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Two grazing experiments were carried out to investigate the effects of 1. Compartmented continuous grazing 2. Strip grazing and 3. Protein supplementation strategy (Low and High rumen degradable protein (RDP) and high RDP plus additional metabolisable protein) on pasture intake, milk and milk solids yield in spring calving dairy cows. Neither grazing system nor protein supplementation strategy influenced pasture dry matter intake. However, high RDP resulted in higher milk yield and milk protein outputs. Additional high RDP plus additional metabolisable protein did not result in further improvement of milk performance. High RDP and high RDP plus additional metabolisable protein resulted in reduced nitrogen use efficiency. Despite similar diet compositions in both experiments, there were large differences in rumen NH_3 and apparent OMD between experiments, suggesting strong year to year effects in rumen fermentation and rumen digestion which were not reflected in the feeding values.

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Preface

In the Netherlands the growing interest in grazing dairy cows increases the need for developing more profitable grazing strategies. The profitability of grazing is related to pasture utilization and pasture intake per cow and therefor strategies aim to stimulate the intake of pasture grass in dairy cows. Pasture intake seems related to regulatory mechanisms in dairy cows. Cows are able to detect digestive and metabolic changes after feed intake, causing changes in diet selection. For example, cows might make feed choices based on a potential protein or energy imbalance. If this applies to grazing dairy cows this mechanism might be used to motivate dairy cows to increase pasture intake.

This hypothesis was tested for a common Dutch part-time grazing system with supplemental feeding indoors, where cows graze during day time and are kept indoors during night time. Two grazing experiments (Exp 1 in 2016 and Exp 2 in 2017) were carried out by the Feed4Foodure (F4F) consortium. The F4F experiments were embedded in a larger framework of grazing experiments of the Amazing Grazing project. Amazing Grazing is an initiative to promote grazing in the Netherlands and to address the major constraints of grazing in intensive dairy farming in the Netherlands (Schils et al., 2018). The Amazing Grazing experiments were having multiple objectives. These objectives involved:

- comparison of two grassland management systems (i.e. compartmented continuous grazing (C) vs. strip-grazing (S) on pasture dry matter yield and pasture utilization,
- study the complex relationships between animal behaviour, pasture allowance, pasture intake (i.e. the interaction of the animal and grass)
- study the effects of the level of dietary rumen degradable protein (RDP) (2016 and 2017) and level of intestinal degradable protein (2017) supplementation on grazing intake and milk production of dairy cows grazing during day-time and housed during the night-time.

This report concerns the Feed4Foodure grazing experiments and concern the third objective, the effect of rumen degradable protein (RDP) on grazing intake and milk production.

Summary

Literature shows that ruminants are able to balance their protein intake to meet their requirements. It would be interesting to know if this also applies to grazing dairy cattle and if it could be used to increase pasture intake. Our question was: Can we motivate grazing dairy cows to increase their intake of protein-rich grass by supplementing them with low protein concentrates? Two experiments were carried out in 2016 and 2017 (Exp 1 and Exp 2 respectively). In both experiments, sixty Holstein-Friesian dairy cows were allocated to two contrasting grazing systems: 30 cows in compartmented continuous grazing (C) versus 30 cows in strip grazing (S).

Design
In Exp 1, a 2×2 factorial design was used, in which the cows within grazing system C and S, were assigned to two levels of protein supplementation Low (L) vs High (H). Treatment L and H received 5.5 kg DM concentrate cow/d, which were different in rumen degradable protein balance (OEB) (approx. -50 vs +50 g OEB/kg DM, respectively), but were equal in intestinal digestible protein (DVE, 105 g DVE/kg) and net energy content (approx. 1130 VEM/kg DM).
In Exp 2, a 2×3 factorial design was used, in which the cows within grazing system C and S were assigned to three dietary protein treatments. Two dietary protein treatments were identical replicates of the treatments L and H in Exp 1. A third additional dietary protein treatment (HH) consisted of a treatment with a similar level of rumen degradable protein (approx. + 50 g OEB/kg DM) and net energy as treatment H but with an increased level of intestinal digestible protein (DVE, 150 g DVE/kg). In both experiments, pasture composition, supplementary feed intake, milk yield and composition was recorded during the whole grazing season. During three measurement periods, individual pasture dry matter intake (PDMI), apparent digestibility was measured using the n-alkane technique. Rumen pH, VFA and NH3 were measured during 2 (July, September) and 3 measurement periods (May, July, September), in Exp 1 and Exp 2, respectively.

Results
In both experiments there was no effect of grazing system on accumulated milk and milk constituent yield. In Exp 1 and Exp 2, the cows on protein treatment H, had a higher accumulated milk and milk protein yield than the cows on treatment L ($p < 0.05$). In Exp 1, the cows on protein treatment H, had a higher accumulated FPCM yield than the cows on treatment L ($p< 0.05$), whereas in Exp 2 there was a tendency for higher accumulated FPCM yield ($0.05 < p < 0.1$). In Exp 2, there were no differences in milk and milk constituent yield between H and HH.
In both experiments there was no effect of grazing treatment and dietary protein treatment on PDMI. In Exp 1 total DMI was significantly lower with treatment L compared to H. This was due to a reduced voluntary intake of maize silage. The reduced total DMI and nutrient intake explains the reduced milk and protein yield in treatment L. In Exp 2 there were no effects of dietary protein treatment on total DMI. Increased levels of dietary protein resulted in a decline of the nitrogen use efficiency (N in milk/N intake). Approximately, 20% of the extra nitrogen intake was converted into milk protein.
In both experiments rumen pH was not different between treatments, nor was rumen VFA concentration. However, In Exp 1, the non-glucogenic to glucogenic ratio was lower for the H treatments than for treatment L. In Exp 1, the concentrations of rumen NH_3 and the concentrations of milk urea indicated that the level of rumen degradable protein was limiting for microbial protein synthesis for the L and H treatment. The levels of rumen NH_3 were much lower in Exp 1 than in Exp 2, despite similar intakes of net energy, nitrogen and rumen degradable protein in Exp 1 and Exp 2. In Exp 2, the levels of rumen NH_3 suggest that they were not or at least less limiting for microbial protein synthesis. There were no effects of grazing and protein treatment on the calculated apparent organic matter digestibility (OMD). However, OMD was lower in Exp 1 (approx. 0.63) than in Exp 2 (approx. 0.72%). The low levels of rumen NH3 concentrations for treatments L and H, together with low OMD in Exp 1 suggest large differences in diet digestibility between years.

Conclusions

There were no significant effects of grazing system on pasture dry matter intake. Also feeding low protein supplements to dairy cows did not result in increased pasture dry matter intake. However, feeding supplements with high rumen degradable protein resulted in increased milk and milk protein yields with a lower nitrogen use efficiency. Despite similar diet compositions, there were large differences in rumen ammonia concentration and apparent organic matter digestibility between experiments, suggesting strong year to year effects rumen fermentation and rumen digestion which were not reflected in the feeding values.

1 Introduction

In order to make grazing more profitable, pasture intake and utilization per cow should be increased (van den Pol - van Dasselaar et al., 2013). Therefore, it is of interest to develop strategies which could stimulate the intake of pasture grass in dairy cows. Pasture intake might respond to regulatory mechanisms in dairy cows. Ruminants are able to detect internal digestive and metabolic changes after feed intake, causing changes in diet selection (Kyriazakis et al., 1999). For example, ruminants might make feed choices based on a potential protein or energy imbalance (Scott and Provenza, 2000). Heublein et al. (2017) found that cows supplemented with low protein feeds selected for high protein plants. According to the study of (Tolkamp et al., 1998), cows select their diets based on the rumen degradable protein (RDP) content of the feed, avoiding both a deficiency and an excess of RDP by diet choice. If this also applies to grazing dairy cows this mechanism might be used to motivate dairy cows to increase pasture intake. This hypothesis was tested for a common Dutch part-time grazing system with supplemental feeding indoors, where cows graze during day time and are kept indoors during night time. The idea was that a low RDP intake during night time would stimulate cows to increase their RDP intake during day time by increasing pasture intake. If this strategy is successful it would be applicable to a majority of dairy farms in the Netherlands.

In 2016 and 2017 two experiments (Exp1 and Exp2, respectively) were carried out with lactating dairy cows to study the effect of a low level of RDP supplementation during night time on pasture dry matter intake during day time, and simultaneously on feed digestibility, rumen fermentation characteristics and milk production. The experiments comprised two RDP treatments (two levels of RDP supplementation) Both experiments were not an exact repetition of the same trial. Based on results of Exp1, not only the response to RDP allowance was tested in Exp2 but also the allowance of intestinal degradable protein. In Exp2 the RDP treatments of Exp1 were repeated, but the trial was extended with an extra treatment offering additional intestinal degradable protein.

Both experiments were replicated under two contrasting grazing systems: strip-grazing and compartmented continuous grazing. In the Netherlands, rotational grazing and continuous grazing are the most common grazing systems. However, the drawbacks of strip-grazing are larger investments in terms of labour required for moving fences, water supply and grassland planning. In controlled experiments, continuous grazing resulted a definite reduction in labour input of 50% and a reduction in investment and maintenance costs for fencing and water supply compared to intensive rotational grazing (Ernst et al., 1980). However, continuous grazing requires more grazing management skills to maintain an equilibrium between pasture growth and pasture intake (Ernst et al., 1980).

