery
17-9-2024	2209	160CP	L3	1-28	Practical	Wrong allocation of treatment concentrates
7-10-2024	1448	130CP	L2	262	Acute disease	Lameness / artritis (247, 250 DIM) en Bluetongue
24-10-2024	2373	160CP	L2	319	Practical	Abortion day before dry-off
15-11-2024	1077	145CP	L3	1-28	Practical/disease	No data (entire period in infirmary), digital dermatitis (1< DIM), fever (13-28)
28-1-2025	605	130CP	L3	8	Practical	No milk production after calving (stillbirth with deformed calf/abortion)
28-1-2025	1108	145CP	L2	462	Practical	Not pregnant after 305 DIM, producing less than 12 kg milk/d

13.4 Appendix 4: Least Significant Differences

Table 13.4.1. Least significant differences (LSD) of animal performance during the first 44-wk lactation of the experiment.

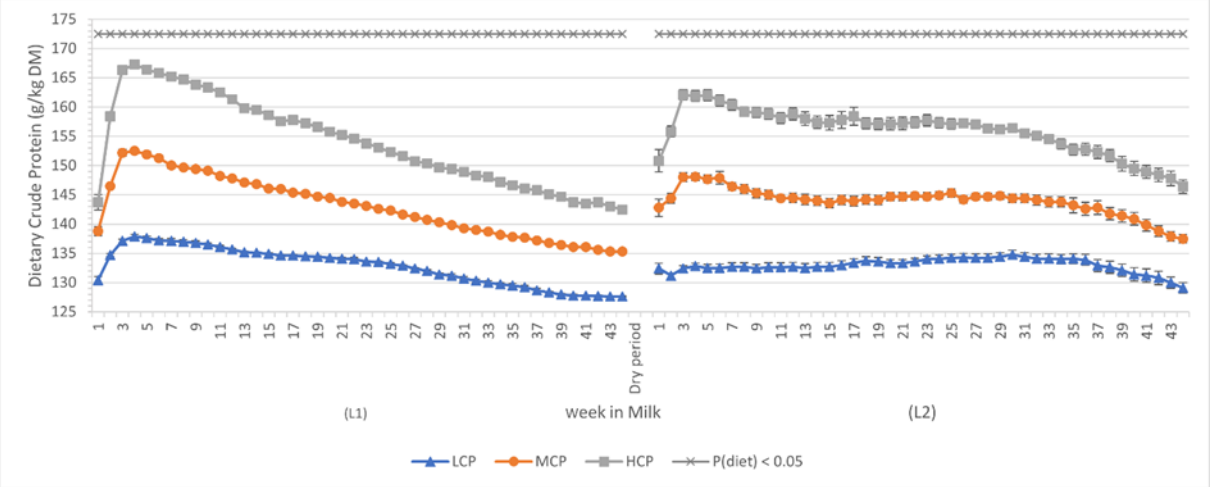
		Diet				*P*-value	LSD			
		LCP	MCP	HCP	SEM	Diet	Average	LCP-MCP	LCP-HCP	MCP-HCP
CP	g/kg DM	133[c]	143[b]	154[a]	0.2	<0.01	0.7	0.7	0.7	0.7
DMI total	kg/d	21.7[b]	23.9[a]	23.9[a]	0.05	<0.01	0.83	0.83	0.83	0.82
DMI PMR	kg/d	13.9[b]	16.2[a]	16.4[a]	0.04	<0.01	0.77	0.77	0.77	0.76
DMI concentrates	kg/d	7.8	7.7	7.5	0.04	0.07				
Milk yield	kg/d	29.9[b]	33.8[a]	34.2[a]	0.15	<0.01	2.38	2.39	2.39	2.36
Fat	g/100g milk	4.41	4.48	4.60	0.011	0.21				
Protein	g/100g milk	3.48	3.50	3.57	0.007	0.19				
Lactose	g/100g milk	4.47	4.48	4.49	0.004	0.90				
Urea	mg/dL	9.2[c]	12.1[b]	16.3[a]	0.09	<0.01	1.23	1.23	1.23	1.21
SCC	× 1,000 cells/ml	253	317	290	11.3	0.83				
SCC(log10)		2.03	2.02	1.87	0.011	0.35				
FPCM yield	kg/d	31.3[b]	35.8[a]	36.8[a]	0.15	<0.01	2.56	2.57	2.57	2.54
Fat yield	kg/d	1.302[b]	1.499[a]	1.552[a]	0.0065	<0.01	0.115	0.116	0.116	0.114
Protein yield	kg/d	1.026[b]	1.165[a]	1.203[a]	0.0044	<0.01	0.079	0.079	0.079	0.078
N intake	g/d	462[c]	547[b]	590[a]	1.6	<0.01	16.9	17.0	17.0	16.7
N excretion in milk	g/d	161[b]	183[a]	189[a]	0.7	<0.01	12.3	12.4	12.4	12.2
N efficiency		0.350[a]	0.334[b]	0.321[b]	0.0012	<0.01	0.017	0.017	0.017	0.016

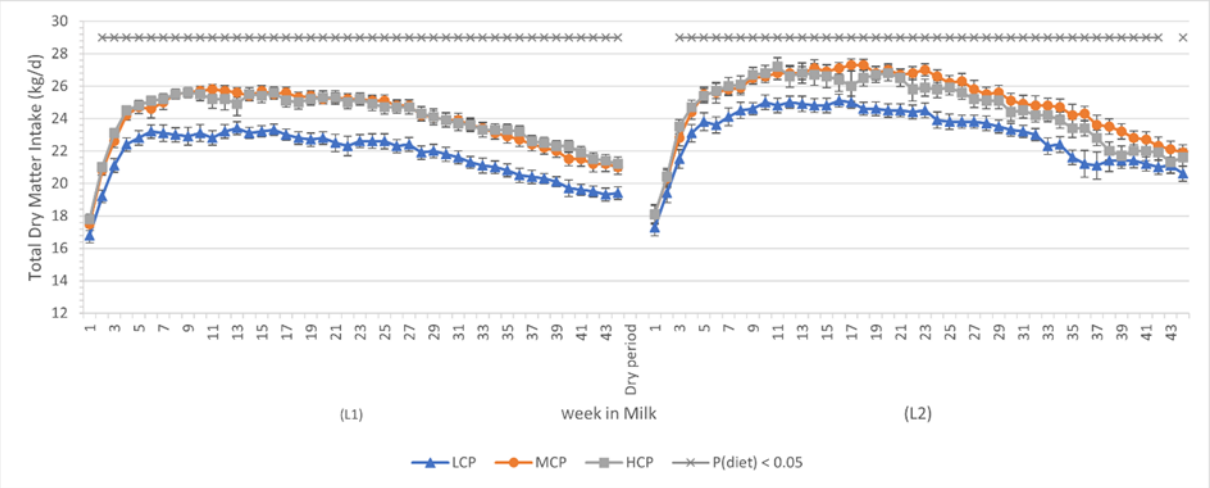
Table 13.4.2. Least square differences (LSD) of animal performance during the second 44-wk lactation of the experiment.

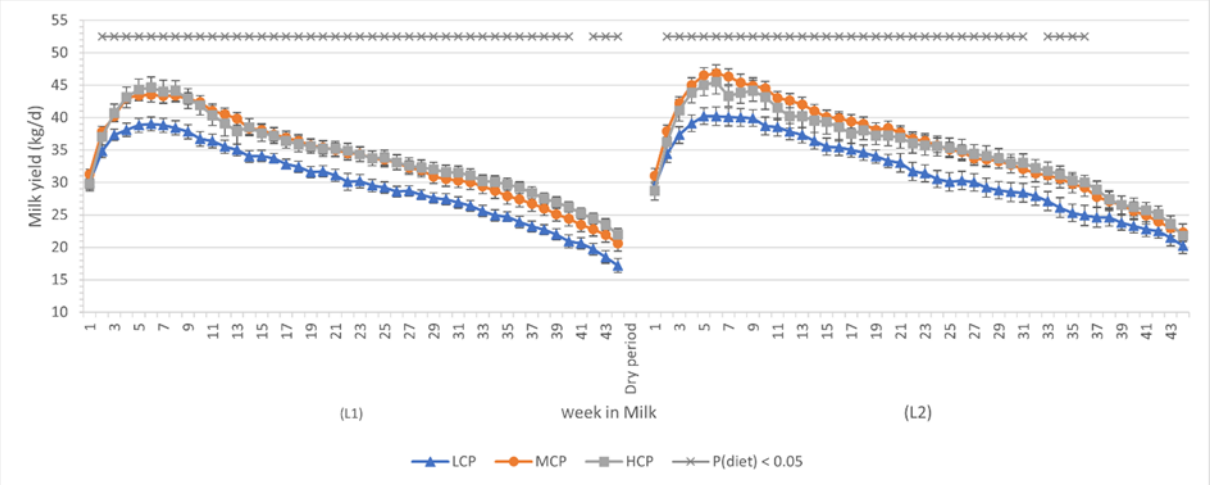
		Diet				*P*-value	LSD			
		LCP	MCP	HCP	SEM	Diet	Average	LCP-MCP	LCP-HCP	MCP-HCP
CP	g/kg DM	133[c]	144[b]	156[a]	0.2	<0.01	1.2	1.2	1.2	1.2
DMI total	kg/d	23.1[b]	25.1[a]	24.6[a]	0.06	<0.01	1.16	1.16	1.19	1.14
DMI PMR	kg/d	15.2[b]	17.1[a]	17.0[a]	0.05	<0.01	1.07	1.07	1.09	1.05
DMI concentrates	kg/d	7.9	7.9	7.6	0.04	0.06				
Milk yield	kg/d	31.4[b]	35.7[a]	35.2[ab]	0.17	0.03	3.42	3.41	3.49	3.36
Fat	g/100g milk	4.62	4.59	4.64	0.012	0.88				
Protein	g/100g milk	3.57	3.57	3.60	0.007	0.84				
Lactose	g/100g milk	4.54	4.56	4.57	0.004	0.85				
Urea	mg/dL	10.9[c]	14.1[b]	19.3[a]	0.11	<0.01	2.03	2.03	2.08	2.00
SCC	× 1,000 cells/ml	523	336	291	17.1	0.31				
SCC(log10)		2.12	2.05	1.95	0.012	0.52				
FPCM yield	kg/d	33.8[b]	38.3[a]	38.0[a]	0.17	0.02	3.40	3.39	3.47	3.34
Fat yield	kg/d	1.430[b]	1.617[a]	1.604[ab]	0.0074	0.03	0.152	0.151	0.155	0.149
Protein yield	kg/d	1.106[b]	1.251[a]	1.251[a]	0.0050	0.01	0.105	0.105	0.108	0.104
N intake	g/d	492[c]	577[b]	614[a]	1.8	<0.01	24.6	24.2	24.8	24.9
N excretion in milk	g/d	173[b]	196a	196[a]	0.8	0.01	16.5	16.5	16.9	16.2
N efficiency		0.357[a]	0.344[ab]	0.321[b]	0.0013	<0.01	0.021	0.021	0.021	0.020

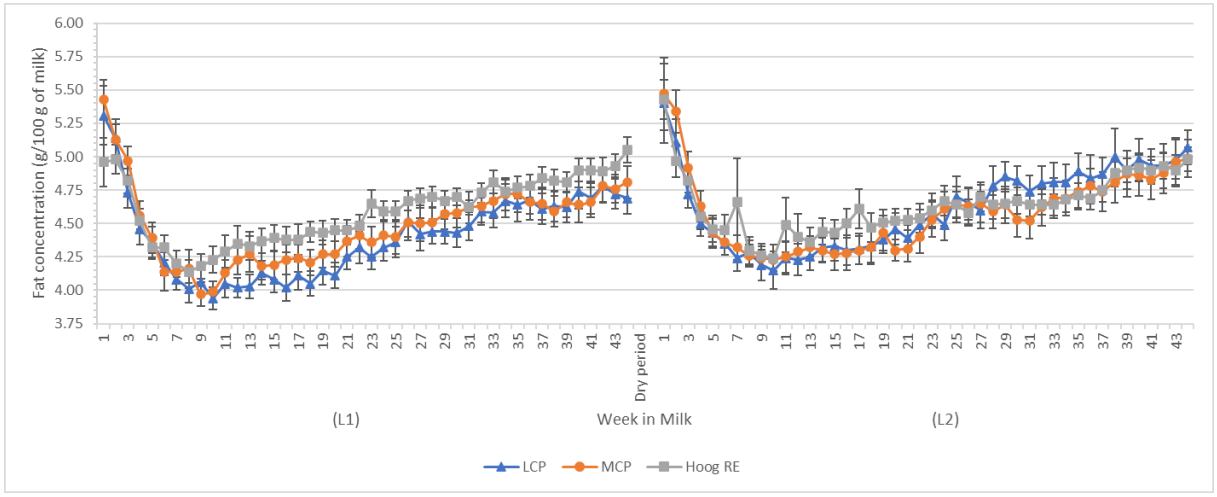
13.5 Appendix 5: Additional graphs

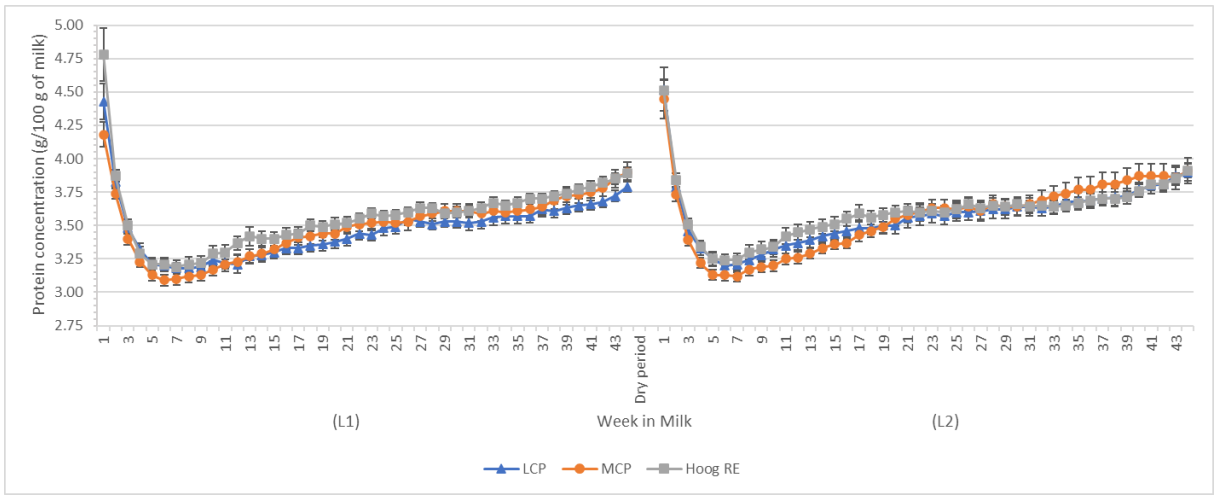
13.5.1 Feed intake and milk production variables during both lactation 1 and 2 (L1 and L2)