A recent farmers invention is compartmented continuous grazing (C). With C the grazed area is compartmented in 5 to 8 paddocks. The paddocks are grazed rotationally in which each paddock is grazed for one day, then the cows are moved to the next paddock and so on. Compartmentation of the grazed area in a continuous grazing system has the following advantages: 1) reduced losses of grass and damage to the sward through poaching around entrance gates and watering points; 2) the smaller area reduces the walking distances of the cows and thereby reducing the loss of pasture through trading and trampling; 3) allows closure of paddocks for cutting (silage cut or topping) or adding paddocks in order to match grass growth with grass intake; 4) reduced selective grazing; 5) a more equal distribution of dung and urine spots, which may improve nutrient cycling; 6) reduced time to fetch the cows. Thus, compartmentation may improve pasture utilization compared with conventional continuous grazing.

Currently there a lack of knowledge regarding technical data regarding grassland production and animal performance in a compartmented continuous grazing system. Such data are needed for a to evaluate compartmented continuous grazing and to assess whether is viable option for intensive dairy farming systems. The objectives of the present study were 1) investigate the effects grazing system and protein supplementation strategy on pasture intake, milk and milk solids yield in spring calving dairy cows. Therefore, two experiments were carried out to investigate the effects of either a low or high level of RDP and additional intestinal degradable protein on pasture intake and milk production under compartmented continuous grazing and strip grazing management.

2 Material and methods

2.1 Experimental site

The experiments were carried out at the research farm Dairy Campus of Wageningen University and Research, Goutum, Friesland, the Netherlands (53.175° N, 5.762° E). The soil type was a heavy marine clay soil with a 40% elutriable fraction.

2.2 Experimental design

2.2.1 Design

Two grazing experiments were carried out in a continuous randomized block design:

- Experiment 1 (Exp1) from April 25 until October 27 in 2016
- Experiment 2 (Exp2) from April 24 until September 3 in 2017.

Both Exp1 and Exp2 consisted of 3 repeated measurements in 3 consecutive measurement periods (P1, P2 and P3) in which the effect was studied of feeding different levels of rumen degradable protein (RDP) on dry matter intake (DMI) from both pasture and supplemental roughage and on production performance (see Figure 1).

Figure 1 *Experimental design and codes used*

Grazing system	Code	Protein level			Experimental year	Code	Treatment Code
		RDP	DVE	Code			
Experiment 1							
Compartmented Continuous Grazing	C	Low	Regular	L	2016	1	LC1
		High	Regular	H	2016	1	HC1
Strip Grazing	S	Low	Regular	L	2016	1	LS1
		High	Regular	H	2016	1	HS1
Experiment 2							
Compartmented Continuous Grazing	C	Low	Regular	L	2017	2	LC2
		High	regular	H	2017	2	HC2
		High	High	HH	2017	2	HHC2
Strip Grazing	S	Low	Regular	L	2017	2	LS2
		High	Regular	H	2017	2	HS2
		High	High	HH	2017	2	HHS2

2.2.2 Animals

In both experiments, 60 cows were selected from the 550 cows in the Holstein Friesian dairy herd of research farm Dairy Campus. The use of animals and experimental handling was approved by the Animal Experiments Committee of Wageningen University and Research and the experiments were carried out under the Dutch law on Animal Experimentation, licence no. AVD4010002016468 issued by the Central Committee on Animal Experiments.
In Exp1 and Exp2 the cows were paired in blocks of four cows and six cows, respectively.
Blocks of cows were formed based on equality in parity (first, second and higher parity number), days in milk, milk constituent yield, and fat and protein corrected milk yield (FPCM, CVB 2012) of the animals. Within blocks, cows were randomly allocated to one of the experimental treatments. At the start of the experiments the cows were on average (mean ± SD):

Exp1: 53 ± 25 days in milk (DIM), 2.5 ± 1.2 of lactation number and their average yields of milk, fat, protein, lactose and FPCM were 38.4 ± 7.5 kg/d, 1608 ± 368 g/d, 1206 ± 206 g/d, 1749 ± 344 g/d and 38.8 ± 7.8 kg/d, respectively.
Exp2: 81 ± 16 days in milk (DIM), 2.6 ± 1.4 of lactation number and their average yields of milk, fat, protein, lactose and FPCM were 35.5 ± 5.1 kg/d, 1513 ± 253 g/d, 1125 ± 160 g/d, 1609 ± 227 g/d and 36.2 ± 5.2 kg/d, respectively.

In both experiments, the cows were milked at 0500 h and 1630 h in a 40-cow rotary milking parlour with automatic cow identification and milk weight recording (GEA Group Düsseldorf, Germany), and they were on pasture from 0800 h to 1600 h. In between, the cows were housed in a free stall with cubicles. Water was freely available during the whole day, indoors and at pasture as well. The research unit was equipped with 4 transponder controlled automatic concentrate dispensers and a roughage intake control (RIC) system that consisted of 32 transponder controlled weighing troughs with access gates (Hokofarm, Marknesse, Netherlands). During the pre-treatment periods of both experiments, the cows were allowed to graze as single herds during the daytime on pasture. Indoors, the cows were supplemented with 5.4 kg DM/d and were fed a roughage mixture composed of grass silage and maize silage.

2.2.3 Pasture and grazing management

2.2.3.1 Pastures

In both experiment Exp1 and Exp2 the same pasture plots were used. The pasture plots consisted of 8 hectares (ha) of adjacent fields. The botanical composition of the grazing plots (predominantly perennial ryegrass) was determined in early March of both experimental years by visual assessment. The plots had a similar botanical composition consisting of perennial ryegrass (Lolium perenne, 73.6%), timothy (Phleum pratense, 13.8%), smooth meadow-grass (Poa pratensis, 7.6%) and annual meadow grass (Poa annua, 4.6%), miscellaneous herbs and grasses (0.4%), and total ground cover was 95%.
The 8-ha pasture was divided in 4 plots of 2 ha. Two plots were assigned to the strip-grazing treatment (S) and two plots to compartmented continuous grazing treatment (C).

2.2.3.2 Grazing management strip-grazing

In treatment S two plots of 2 ha were divided in 31 strips of 645 m^2. Daily, the cows were given access to a new strip, with access to the strip of the previous day. So, on each plot the cows were always grazing on to 2 adjacent strips (1290 m^2; 86 m^2/cow). For grazing treatment S, the weekly sward surface height (SSH) measurements were used as inputs for a so called 'grazing wedge' grazing planning tool (Eastes and van Bysterveldt, 2009). The grazing was planned for a rotation of 31 days (number of strips) and updated weekly.
When the grazing planning tool indicated an excess of pasture (grazing rotation shorter than 31 days), then some strips were taken out of the grazing rotation and cut for silage making. Strips used for cutting, were those with the poorest sward structure (highest heterogeneity, patchiness and proportion of heading tillers), highest grass cover (kg DM/ha) and days of regrowth. Strips were also rejected for grazing and cut for silage making when the pre-grazing SSH indicated a pre-grazing pasture mass (preGPM) higher than 2500 kg DM/ha above 5 cm sample height (gross allowance 21 kg DM/cow/d). The mean post grazing SSH on the strips was maintained around 6 cm above ground level. The allowance of supplemental roughage was reduced when pasture utilization tended to decline as indicated by an increase of the rejected area and the mean post-grazing SSH.

2.2.3.3 Management compartmented continuous grazing

In treatment C two plots of 2 ha were divided in 6 compartments of 3333 m^2 (222 m^2/cow). These compartments were grazed during one day (one AM to PM milking interval). The following day the cows were moved to the next compartment and so on. In this way the cows grazed the same pasture continuously during the whole grazing season at a SSH that was maintained throughout the grazing season within a range of 7 to 12 cm above ground level. The pasture mass (PM; kg DM/ha), was managed such that the total pasture accumulation (kg DM/ha/d) equalled the total quantity of PM consumed by the grazing animals.

2.2.3.4 Fertilization with manure and nitrogen fertilizer

In both experiments, the nitrogen fertilization was planned to be 345 kg N/ha/year in compliance with the Dutch environmental legislation (www.rvo.nl).
In Exp1, all grazing plots were fertilized on February 16, 2016, with approximately 40 m^3 of cattle slurry providing 50 kg effective N/ha using a trialling shoe sod injector. Before the start of the grazing experiment, the plots were grazed from March 25 until April 24, 2016 with dairy cows in order to create growing-steps. On April 5, the compartments of treatment C and strips of treatment S were fertilized with 40 and 60 kg effective N/ha respectively using calcium ammonium nitrate with sulfate (CAN+S, 24% N, 15% SO_3).

In Exp2, all grazing plots were fertilized on February 16, 2017, with 40 m^3 of cattle slurry providing 60 kg effective N/ha using a trialling shoe sod injector. Previous to the start of experimental period, the plots were grazed from March 29 until May 1 with dairy cows in order to create growing-steps. On March 29 the compartments of treatment C and strips of treatment S were fertilized with 40 and 60 kg effective N/ha respectively using calcium ammonium nitrate with sulfate (CAN+S, 24% N, 15% SO3). In both experiments, during second and later N fertilizer application consisted of calcium ammonium nitrate (CAN, 27% N). The second and later applications of nitrogen fertilizer were as follows: the compartments of treatment C were fertilized every 3 or 4 weeks depending on the growth and weather conditions, whereas the strips of treatment S were fertilized within a week after each grazing or cutting event with 40 kg effective N/ha. More detailed information on pasture management and pasture fertilization is reported by Holshof (2019 in progress).

2.2.4 Experimental treatments

2.2.4.1 Experiment 1

In Exp1 the cows received two levels rumen degradable protein (RDP, L: low and H: high) and were under two grazing regimes (S: strip-grazing and C: compartmented continuous grazing) in a 2×2 arrangement: low rumen degradable protein – strip-grazing (LS1), low rumen degradable protein – compartmented continuous grazing (LC1), high rumen degradable protein – strip-grazing (HS1), and high rumen degradable protein – compartmented continuous grazing (HC1). Two grazing plots were managed according to grazing treatment S and grazing treatment C. Each grazing plot was grazed with 30 cows and these cows were allocated to either the dietary protein treatment L or H according to a randomized block design (i.e. 15 cows per dietary protein treatment).
The differences in RDP intake were created by supplementing the cows 5.4 kg DM/d of concentrates differing in rumen degradable protein balance (OEB; aimed at -50 vs. +50 OEB/kg DM), but similar in intestinal digestible protein (DVE; aimed at 105 DVE/kg DM) according to the DVE/OEB system of Tamminga et al. (1994) revised by Van Duinkerken et al. (2011). The dietary treatments resulted in a contrast in OEB intake of approximately 540 g OEB/d.
The ingredients, chemical composition and feeding values of the experimental concentrates are presented in Annex 1.1a and Annex 1.1b. A total of 60 cows was blocked into 4 separate groups of 15 cows with each group following either the LS1, LC1, HS1 or HC1 treatment, according to a randomized block design.

2.2.4.2 Experiment 2

In Exp2 the cows were subjected to two grazing regimes (S: strip-grazing and C: compartmented continuous grazing) and three dietary treatments (L, H, HH) in a 2×3 arrangement: low rumen degradable protein – strip-grazing (LS2), low rumen degradable protein – compartmented continuous grazing (LC2), high rumen degradable protein – strip-grazing (HS2), high rumen degradable protein – compartmented continuous grazing (HC2), high rumen degradable protein plus high intestinal digestible protein – strip-grazing (HHS2), and high rumen degradable protein plus high intestinal digestible protein – compartmented continuous grazing (HHC2).
The grazing treatments were identical to grazing treatments S and C in Exp1. The L2 and H2 diet treatments were similar to the treatments L1 and H1 in Exp1. A third treatment (HH2) consisted of 5.4 kg DM/d of concentrates with a similar OEB as treatment H in Exp1 and Exp2, but with an increased level of DVE (DVE; approximately 150 DVE/kg DM).
The ingredients, chemical composition and feeding values of the supplemented roughages, grass swards and experimental concentrates are presented in Annex 1. In Exp2 the cows LS2, LC2, HS2,

HC2, HHS2, and HHC2 were grazing in 4 separate groups. Two grazing plots were managed according to either the grazing treatment S or the grazing treatment C. Each grazing plot was grazed with 15 cows of which 5 cows were allocated to each dietary protein treatment (L, H and HH) according to a randomized block design.

2.3 Experimental diets

2.3.1 Concentrates

In both Exp1 and Exp2 allowance of all concentrates was 5.4 kg DM/cow/d. The concentrates were manufactured by Agrifirm, Apeldoorn, the Netherlands, the ingredients, chemical composition and feeding value of the concentrates is given in Annex 1.1a and Annex 1.1b for Exp1 and Exp2, respectively.

2.3.2 Supplementary roughage feeding

2.3.2.1 Level of supplementary roughage

The access time to pasture was restricted in both experiments, therefore maize silage was offered indoors as supplementary roughage to ensure that total DMI was not compromised. The minimum amount of supplementary roughage was set at 5 kg DM/cow/d in Exp1 and Exp2. This amount was based on an expected daily maximum pasture growth rate of approximately 80 kg DM/ha above 5 cm stubble height at an N-fertilization rate of 345 kg N/ha/year (www.handboekmelkveehouderij.nl, Wageningen Livestock Research). A pasture growth rate of 80 kg DM/ha would result in a daily mean pasture mass (PM) growth of 160 kg/ha on each grazed plot of 2 ha. This would provide 10.6 kg DM pasture/cow/d above 5 cm stubble height (160 kg DM/15 cows). A pasture allowance of 10.6 kg DM/cow/d together with 5.4 kg DM/cow/d of concentrate and 5 kg DM/cow/d supplementary roughage would therefore enable a theoretical TDMI of 21 kg DM/cow/d.
In both experiments and with both grazing treatments, the amount of maize silage as supplementary roughage was maximized at 8 kg DM/cow/d. When more than 8 kg DM/cow/d of supplementary roughage was needed in case of low available pasture, additional roughage above this amount consisted of grass silage.

Matching the amount of supplementary roughage and pasture allowance is delicate. On one hand, to achieve an efficient pasture utilisation excessive supplementary roughage must be avoided. Excessive supplementary roughage to grazing cows would have resulted in reduced pasture intake (substitution) and pasture utilization. This could have a cascading effect resulting in more selective grazing and larger rejected areas and a patchy sward structure, deterioration of the sward and a reduced nutritive value of the pasture during successive grazing rotations with a negative effect on pasture intake. On the other hand, insufficient supplementary roughage would have compromised total DMI and have resulted in too severe grazing. Too severe grazing and defoliation impairs pasture production. Therefore, pasture growth, pasture utilisation and intake and refusals of supplementary roughage were all taken in to account while decision making for grazing management and feeding of supplementary roughage. In order to do so, pasture growth and accumulation on each plot was estimated from daily measurements of the pre- and post-grazing sward surface height (SSH) of the C and S grazing treatments using a rising plate pasture meter (Jenquip, Feilding, New Zealand). On each plot, pre- and post-grazing SSH measurements were performed on at least 50 and 35 points for C and S, respectively. The PM, pre-grazing pasture mass (preGPM; kg DM/ha) and post grazing pasture mass (postGPM; kg DM/ha) were estimated from pre- and post-grazing SSH using an equation developed by Klootwijk et al. (Submitted 2019)in a separate study using the same pastures as the present study.

2.3.2.2 Decision rules supplementary roughage with compartmented continuous grazing

For grazing treatment C, supplementary roughage feeding was controlled on the basis mean PM. An increasing mean PM indicates that pasture growth (kg DM/ha/d) exceeds pasture intake (kg DM/ha/d), whereas a declining PM indicates that pasture intake (kg DM/ha/d) exceeds pasture growth (kg DM/ha/d). In case of an increasing PM, the daily allowance of supplemental roughage was reduced. In

case of a declining PM, the daily allowance of supplemental roughage was increased. However, supplementary roughage was not further increased when the cows did not consume their supplementary roughage completely, as indicated by a refusal weight of more than 5% of the amount at offer.
For grazing treatment S, supplementary roughage feeding was controlled on the basis of the development of the pasture stocks (grazing wedge planning tool, see section pasture management), pre- and post-grazing SSH and visual estimates of the size of the rejected area. On a weekly basis, the allowance of supplemental roughage was reduced when pasture utilization tended to decline as indicated by an increase of the rejected area and the mean post-grazing SSH. Supplementation with roughage was increased when the pastures were depleted completely and the refusal weight of the supplementary roughage was less than 5% of the amount at offer.

2.4 Measurements

2.4.1 Individual supplementary feed intake

2.4.1.1 Concentrate intake

In Exp1 concentrates were fed in two portions of 3 kg per day during milking in a rotary milking parlour. Before the cows entered the rotary milking parlour, it was ensured that the feed troughs were empty and clean. The allowance and refusals were monitored by the staff of the farm. When necessary, the speed of the rotary milking parlour was reduced in order to ensure that all cows had sufficient time to consume their concentrates.
In Exp2 concentrates were fed using transponder controlled concentrate dispensers and feed was dosed in portions at a rate of 300 g/min. The amounts of concentrates fed were recorded automatically.

2.4.1.2 Supplementary roughage intake

Indoors between the PM and AM milking interval, the cows were individually fed controlled amounts of roughage using the RIC system and the individual intakes of fresh weights of supplemental roughage were recorded daily.

2.4.1 Pasture intake

2.4.1.3 Pasture intake measurement periods

In both experiments, individual pasture intake was measured with the alkane marker technique (to be explained in 2.4.1.4) during the three measurement periods P1, P2 and P3. In Exp1, P1, P2 and P3 were carried out during the periods of 12-19 June, 24-31 July, and 4-11 September, respectively. In Exp2, the pasture measurements in P1, P2 and P3 were carried out during the periods of 21-27 May, 23-30 July, and 27 August - 2 September, respectively.

2.4.1.4 Alkane marker technique

In both experiments, individual cow pasture intake and feed digestibility measurements were performed using the alkane marker technique (Dove and Mayes, 2006).

2.4.1.4.1 Production procedure C32 alkane concentrates

Experiment 1

First a batch of 250 kg soybean meal (50 bins with 5 kg soybean meal) was heated during 24 hours in a forced air oven at 70 °C. Then, 50 portions of 50 g C32 alkane flakes (dotriacontane, Sigma-Aldrich, Netherlands) were prepared. Each portion was dissolved in 700 ml in n-heptane which was heated 'au bain marie' at 70 °C. After that, 5 kg of the heated soybean meal was taken out of the oven and mixed with the 700 ml hot solution of heptane and C32 alkane in a 20 L paddle mixer until the mixture appeared to be visually dry. To evaporate the heptane, the mixture of soybean meal and C32 alkane was left drying for 2 days while turned regularly. Subsequently, the dried mixture remained in an oven for two days at 70 °C in order to achieve good cohesion of the C32 alkane to the soybean meal. Then, soybean meal labelled with C32 alkane was mixed with approximately 2400 kg of concentrate ingredients and mixed extensively using a ribbon mixer. Finally, the C32 alkane enriched compound

concentrate mixture was pelleted (4 mm) using a pellet press at 70°C. Details on the ingredients, chemical composition and feeding values are given in Annex 1.