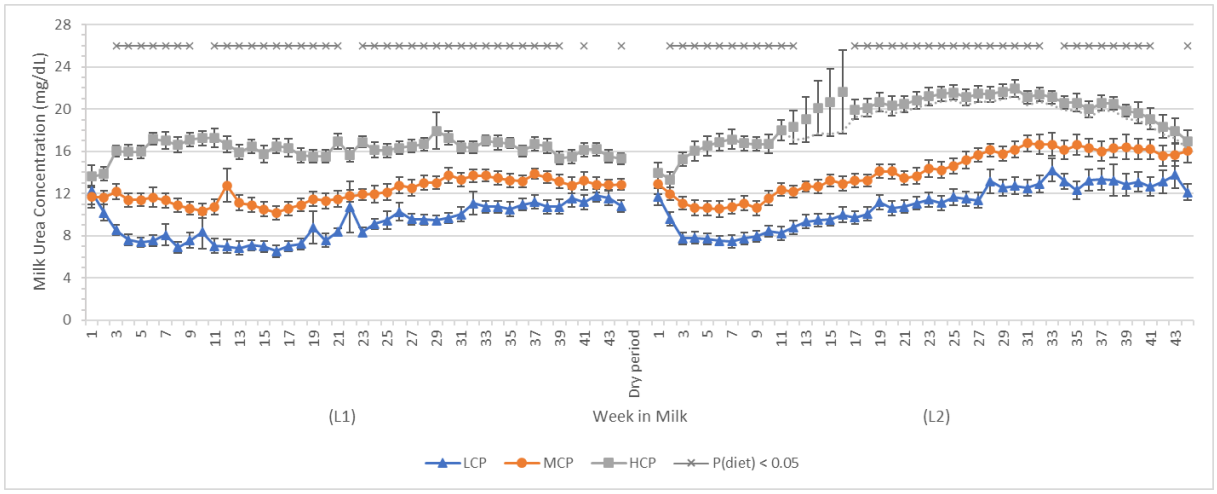


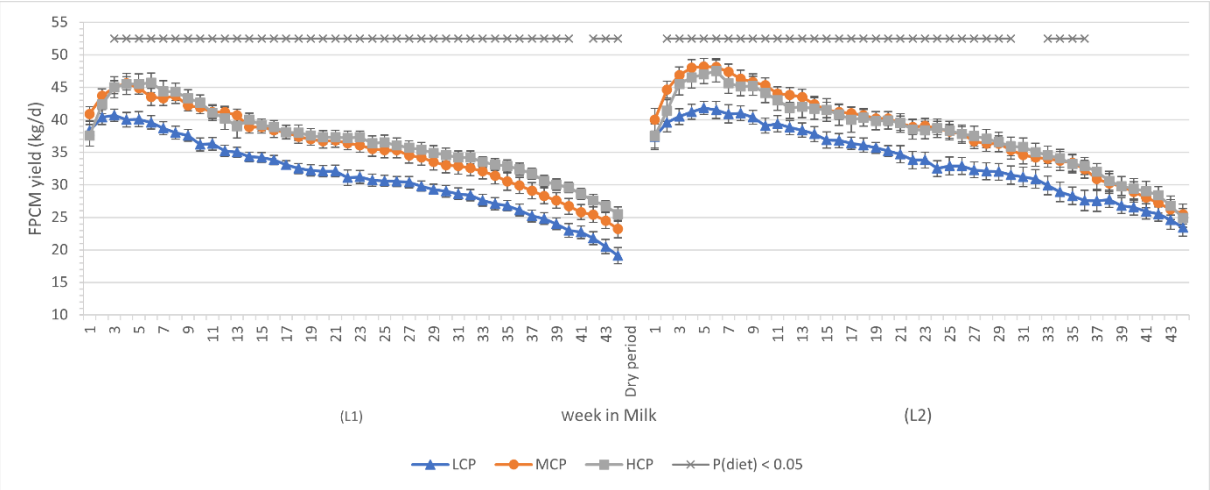


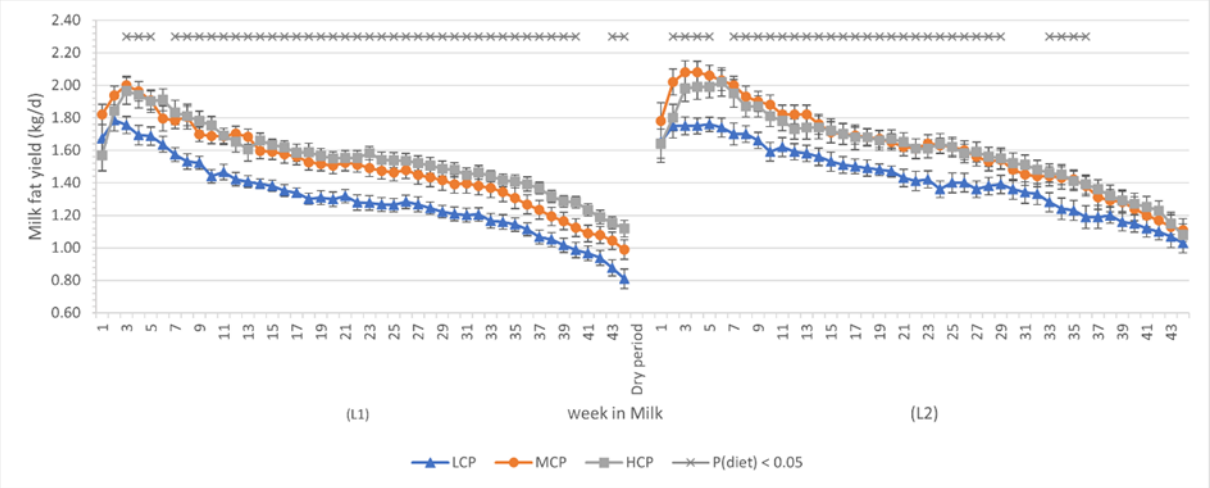


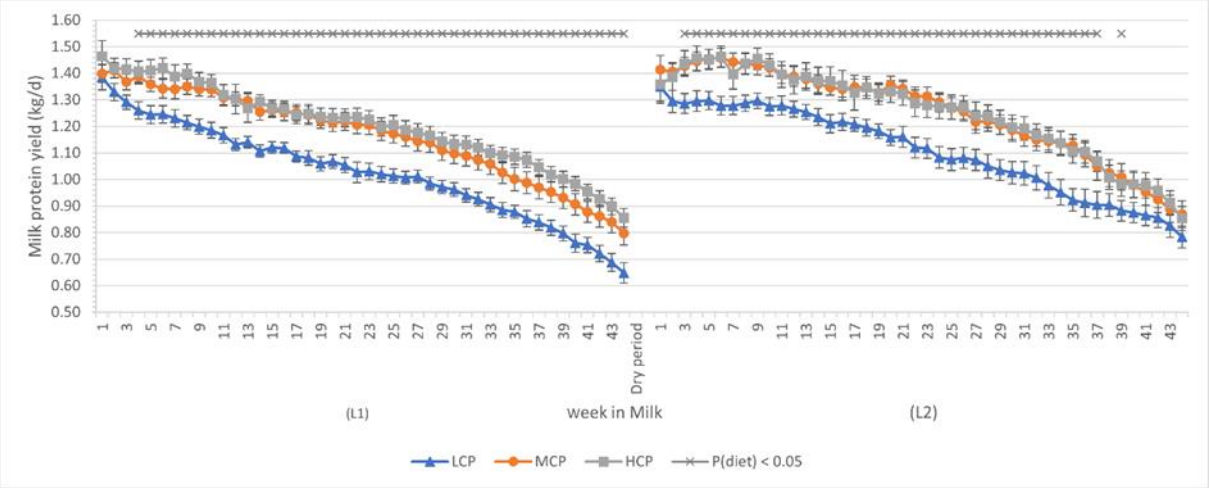

Fat concentration (g/100 g of milk)
6.00
5.75
5.50
5.25
5.00
4.75
4.50
4.25
4.00
3.75
Dry period
(L1)
Week in Milk
(L2)
LCP
MCP
Hoog RE