Experiment 2
It was intended to produce 2500 kg of C32 alkane labelled concentrate according to the same procedure as in Exp1. However, the labour safety authority regarded dissolving n-alkanes in warm heptane as unsafe because of risk on fire and explosion, and health damage due to the inhalation of the harmful vapour. Therefore, a novel production process was developed: the melting procedure. A second batch of approximately 2500 kg with 955.7 mg C32 alkane (dotriacontane, Matrix Fine Chemical, Sevelen, Switzerland) was made according to the following procedure. Rectangular bins (app. 25 cm width, 35 cm long, 10 cm high) which were filled with an 8 cm thick layer of soybean meal. Twenty five grams of dotriacontane flakes were carefully spread on top of the soybean meal. The bins with soybean meal, top-dressed with dotriacontane flakes, were covered with aluminium sheets and placed in a forced air oven at 70 °C for 48 hours to melt the C32 alkane. Thereafter, the bins were removed from the oven and cooled down at room temperature. The chunks of soybean meal and melted C32 formed during cooling were crumbled in a paddle mixer. Details on the ingredients, chemical composition and feeding values are given in Annex 1.

2.4.1.4.2 Alkane dosing and faecal sampling
In both Exp1 and Exp2, the cows were dosed with C32 labelled concentrate during 14 consecutive days. The C32-alkane labelled concentrates were offered in two equal portions during milking in the rotary milking parlour. In Exp1, doses of C32 alkane concentrate during measurement periods P1, P2 and P3 were 0.89, 0.80 kg and 0.80 kg DM/cow/d during measurement periods P1, P2 and P3, respectively. In Exp2, the dose of C32 alkane concentrate was 0.85 kg DM per cow per day in all three experimental periods P1, P2 and P3.
The speed of the rotary milking parlour was adjusted to allow the cows sufficient time to consume their C32 labelled concentrates. Nevertheless, incidentally some cows had small concentrate refusals. These refusals were weighed and recorded. During day 7 to 14 of each dosing period, individual faecal samples were collected after AM and PM milking, and stored at -20°C.
During day 7 to 14 of each dosing period, individual faecal grab samples were collected after AM and PM milking. The faecal samples were pooled on the basis of fresh weight to one composite faecal sample for each cow. Until analysis faecal samples were stored in freezer at -20°C. The samples were analysed on dry matter content, ash, nitrogen, phosphorus and n-alkane concentrations as described by Klootwijk et al. (2019, in preparation).

2.4.1.4.3 Analysis of the n-alkane concentrations
The oven dried samples of pasture, maize silage and concentrates were ground using a hammer mill with a 1 mm screen (Peppink 100AN Peppink, Olst, The Netherlands). The ground pasture samples were pooled to one composite sample per pasture measurement period and per grazed plot. These pooled pasture samples were sub-sampled for analysis of the n-alkanes concentrations.
The ground samples of maize silage were pooled to one composite sample per pasture intake measurement period for analysis of the n-alkanes concentrations.
The individual AM and PM faecal samples were thawed and pooled and homogenized to one individual composite sample per cow per intake measurement period. The individual composite faecal samples were sub-sampled and dried at 70°C before analysis. The faecal samples were ground using a Retch ZM200 centrifugal mill (Retsch Technology GmbH, Haan, Germany) with a 1 mm screen.
Prior to analysis, the ground samples of pasture, feed and faeces were pulverized using a Retch MM200 ball mill (Retsch Technology GmbH, Haan, Germany). The analysis of n-alkanes were carried out at the laboratory of the Animal Nutrition Group of Wageningen University & Research, The Netherlands. Alkane extraction and analysis of the concentration of n-alkanes was based on the procedures as described by Smit et al. (2005).

2.4.1.4.4 Calculation of individual pasture DMI and digestibility
The ratio of the natural occurring C33 (trititracontane) in pasture to dosed C32 (dotriacontane) was used to estimate pasture DMI (I_P) using equation [1]. The intakes were estimated with and without using faecal recoveries for alkanes. Dry matter faecal output (FO) was calculated using equation [2], and dry matter digestibility (DMD%) was calculated using equation [3].

$$\text{Pasture DMI } (I_P;\ \text{kg DM/d}) = \frac{\left(\frac{F_{C32}}{F_{C33}} * (I_C \times C_{C33} + I_R \times R_{C33} + I_D \times D_{C33}) - (I_C \times C_{C32} + I_R \times R_{C32} + I_D \times D_{C32})\right)}{\left(P_{C32} - \frac{F_{C32}}{F_{C33}} \times P_{C33}\right)} \quad [1]$$

$$\text{Faecal output (FO; kg DM/d)} = \frac{(I_C \times C_{C32} + I_R \times R_{C32} + I_D \times D_{C32})}{F_{C32}} \quad [2]$$

$$\text{DMD\%} = (I_C + I_D + I_R + I_P)/FO \times 100 \quad [3]$$

where
F_{C32}, C_{C32}, D_{C32}, R_{C32}, P_{C32} are the concentrations of C32 n-alkane (mg/kg DM) in faeces, concentrate, C32 labelled concentrate, roughage supplement and pasture, respectively; F_{C33}, C_{C33}, D_{C33}, R_{C33}, P_{C33} are the concentrations of C33 n-alkane (mg/kg DM) in faeces, concentrates, C32 labelled concentrate, roughage supplement and pasture, respectively; I_C, I_R, I_D, are the intakes (kg DM/d) of concentrate, roughage supplement and C32 labelled concentrate.

The same equations were used to calculate organic matter intake, faecal organic matter output and organic matter digestibility. To perform these calculations, the concentration C32 and C33 alkane in faeces, concentrates, C32 labelled concentrate, roughage supplement and pasture were expressed in g per kg organic matter by multiplying the formula inputs (F_{C32}, C_{C32}, D_{C32}, R_{C32}, P_{C32}, F_{C33}, C_{C33}, D_{C33}, R_{C33}, P_{C33}, I_C, I_R, I_D) with 1000/(1000-ash content).

2.4.2 Milk production, body weight and body condition score

Individual milk weights (kg) were automatically recorded at each milking. Weekly, individual milk samples were collected during 4 consecutive milkings. Two aliquot AM and PM milk samples were pooled to one composite AM milk sample and one composite PM milk sample, respectively. The composite AM and PM milk samples, were analysed for milk fat, milk protein, lactose and urea concentration and somatic cell count at the Qlip laboratory (Zutphen, the Netherlands). Fat- and protein-corrected milk (FPCM) yield (kg/d) was calculated as milk yield × (0.337 + 0.116 × fat%+ 0.06 × protein %) (kg/d; CVB, 2012)].
Body weight of each cow was recorded automatically after milking.

2.4.3 Feed sampling and analysis

2.4.3.1 Concentrates

During the compounding process each batch of concentrate (L, H, HH, C32 alkane labelled concentrate) was sampled at the feed mill. The samples were stored in a freezer at -20°C pending for further analysis on n-alkane concentrations and chemical composition.
During the whole experimental period, experimental concentrates were sampled weekly for determination of dry matter content after 24 hours oven drying at 104°C. For the C32 alkane concentrates individual feed refusals were collected daily and weighed after oven drying at 104°C.

In Exp1, the chemical composition, feeding value and digestibility of each batch of concentrate were obtained from the feed manufacturer.
In Exp2, the samples of concentrates collected during the pasture intake measurement periods were analysed on dry matter, crude protein, ether extract, crude fibre, sugars (except maize silage), starch (except pasture), NDF, ADF, and ADL concentrations using wet chemical analysis at Eurofins Agro (Wageningen, Netherlands) and organic matter digestibility was determined in-vitro according to Tilley and Terry (1963) at Eurofins Agro (Wageningen, Netherlands). The chemical composition and feeding and digestibility of each batch of concentrate were obtained from the feed manufacturer.

2.4.3.2 Pasture grass

During the entire experiment pasture samples were collected from calendar week 18 until 42 and from calendar week 14 until 37, in Exp1 and Exp2, respectively. The pasture samples were taken from each

of the grazed compartments or strips by hand plucking randomly along a zigzag transect at intervals of approximately 5 meters. The pasture samples were dried and at 70°C and stored awaiting for further analysis on dry matter, crude protein, ether extract, crude fibre, sugars, NDF, ADF, and ADL concentrations and organic matter digestibility. The analysis of chemical composition and feeding value was carried out at the Eurofins-Agro (Wageningen, the Netherlands) using NIRS analysis.
During the three subsequent measurement periods (P1, P2 and P3) extra pasture samples were taken daily by hand plucking from each grazed strip (grazing treatment S) or compartment (grazing treatment C). In order to obtain representative samples, the grazing behaviour of the cows was observed, and similar material as selected and grazed by the cows was collected on at least 15 different locations in each of the grazed strips or compartments. The pasture samples were oven dried for 24 hours at 70°C and stored awaiting for further analysis on dry matter, crude protein, ether extract, crude fibre, sugars, NDF, ADF, and ADL concentrations and organic matter digestibility using wet chemical analysis at Eurofins Agro (Wageningen, Netherlands) and organic matter digestibility was determined in-vitro according to Tilley and Terry (1963) at Eurofins Agro (Wageningen, Netherlands). The feeding values of pasture grass were calculated according to the prescriptions of the Centraal Veevoeder Bureau (CVB, 2012).

2.4.3.3 Supplementary roughage

Throughout the whole grazing season, supplementary roughages were sampled daily for determination of dry matter content after 24 hours oven drying at 104°C. Week samples were analysed on dry matter, crude protein, ether extract, crude fibre, sugars, NDF, ADF, and ADL concentrations and organic matter digestibility. The analysis of chemical composition and feeding value was carried out at the Eurofins-Agro (Wageningen, the Netherlands) using NIRS analysis.
During the measurement periods P1, P2 and P3 extra triplicate samples were taken from the roughage supplement (maize silage). One sample was for analysis of dry matter content by oven drying for 24 hours at 104°C in order to calculate daily dry matter intake. One sample was oven dried for 24 hours at 70°C and stored awaiting for n-alkane analysis. One sample was stored in a freezer at -20°C awaiting for analysis on chemical composition and digestibility.
These samples were analysed on dry matter, crude protein, ether extract, crude fibre, starch, NDF, ADF, and ADL concentrations using wet chemical analysis at Eurofins Agro (Wageningen, Netherlands) and organic matter digestibility was determined in-vitro according to Tilley and Terry (1963) at Eurofins Agro (Wageningen, Netherlands).
The feeding value of roughages were calculated according to the prescriptions of the Centraal Veevoeder Bureau (CVB, 2012).

2.4.4 Rumen fluid sampling

Rumen fluid sampling was carried out during intake measurement periods P2 and P3 of Exp1 and during intake measurement periods P1, P2 and P3 of Exp2 for measurement of rumen pH and analysis of rumen NH_3 and volatile fatty acid composition. In both experiments, rumen sampling was carried out immediately following the pasture intake measurements in order to avoid disturbance of intake and intake behaviour. Individual rumen fluid samples were taken at 4 time points (0400 h, 1100 h, 1500 h, 2100 h) using an oesophagus sampling device (H. Hauptner & Richard Herberholz GmbH & Co. KG, Solingen, Germany). In order to minimize the impact on the animals, rumen fluid sampling was carried out on 2 consecutive days and on 2 time points per day. The cows were fixated in a headlock feed barrier. One person inserted a flexible stainless steel technique tube with a stainless steel suction head (H. Hauptner & Richard Herberholz GmbH & Co. KG, Solingen, Germany) through the mouth and oesophagus into the rumen. During oesophageal sampling, the head of the cow was kept downwards in order to prevent saliva flowing to the rumen. One person operated a hand-driven suction pump to collect rumen fluid. In order to obtain a representative sample and to avoid contamination with saliva, first a rumen fluid sample of at least 500 ml was taken and discarded. Then, a second sample of at least 500 mL was taken. Immediately after sampling, the pH of rumen fluid samples was measured using a handheld pH meter (pH electrode InLab 413 SG/2m IP 67, Seven2GoPro, Mettler-Toledo) and a duplicate 10 ml sub-sample was taken using a syringe and transferred to 15 ml tubes with screw top lid. The duplicate samples were immediately covered with ice and transferred to a freezer and stored at -20°C awaiting for ammonia and VFA (acetate, butyrate, isobutyrate, propionate, valerate, isovalerate) analysis, according to the procedures described by

Riede et al. (2013) and Oeztuerk et al. (2005) respectively. Analysis of the rumen fluid samples was carried out at the Physiological Institute of the University of Veterinary Medicine (Hannover, Germany).

2.5 Statistical analysis

For each cow, weekly means of milk yield and milk constituents yield were calculated from the daily milk yields and weekly milk recording measurements of milk constituents concentration. Mixed model analysis was performed using the REML procedure in Genstat 18th (Genstat, 2017). A polynomial curve model with a subject-specific general slope and intercept $y = a + bx + cx^2$ was used to describe the time curves of milk yield and milk constituent yield during the whole grazing season. The grazing treatment G_j (with j=1,2), and dietary protein treatments P_k (with protein levels k=1,2 in Exp1, and k = 1,..3 in Exp2), experimental week (both linear, W_i, and quadratic $(W_i)^2$, *with i=1,..6*), and their interactions were included as fixed effects in the model. Repeated measurements within the same cow were considered to be correlated and, therefore, an autoregression term was included in the model. Cow, block, experimental week W_i, and the cow by week interaction were included as random effects in the model.

$$Y_{ijk} = \beta_0 + \beta_1 \times W_i + \beta_2 \times W_i^2 + \beta_3 \times (W_i \times G_j) + \beta_4 \times (W_i \times P_k) + \beta_5 \times (W_i \times G_j \times P_k) + \beta_6 \times (W_i^2 \times P_k) + \varepsilon_{ijk}$$

where
Y_{ijk} = the response in milk yield, milk constituent yield, milk constituent concentration BW or BCS
β_0 = the average experimental value
β_1 = the fixed effect for experimental week W_i
β_2 = the fixed quadratic effect of week W_i
β_3 is the fixed effect of the interaction of grazing treatment G_j and week W_i
β_4 is the fixed effect of the interaction of dietary protein treatment P_k and week *Wi*
β_5 is the fixed effect of the interaction of dietary protein treatment P_k, grazing treatment G_j and week W_i
β_6 is the fixed effect of the interaction of dietary protein treatment P_k and the quadratic effect of week $(W_i)^2$ εijk = the residual variance.
Differences in the shape of the curve are indicated by significant treatment effects on parameters β_3, β_4, β_5 and β_6 between the treatment groups, as indicated by the contrasts in $\Delta\beta_3$, $\Delta\beta_4$, $\Delta\beta_5$, and $\Delta\beta_6$, respectively.

The cumulative effects on milk, milk constituents and FPCM yield were analyse using the ANOVA procedure of GenStat 19th edition.

Data from the pasture measurement periods was analysed using GenStat 19th edition. A linear mixed model with repeated measurements was used to analyse the effect of the treatments on total DM intake, TDMI, and average DM intake, GDMI, milk performance, grazing behaviour characteristics and rumen characteristics:
$Y_{ijklm} = \mu + P_k + G_j + M_n + C_l + B_m + (P_k \times G_j \times P_n) + \varepsilon_{ijklm}$

where
Y_{ijklm}, represents the analysed of the response variables (TDMI, GMDI, milk yield, milk composition, grazing behaviour characteristics and rumen characteristics)
μ, the average experimental mean
P_k (k = 1,..2 in Exp1, or k = 1,..3 in Exp2), the fixed treatment effect of dietary protein treatment
G_j (j = 1,2), the fixed treatment effect of grazing treatment
M_n (n = 1,..3), the fixed measurement period effect
C_l (l = 1,..60), the random cow effect
B_m (m = 1,..15), the random block effect
P_k x G_j x M_n, , the interactions between dietary protein treatment, grazing treatment and measurement periods.
ε_{ijklm}, the residual

Least significant differences (LSD's) were used to study the differences between the methods with a significance level of $\alpha=0.05$.

3 Results

3.1 Intended and achieved dietary treatments

The experiments were designed to investigate whether or not a difference in rumen degradable protein (RDP) offered with supplementary feed during night time would increase pasture intake during day time. The RDP treatments were imposed by offering equal amounts of concentrates differing in OEB (Exp1) and in rumen degradable protein balance OEB and/or DVE (intestinal digestible protein) (Exp2). Table 1 provides an overview of the intended and achieved concentrate RDP and intestinal digestible protein concentration.

Table 1 *Experiment 1 and 2: Intended and achieved (between brackets) concentration of low rumen degradable protein (RDP) expressed as rumen degradable protein balance (OEB) and intestinal digestible protein (DVE) in the experimental concentrates, including the treatment effect in respectively g OEB and DVE ingested per animal per day.*

Exp1			Concentrate offered			Treatment difference relative to treatment LC	
Pasturing System	RDP level	Treatment Code	kg DM/d	g OEB/kg DM	g DVE/kg DM	g OEB/d	g DVE/d
C	L	LC1	5.4	-50 (-45)	105 (101)	-	-
	H	HC1	5.4	+50 (+57)	105 (107)	+540 (+551)	- (32)
S	L	LS1	5.4	-50 (-45)	105 (101)	-	-
	H	HS1	5.4	+50 (+57)	105 (107)	+540 (+551)	- (32)

Exp2			Concentrate offered			Treatment difference relative to treatment LC	
Pasturing System	RDP level	Treatment Code	kg DM/d	g OEB/kg DM	g DVE/kg DM	g OEB/d	g DVE/d
C	L	LC2	5.4	-50 (-47)	105 (110)	-	-
	H	HC2	5.4	+50 (+64)	105 (109)	+540 (+599)	- (-5)
	H	HHC2	5.4	+50 (+63)	150 (146)	+540 (+594)	243 (194)
S	L	LS2	5.4	-50 (-47)	105 (110)	-	-
	H	HS2	5.4	+50 (+64)	105 (109)	+540 (+599)	-
	H	HHS2	5.4	+50 (+63)	150 (146)	+540 (+594)	243 (194)

3.2 Treatment effects during the whole grazing season

3.2.1 Chemical composition and feeding value of pasture grass

Within years the chemical composition and feeding values of pasture grass were similar for the grazing treatments S and C. Between years and within grazing system, the chemical composition of pasture grass was slightly different. The crude protein and sugar concentrations were more variable in 2016 than in 2017. Annex 2 provides information of the development of the chemical composition and feeding value of pasture grass during the grazing season. In Exp1, pasture measurements started calendar week 18 of 2016 and ended in week 42 when the experiment was terminated because the pastures were depleted completely. The chemical composition, organic matter digestibility (OMD), VEM (1 VEM = 6.9 kJ NEL), DVE and OEB of the pasture during the whole grazing season was similar between grazing treatments and dietary protein treatments. In the early grazing season of Exp1, the

pastures were characterized by a high OMD and extremely high sugar concentrations and low CP and NDF concentrations resulting in high net energy content but with a low (negative) OEB. This is probably the result of low nitrogen uptake of the pasture due to a combination of drought, high solar radiation and low night temperatures.
In week 33, unusual low values of OMD were observed in all grazing treatments. These OMD values could not be traced back to erroneous sampling or analysis, neither to extraordinary weather and growing conditions. Therefore, the cause of this phenomenon remains unclear.
In Exp2, pasture measurements started calendar week 14 and were terminated in week 36 because the pastures could not be grazed due to poor soil conditions caused by heavy rainfall. The chemical composition, OMD, VEM (1 VEM = 6.9 kJ NEL), DVE and OEB of the pasture during the whole grazing season was similar between grazing treatments and dietary protein treatments. The concentrations CP, Sugar, NDF, VEM, DVE and OEB in pasture during the grazing season are displayed in Annex 2. Details on the weather conditions, pasture management, pre- and post-grazing SSH, PreGPM, post-grazing pasture mass (PostGPM) and pasture production (kg DM/ha) of both grazing treatments is published by Holshof et al. (2019, in preparation).