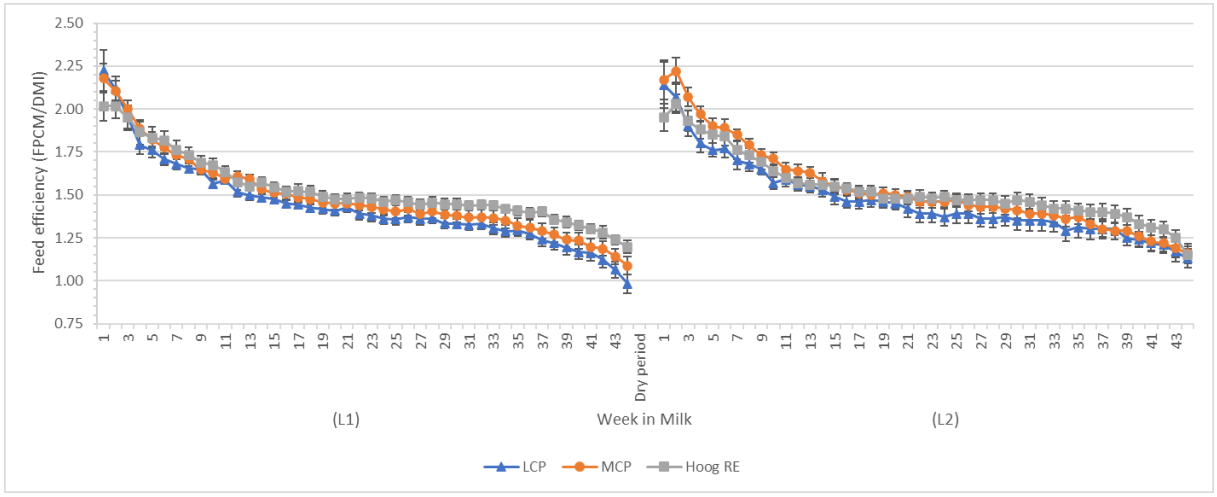

Protein concentration (g/100 g of milk)
5.00
4.75
4.50
4.25
4.00
3.75
3.50
3.25
3.00
2.75
Dry period
(L1)
Week in Milk
(L2)
LCP
MCP
Hoog RE

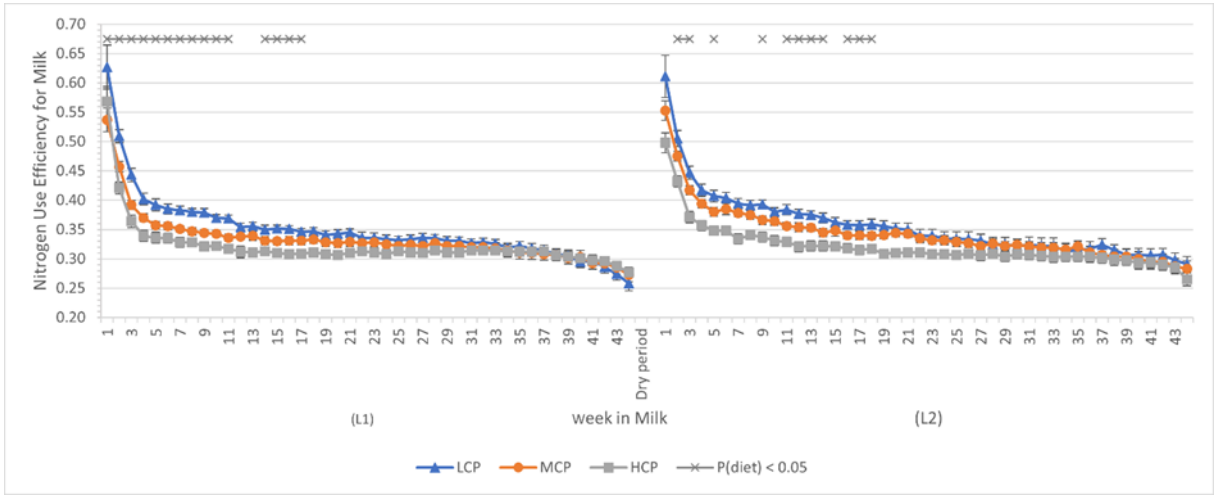

Milk Urea Concentration (mg/dL)
28
24
20
16
12
8
4
0
Dry period
(L1)
Week in Milk
(L2)
LCP
MCP
HCP
P(diet) < 0.05

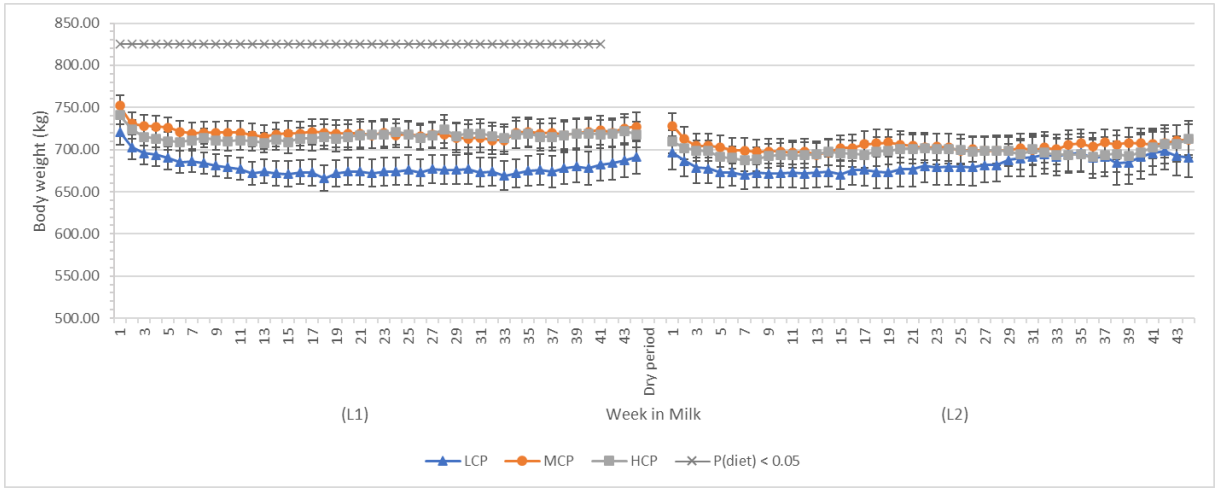

FPCM yield (kg/d)
55
50
45
40
35
30
25
20
15
10
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43
Dry period
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43
(L1)
week in Milk
(L2)
LCP
MCP
HCP
P(diet) < 0.05