3.2.2 Effects on milk production whole grazing season Experiment 1

The analysis of the effects on milk production during the course of the grazing season of Exp1 in Tables 2 and 3. In Exp1, there was a significant effect of week (W) on the production of milk, fat, protein, lactose and FPCM yield, but no effect of squared Week effect. This indicated a linear decrease milk and milk constituent yield during the course of the experiment.
In Exp1, there were no effects of grazing system, as indicated non-significant contrasts of grazing system (Δβ3, contrast between grazing systems). There was a significant effect of dietary protein treatments on milk yield, protein yield, and lactose yield as indicated by significant values of Δβ4 the contrast between dietary protein treatment L and H. There were no significant effects of dietary protein treatment on milk fat and FPCM yield. In Exp 1 there were no linear week by grazing system by dietary protein treatment interactions.
In Exp1, there were significant effects of the quadratic week by grazing system by dietary protein treatment (contrast Δβ6) on milk yield, milk fat yield and lactose yield. This means that the lactations curves of the high (H) and low (L) dietary protein treatment were shaped differently.
The cumulative milk, fat and protein yield (Table 3) were not significantly different for grazing treatment C and S. The cumulative milk, protein and FPCM yields of cows on dietary protein treatment H were significantly higher compared with L.

3.2.3 Effects on milk production whole grazing season Experiment 2

In Exp2, there was a significant effect of week (W) on the production of milk, fat, protein, lactose and FPCM yield, but no effect of squared week (W) effect (Table 4). The squared week effects were removed from models for the curves of lactose and FPCM yield because they were non-significant, indicating a more linear decrease milk and milk constituent yield during the course of the experiment.
In Exp2 there was a significant effect of dietary protein treatments on milk yield, protein yield, and lactose yield as indicated by significant values of Δβ4 the contrast between dietary protein treatment L and H. There were no significant effects of dietary protein treatment on milk fat and FPCM yield.
The cumulative milk, fat and protein yield were not significantly different for grazing treatment C and S. The cumulative milk were protein of cows on dietary protein treatments H and HH were significantly higher compared with L (Table 5). There was a tendency for higher cumulative FPCM yields with treatment H and HH than for L. There were no significant effects of dietary protein treatment on the cumulative fat yield.

Table 2 *Experiment 1: The effect of grazing treatment (G) (compartmented continuous grazing (C) vs. strip-grazing (S)) and dietary protein treatment (P) (low rumen degradable protein, L vs. high rumen degradable protein, H) during subsequent measurement weeks (W) on milk production in 2016,*
using the statistical model $Y_{ijk} = \beta_0 + \beta_1 \times W_i + \beta_2 \times W_i^2 + \Delta\beta_3 \times (W_i \times G_j) + \Delta\beta_4 \times (W_i \times P_k) + \Delta\beta_5 \times (W_i \times G_j \times P_k) + \Delta\beta_6 \times (W_i^2 \times P_j) + \varepsilon_{ijk}$

					W×G	W×P	W×G×P	W^2×P
		β_0	β_1	β_2	Contrasts			
		Constant	Week	$Week^2$	$\Delta\beta_3$	$\Delta\beta_4$	$\Delta\beta_5$	$\Delta\beta_6$
Milk Yield (kg/d)	Estimate	35.3	-1.1	0.03	-0.008	0.314	0.045	-0.010
	SED	1.2	0.26	0.10	0.043	0.066	0.059	0.005
	P-value		<0.001	0.09	0.653	<0.001	0.449	<0.001
Milk Fat Yield (g/d)	Estimate	1500	-16	0.03	-1.174	-11.543	2.663	0.379
	SED	241	51.1	0.10	2.301	3.465	3.216	0.113
	P-value		<0.001	0.09	0.912	0.400	0.411	<0.001
Protein Yield (g/d)	Estimate	1306	-45	1.14	-1.078	0.864	2.231	-0.001
	SED	294	62.7	2.32	1.723	2.599	2.344	0.085
	P-value		<0.001	0.65	0.973	0.094	0.647	0.993
Lactose Yield (g/d)	Estimate	1256	18	-1.40	0.430	16.607	2.353	-0.5571
	SED	879	187.4	2.32	2.207	3.401	3.042	0.1123
	P-value		<0.001	0.65	0.331	0.002	0.647	<0.001
FPCM Yield (kg/d)	Estimate	37.3	-1.269	0.03	-0.023	-0.030	0.059	0.0015
	SED	0.83	0.18	0.07	0.048	0.007	0.066	0.0024
	P-value		<0.001	0.66	0.850	0.302	0.337	0.551

$\Delta\beta_3$ contrast C vs. S; $\Delta\beta_4$ contrast L vs. H; $\Delta\beta_5$ contrasts HS1 vs. LS1 and HS1 vs LC1 and HS vs HC1; $\Delta\beta_6$ contrasts L vs. H

Table 3 *Experiment 1: Cumulative milk, fat, protein and FPCM yield during the whole grazing season of 185 days in 2016 with two grazing management treatments (G) and two rumen degradable dietary protein treatments (P). Grazing management treatments strip-grazing (S); compartmented continuous grazing (C);Dietary protein treatments low rumen degradable protein (L); high rumen degradable protein (H). Grazing and dietary protein treatments in a 2×2 arrangement: low rumen degradable protein – strip-grazing (LS1), low rumen degradable protein – compartmented continuous grazing (LC1), high rumen degradable protein – strip-grazing (HS1), and high rumen degradable protein – compartmented continuous grazing (HC1)*

	C	S	lds	L	H	lsd	LC1	HC1	LS1	HS1	lsd	G	P	P×G
Milk yield (kg)	4964	4934	236.7	4624	5273	236.7	4683	5245	4565	5302	334.7	0.796	<0.01	0.461
Fat yield (kg)	192	188	11.3	185	194	11.3	190	194	181	194	15.9	0.412	0.121	0.437
Protein (kg)	178	172	8.5	166	185	8.5	171	162	185	183	12.0	0.197	<0.01	0.450
FPCM (kg)	4966	4859	242.3	4698	5126	242.3	4801	4596	5130	5122	342.7	0.380	<0.01	0.420

Table 4 *Experiment 2: The effect of grazing treatment (compartmented continuous grazing (C) vs. strip-grazing (S)) and dietary protein treatment (level rumen degradable protein, RDP, and intestinal digestible protein, DVE) (low RDP (L), high RDP (H), and high RDP and high DVE (HH)) in subsequent measurement weeks (W) in 2017, using the statistical model* $Y_{ijk} = \beta_0 + \beta_1 \times W_i + \beta_2 \times W_i^2 + \beta_3 \times (W_i \times G_j) + \beta_4 \times (W_i \times P_k) + \beta_5 \times (W_i^2 \times P_j) + \varepsilon_{ijk}$

					Contrasts between main effects				
					W×G	W×P		W²×P	
		Constant	W	W²	C vs. S	H vs. L	HH vs. L	H vs. L	HH vs. L
		β_0	β_1	β_2	$\Delta\beta_3$	$\Delta\beta_4$		$\Delta\beta_5$	
Milk Yield (kg/d)	Estimate	33.8	-1.307	0.034	0.064	0.376	0.686	-0.01569	-0.03447
	SED	0.99	0.200	0.012	0.047	0.017		0.005233	
	P-value		<0.001	0.092	0.179	<0.001		<0.001	
Milk Fat Yield (g/d)	Estimate	1355	-69.470	2.193	2.805	15.3	24.9	-0.7309	-1.2639
	SED	50.9	10.335	0.554	1.695	5.651		0.005207	
	P-value		<0.001	0.009	0.104	<0.001		<0.001	
Protein Yield (g/d)	Estimate	1081	-43.340	1.333	1.197	14.1	28.1	-0.5225	-1.2868
	SED	33.9	7.198	0.378	1.418	3.91		0.2133	
	P-value		<0.001	0.055	0.402	<0.001		<0.001	
Lactose Yield (g/d)	Estimate	1481	-34.050		2.566	4.8	4.5		
	SED	39.1	3.540		2.229	2.73			
	P-value		<0.001		0.255	0.154			
FPCM Yield (kg/d)	Estimate	34.5	-0.748		0.052	0.14	0.17		
	SED	0.858	0.075		0.043	0.052			
	P-value		<0.001		0.226	0.005			