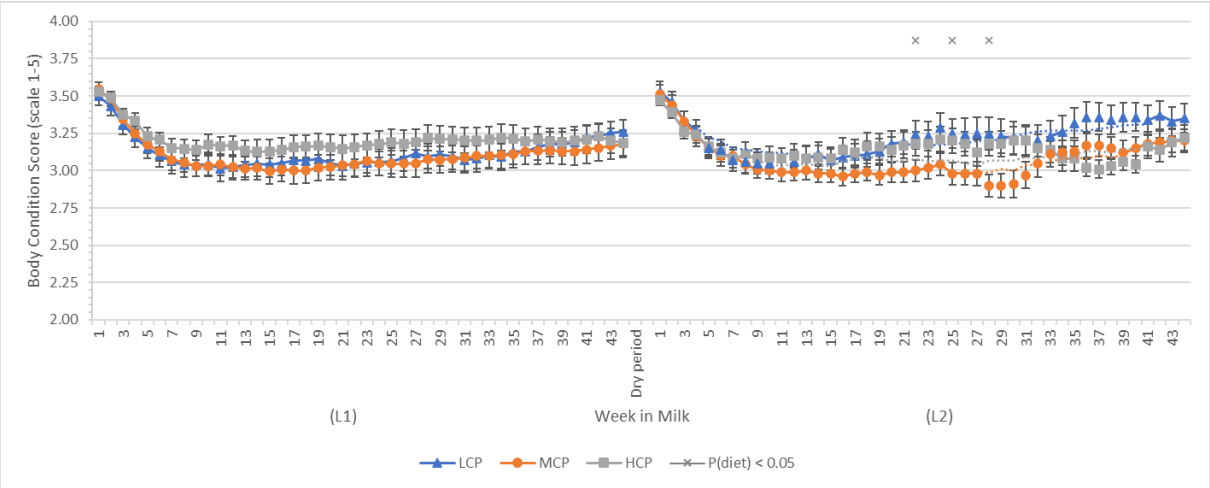

Milk fat yield (kg/d)
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43
Dry period
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43
(L1)
week in Milk
(L2)
LCP
MCP
HCP
P(diet) < 0.05


Milk protein yield (kg/d)
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43
Dry period
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43
(L1)
week in Milk
(L2)
LCP
MCP
HCP
P(diet) < 0.05

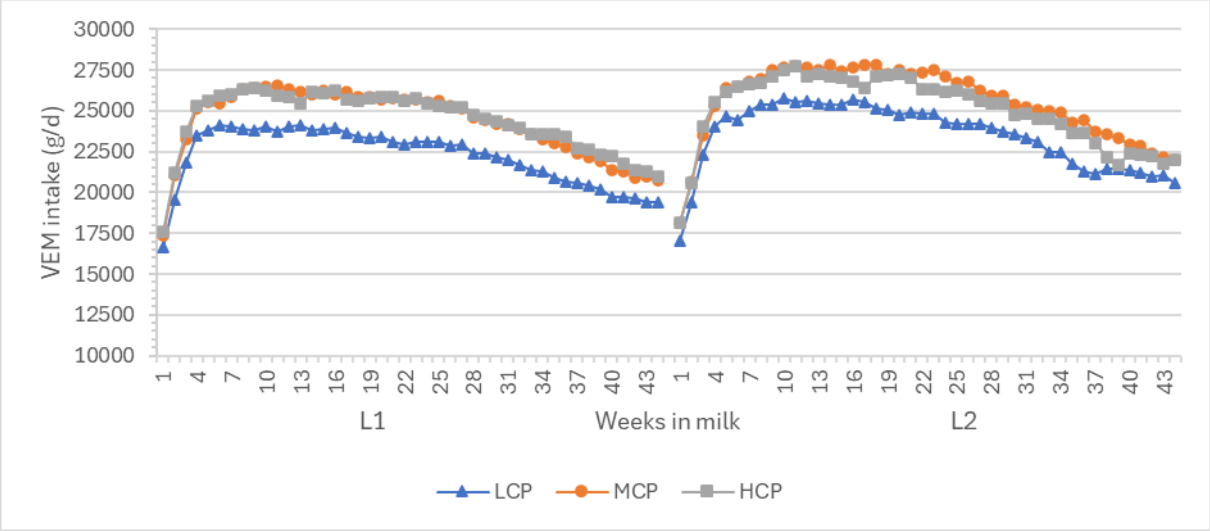

Feed efficiency (FPCM/DMI)
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
Dry period
(L1)
Week in Milk
(L2)
LCP
MCP
Hoog RE

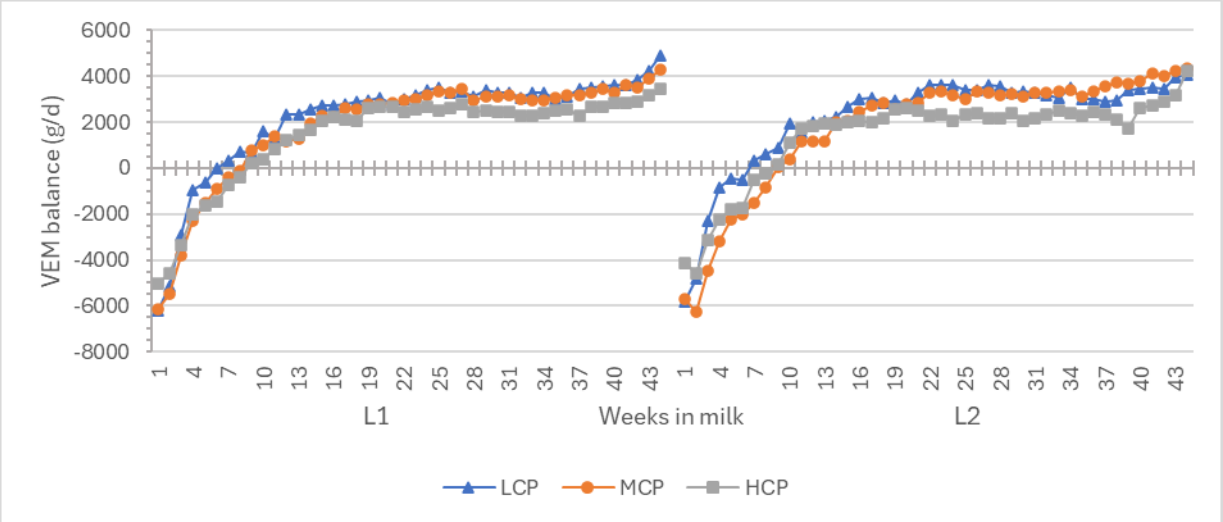

Nitrogen Use Efficiency for Milk
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
Dry period
(L1)
week in Milk
(L2)
LCP
MCP
HCP
P(diet) < 0.05