Table 5 *Experiment 2: Cumulative milk, fat, protein and FPCM yield during the whole grazing season of 132 days in 2017 with two grazing management treatments (G) and three dietary protein treatments (P). Grazing management treatments strip-grazing (S); compartmented continuous grazing (C). Dietary protein treatments: low rumen degradable protein (L); high rumen degradable protein (H); high rumen degradable protein and high intestinal digestible protein (HH). The experiment was performed in a 2×3 arrangement: low rumen degradable protein–strip-grazing (LS2), low rumen degradable protein–compartmented continuous grazing (LC2), high rumen degradable protein–strip-grazing (HS2), and high rumen degradable protein–compartmented continuous grazing (HC2), high rumen degradable protein plus high intestinal digestible protein–strip-grazing (HHS2), high rumen degradable protein plus high intestinal digestible protein–compartmented continuous grazing (HHC2))*

	C	S	lds	L	H	HH	lsd	LC2	HC2	HHC	LS2	HS2	HHS	lsd	G	P	P×G
Milk yield (kg)	3336	3298	219.6	3158	3372	3421	179.3	3254	3399	3353	3062	3344	3488	310.5	0.675	0.047	0.330
Fat yield (kg)	129	130	9.6	124	132	132	7.9	128	132	128	120	133	136	13.6	0.933	0.166	0.227
Protein (kg)	114	110	7.8	104	114	118	6.4	109	117	117	100	112	119	11.1	0.216	0.004	356.000
FPCM (kg)	3419	3367	234.4	3242	3426	3512	191.4	3303	3481	3474	3181	3370	3551	331.4	0.586	0.071	0.635

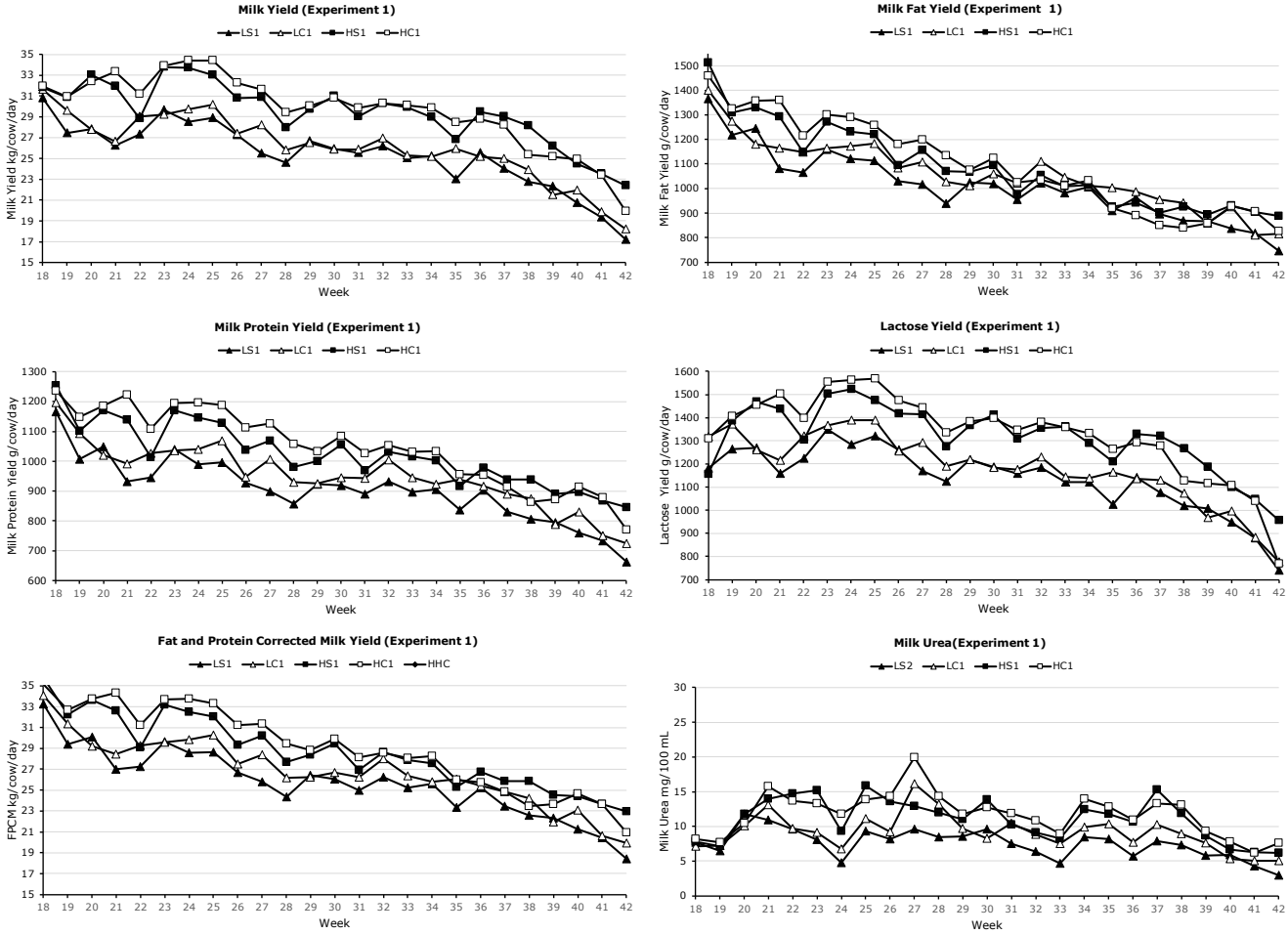


Figure 2a *<u>Experiment 1:</u> Milk production, milk constituent yield and milk urea concentration during the whole grazing season (week 18 - week 42).*

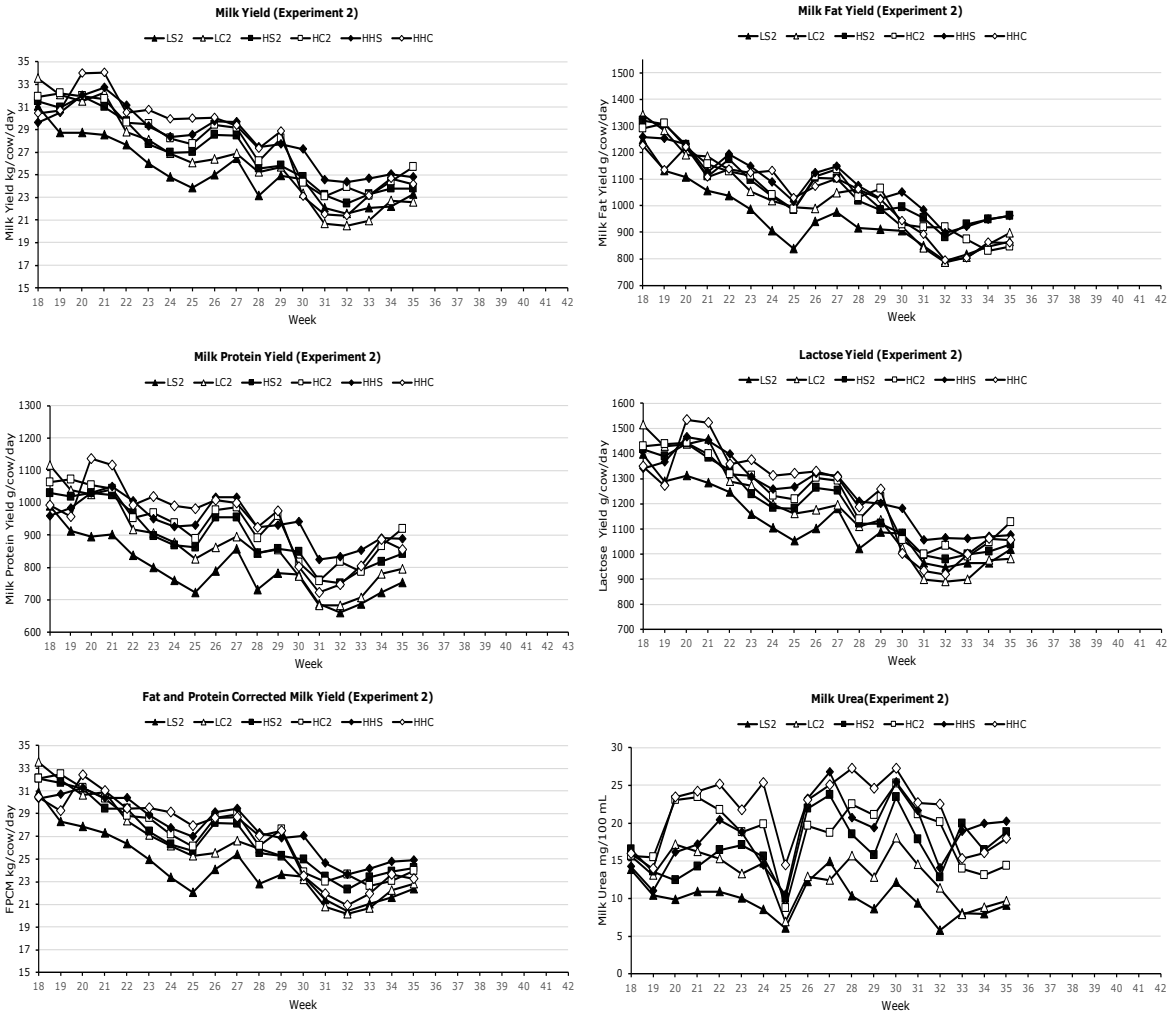


Figure 2b *<u>Experiment 2:</u> Milk production, milk constituent yield and milk urea concentration during the whole grazing season (week 18 - week 42).*

3.3 Pasture intake measurement periods

3.3.1 Composition of the ingested diets

The composition of the ingested diets of each treatment group during the intake measurement periods of Exp1 and Exp2 are presented Tables 6 and 7a&b, respectively.