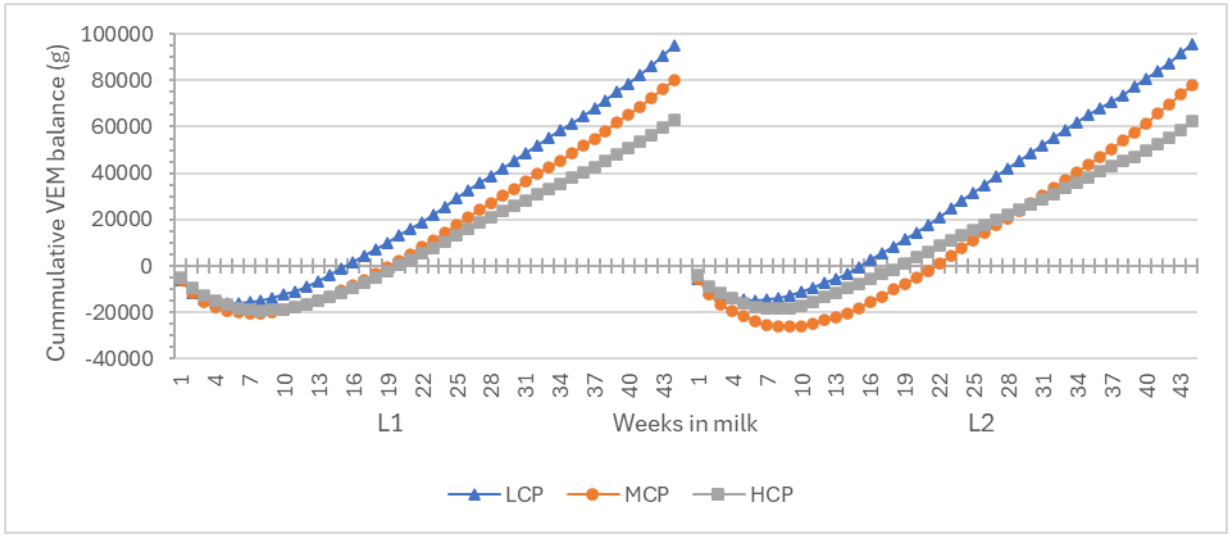

Body weight (kg)
850.00
800.00
750.00
700.00
650.00
600.00
550.00
500.00
Dry period
(L1)
Week in Milk
(L2)
LCP
MCP
HCP
P(diet) < 0.05

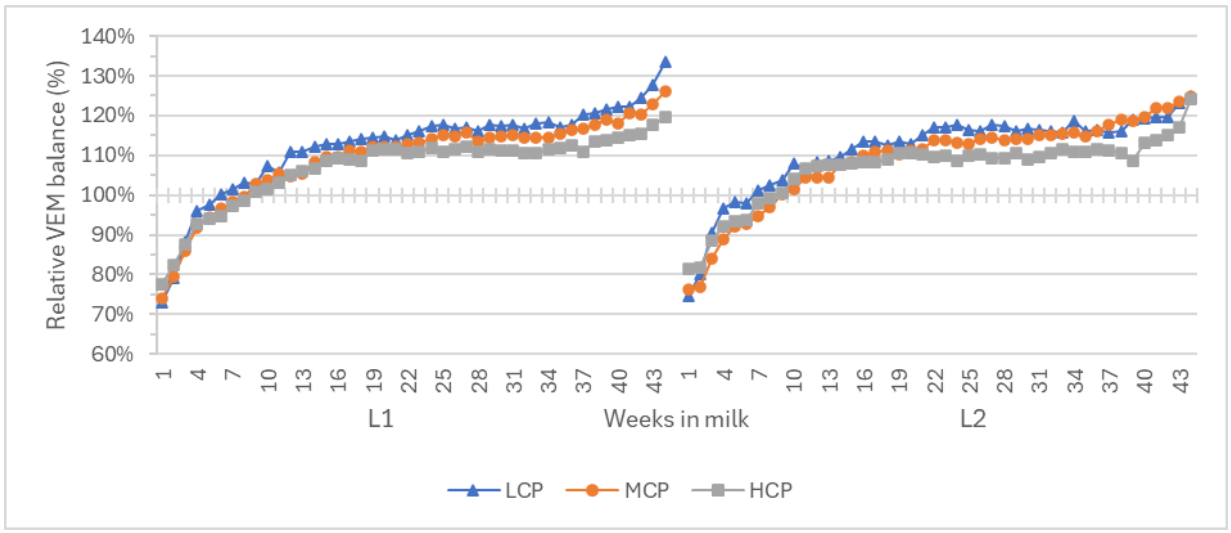


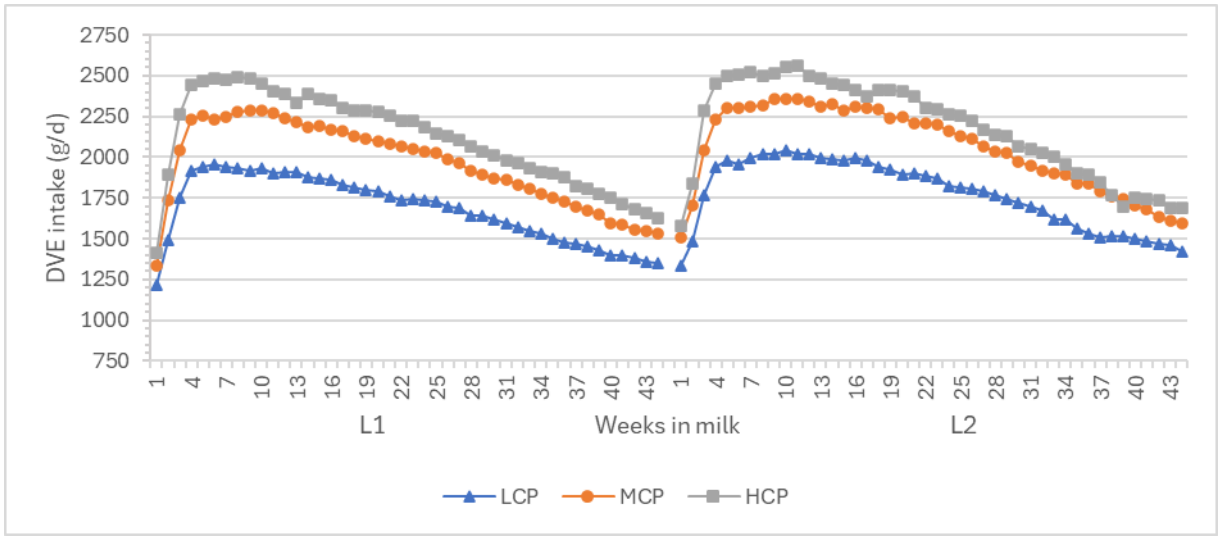
13.5.2 VEM and DVE balance (intake / calculated requirements) for lactation 1 (L1) and 2 (L2). Calculated needs based on CVB Tabellenboekje (2016)

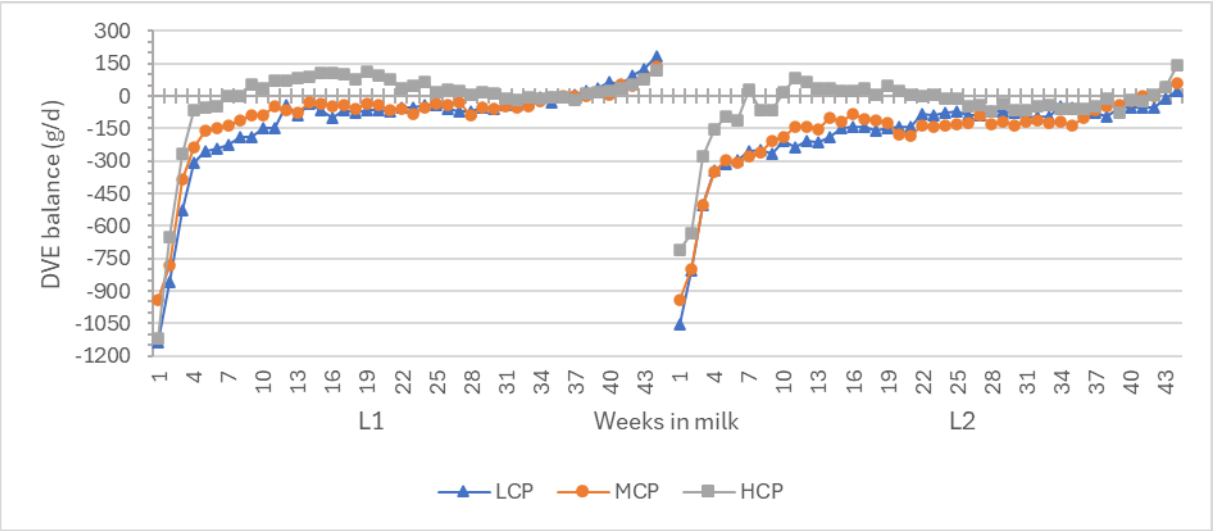


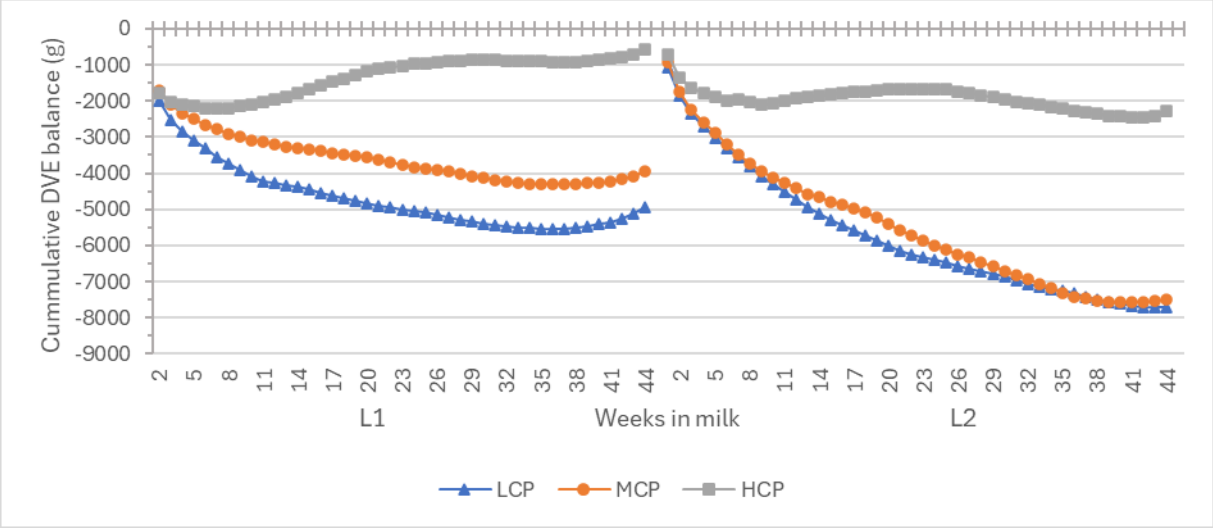


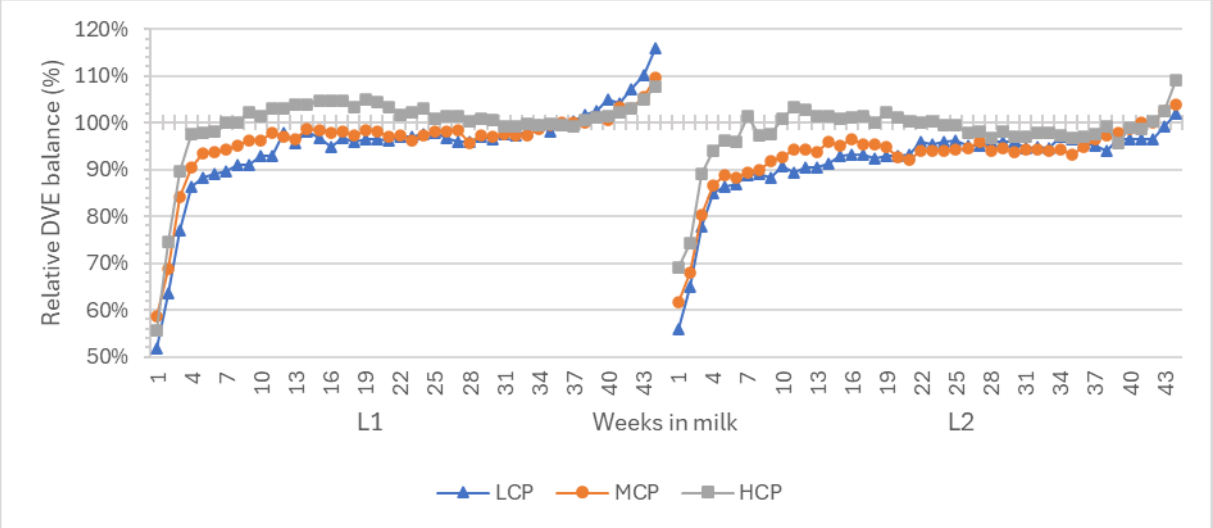

Cummulative VEM balance (g)
100000
80000
60000
40000
20000
0
-20000
-40000
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 1 4 7 10 13 16 19 22 25 28 31 34 37 40 43
L1
Weeks in milk
L2
LCP
MCP
HCP

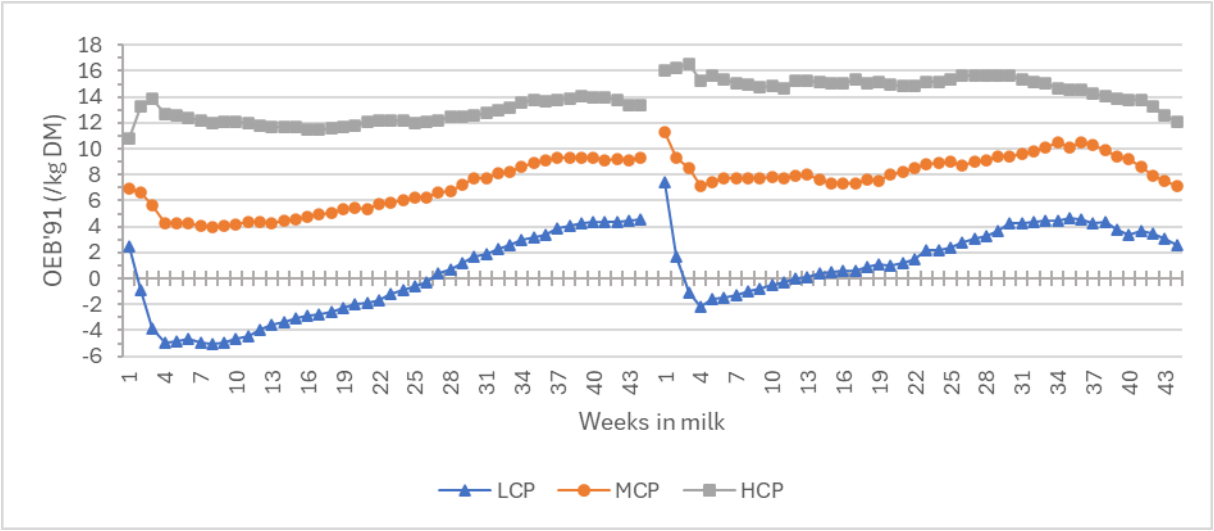

Relative VEM balance (%)
140%
130%
120%
110%
100%
90%
80%
70%
60%
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 1 4 7 10 13 16 19 22 25 28 31 34 37 40 43
L1
Weeks in milk
L2
LCP
MCP
HCP


DVE intake (g/d)
2750
2500
2250
2000
1750
1500
1250
1000
750
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 1 4 7 10 13 16 19 22 25 28 31 34 37 40 43
L1
Weeks in milk
L2
LCP
MCP
HCP


DVE balance (g/d)
300
150
0
-150
-300
-450
-600
-750
-900
-1050
-1200
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43
L1
Weeks in milk
L2
LCP
MCP
HCP


Cummulative DVE balance (g)
0
-1000
-2000
-3000
-4000
-5000
-6000
-7000
-8000
-9000
2 5 8 11 14 17 20 23 26 29 32 35 38 41 44
2 5 8 11 14 17 20 23 26 29 32 35 38 41 44
L1
Weeks in milk
L2
LCP
MCP
HCP


Relative DVE balance (%)
120%
110%
100%
90%
80%
70%
60%
50%
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43
L1
Weeks in milk
L2
LCP
MCP
HCP


OEB'91 (/kg DM)
18
16
14
12
10
8
6
4
2
0
-2
-4
-6
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 1 4 7 10 13 16 19 22 25 28 31 34 37 40 43
Weeks in milk
LCP
MCP
HCP

13.6 Appendix 6: Pregnancy

Table 13.6.1. Performance during first 33 weeks of pregnancy of lactating cows fed LCP, MCP, and HCP during two consecutive lactations (L1 and L2).

		Diet (CP)		
		LCP	MCP	HCP
Pregnancy 1				
CP	g/kg DM	130	139	147
DMI total	kg/d	21.0	22.9	22.9
PMR	kg/d	14.9	16.9	17.1
Concentrates	kg/d	6.1	6.0	5.8
Milk yield	kg/d	24.1	26.5	27.6
Fat	g/100 g milk	4.58	4.70	4.83
Protein	g/100 g milk	3.63	3.75	3.78
Lactose	g/100 g milk	4.44	4.44	4.49
Urea	mg/dL	10.4	12.8	15.9
SCC		288	428	287
SCC.log		2.16	2.16	1.95
FPCM	kg/d	25.8	28.8	30.7
Fat yield	kg/d	1.081	1.214	1.312
Protein yield	kg/d	0.856	0.967	1.022
BW	kg	677	711	696
BCS		3.15	3.15	3.22
Pregnancy 2				
CP	g/kg DM	132	141	152
DMI	kg/d	21.8	24.2	24.3
PMR	kg/d	15.8	17.9	17.7
Concentrates	kg/d	6.0	6.3	6.6
Milk yield	kg/d	25.0	28.4	31.0
Fat	g/100 g milk	4.78	4.80	4.76
Protein	g/100 g milk	3.74	3.80	3.72
Lactose	g/100 g milk	4.43	4.47	4.53
Urea	mg/dL	12.4	15.3	18.7
SCC		613	414	191
SCC.log		2.33	2.16	1.97
FPCM	kg/d	27.5	31.3	34.2
Fat yield	kg/d	1.172	1.329	1.457
Protein yield	kg/d	0.915	1.054	1.137
BW	kg	691	716	707
BCS		3.33	3.19	3.17

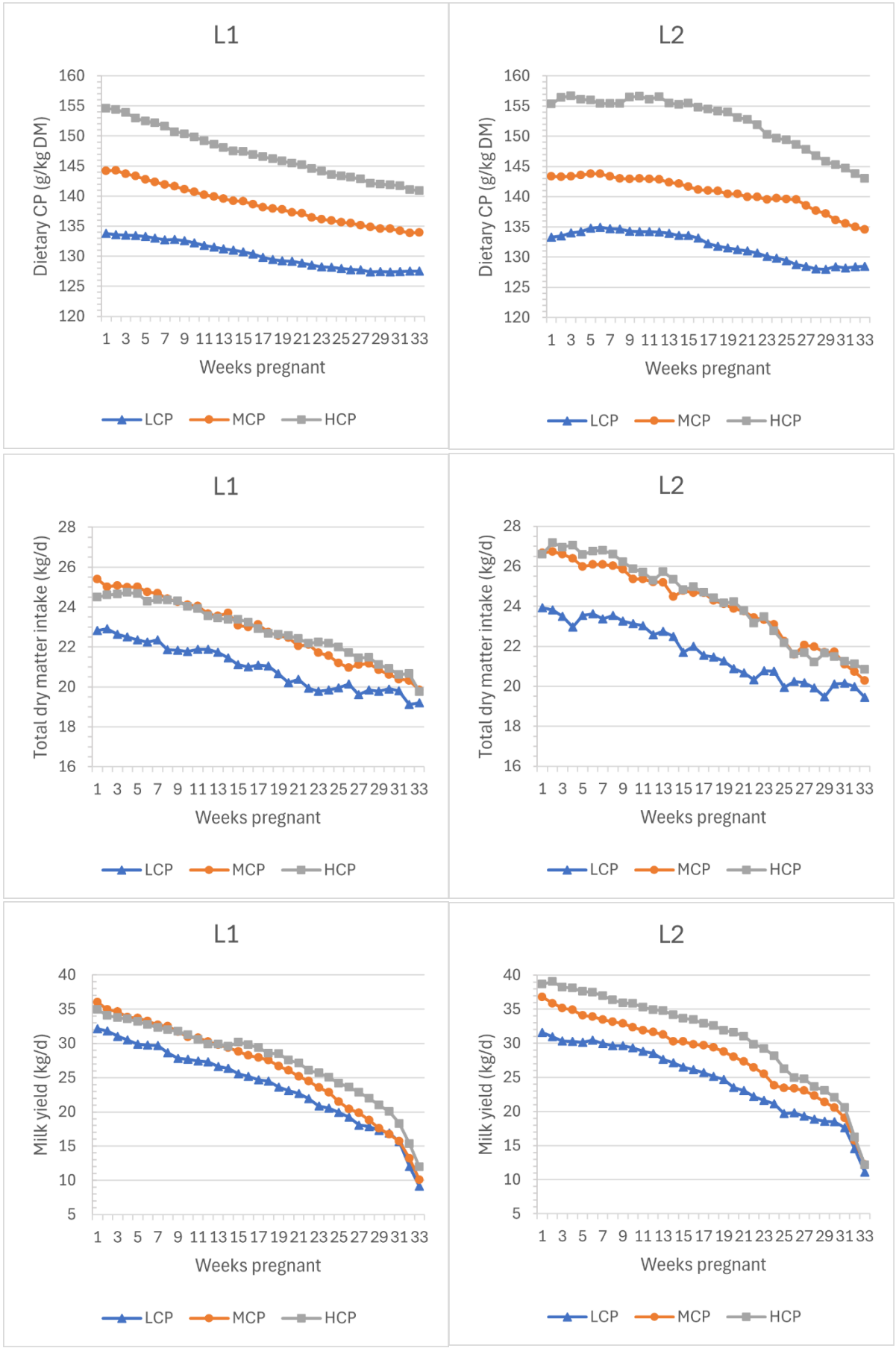


Figure 13.6.1. Performance during pregnancy with dietary crude protein level per week per lactation (Top), total dry matter intake (middle), and milk yield (bottom) for cows fed different levels of CP.