Table 6 *Experiment 1: Diet composition of the Grazing treatments by rumen degradable dietary protein treatment groups. HC1 = High rumen degradable protein with continuous grazing; LC1 = Low rumen degradable protein with continuous grazing; HS1 = High rumen degradable protein with strip-grazing; LS1 = Low rumen degradable protein with strip-grazing.*

Measurement period	P1				P2				P3			
Treatments	HC1	LC1	HS1	LS1	HC1	LC1	HS1	LS1	HC1	LC1	HS1	LS1
All values g/kg DM, unless indicated else												
Ash	69	69	68	73	74	76	70	73	67	71	66	68
Organic Matter	931	931	932	928	926	924	930	926	933	929	934	932
Crude Protein	154	117	148	122	159	133	158	134	157	138	149	126
Ether extract	37	33	38	35	40	37	40	38	40	39	41	38
Crude fibre	189	190	180	182	176	181	167	175	170	171	164	169
Sugars	64	76	72	84	66	79	67	79	59	69	66	75
Starch	200	206	218	203	217	201	241	211	242	228	256	240
NDF	408	400	383	388	387	393	364	385	366	368	345	358
DOM[1)]	731	736	736	743	730	740	736	746	737	747	739	751
Phopohrus	3.6	3.0	3.6	3.1	4.0	3.3	3.9	3.3	3.7	3.3	3.6	3.2
DVE[2)]	81	78	81	81	84	84	86	86	84	86	83	85
OEB[3)]	14	-21	10	-19	17	-10	15	-11	16	-6	10	-17
OEB2h[4)]	14	-5	12	-5	14	-3	15	-3	15	0	13	-4
FOSp[5)]	556	544	557	549	558	550	562	555	562	557	560	557
FOSp2h[6)]	239	240	253	245	245	239	257	244	254	248	263	254
VEM (/kg DM)[7)]	996	996	1009	1011	1003	1009	1017	1021	1013	1021	1020	1030
NEL (MJ/kg DM)[7)]	6.82	6.82	6.96	6.97	6.92	6.96	7.02	7.05	7.01	7.04	7.04	7.11
DVE 1991[8)]	81	77	80	80	82	82	83	84	81	83	80	82
OEB 1991[9)]	13	-19	9	-17	17	-8	16	-9	16	-5	11	-14
FOS 1991[10)]	559	566	558	570	550	566	546	567	546	557	544	560

1) DOM digestible organic matter (Tilley and Terry 1963) 2) DVE intestinal degradable protein 3) OEB degradable protein balance 4) OEB2h degradable protein balance within 2 hours after ingestion 5) FOSp Rumen Fermentable Organic Matter 6) FOSp2h Rumen Fermentable Organic Matter within 2 hours after ingestion, DVE, OEB, OEB2h, FOSp, FOSp2h based on the DVE/OEB system of Tamminga et al.1994, revised by van Duinkerken et al. 2011. 7) VEM Feed Unit Milk 1 VEM =6.9 KJ NEL net energy for lactation (van Es, 1978) 8) DVE 1991 intestinal degradable protein 9) OEB 1991 degradable protein balance 10) FOS 1991 Fermentable Organic Matter DVE 1991, OEB 1991, FOS 1991 based on the DVE/OEB system of Tamminga et al.1994

Table 7a *<u>Experiment 2:</u> Chemical composition and feeding values of the total diet of treatment LC2, HC2 and HHC2 in measurement periods P1, P2, P3. Treatments: LC2 = low rumen degradable protein with compartmented continuous grazing; HC2 = high rumen degradable protein with compartmented continuous grazing, HHC2 high rumen degradable protein plus high intestinal digestible protein with compartmented continuous grazing.*

Measurement period	P1			P2			P3		
Treatment	LC2	HC2	HHC2	LC2	HC2	HHC2	LC2	HC2	HHC2
Diet composition g/kg DM, unless indicated else									
Ash	69	69	73	83	81	83	73	71	73
Organic Matter	931	931	927	917	919	918	927	930	930
Crude Protein	144	164	174	140	162	172	130	151	156
Ether Extract	38	44	42	42	46	45	42	45	44
Crude fibre	161	154	157	175	170	167	168	163	163
Sugar	92	88	91	72	65	73	61	60	62
Starch	180	188	158	187	196	173	234	236	218
NDF	261	263	235	285	288	257	302	296	271
DOM[1)]	788	782	780	757	754	758	774	775	773
DVE[2)]	92	91	98	86	86	96	87	86	94
OEB[3)]	72	73	80	70	71	82	78	78	87
OEB2h[4)]	-23	7	8	-22	8	10	-27	4	4
FOSp[5)]	228	227	227	215	216	195	152	154	130
FOSp2h[6)]	415	423	420	392	401	416	444	454	467
VEM (/kg DM)[7)]	1054	1055	1051	1014	1018	1022	1044	1048	1049
NEL (MJ/kg DM)[7)]	7.27	7.28	7.25	7.00	7.02	7.05	7.21	7.23	7.24
DVE 1991[8)]	95	94	103	89	88	100	88	86	96
OEB 1991[9)]	-7	22	21	-3	25	24	-11	19	16
FOS 1991[10)]	610	599	603	575	566	571	574	565	568

1) DOM digestible organic matter (Tilley and Terry 1963) 2) DVE intestinal degradable protein 3) OEB degradable protein balance 4) OEB2h degradable protein balance within 2 hours after ingestion 5) FOSp Rumen Fermentable Organic Matter 6) FOSp2h Rumen Fermentable Organic Matter within 2 hours after ingestion, DVE, OEB, OEB2h, FOSp, FOSp2h based on the DVE/OEB system of Tamminga et al.1994, revised by van Duinkerken et al. 2011. 7) VEM Feed Unit Milk 1 VEM =6.9 KJ NEL net energy for lactation (van Es, 1978) 8) DVE 1991 intestinal degradable protein 9) OEB 1991 degradable protein balance 10) FOS 1991 Fermentable Organic Matter DVE 1991, OEB 1991, FOS 1991 based on the DVE/OEB system of Tamminga et al.1994

Table 7b *Experiment 2: Chemical composition and feeding values of the total diet in by treatment LS2, HS2 and HHS2 in measurement periods P1, P2, P3. All values in g/kg DM, except when indicated otherwise. Treatments: LS2 = low rumen degradable protein with strip-grazing, HS2 = high rumen degradable protein with strip-grazing; HHS2 = high rumen degradable protein plus high intestinal digestible protein with strip-grazing*

Measurement period	P1			P2			P3		
Treatment	LS2	HS2	HHS2	LS2	HS2	HHS2	LS2	HS2	HHS2
Diet composition g/kg DM, unless indicated else									
Ash	67	69	72	83	82	84	74	74	77
Organic Matter	933	931	928	917	918	916	926	928	926
Crude Protein	138	166	172	131	156	163	135	160	168
Ether Extract	39	45	44	42	46	45	42	46	45
Crude fiber	157	154	153	178	174	174	169	167	166
Sugar	97	98	96	76	69	74	66	64	68
Starch	179	169	154	194	199	175	216	208	186
NDF	259	254	230	293	294	265	294	286	259
DOM[1)]	803	795	793	761	756	756	777	776	773
DVE[2)]	93	93	101	85	85	93	89	88	98
OEB[3)]	71	69	79	67	68	77	78	77	87
OEB2h[4)]	-25	8	9	-25	7	7	-25	8	9
FOSp[5)]	220	255	220	199	209	190	176	195	176
FOSp2h[6)]	419	406	423	403	407	416	427	426	435
VEM /kg DM[7)]	1077	1076	1074	1019	1020	1020	1049	1050	1050
NEL MJ/kg DM[7)]	7.43	7.43	7.41	7.03	7.04	7.04	7.24	7.24	7.24
DVE 1991[8)]	97	97	106	88	87	97	91	90	101
OEB 1991[9)]	-14	20	17	-11	21	19	-9	23	22
FOS 1991[10)]	624	619	617	579	569	571	583	575	578

1) DOM digestible organic matter (Tilley and Terry 1963) 2) DVE intestinal degradable protein 3) OEB degradable protein balance 4) OEB2h degradable protein balance within 2 hours after ingestion 5) FOSp Rumen Fermentable Organic Matter 6) FOSp2h Rumen Fermentable Organic Matter within 2 hours after ingestion, DVE, OEB, OEB2h, FOSp, FOSp2h based on the DVE/OEB system of Tamminga et al.1994, revised by van Duinkerken et al. 2011. 7) VEM Feed Unit Milk 1 VEM =6.9 KJ NEL net energy for lactation (van Es, 1978) 8) DVE 1991 intestinal degradable protein 9) OEB 1991 degradable protein balance 10) FOS 1991 Fermentable Organic Matter DVE 1991, OEB 1991, FOS 1991 based on the DVE/OEB system of Tamminga et al.1994

3.3.2 Feed intake and animal performance

The treatment effects of grazing system and dietary protein on pasture intake, (PDMI), total dry matter intake (TDMI), milk and milk constituent yield, milk composition, organic matter digestibility and nitrogen use efficiency in Exp1 and Exp2 are presented in Tables 8 and 9.

3.3.2.1 Pasture and totale dry matter intake

In both experiments there were no significant effects of dietary protein treatment supplementation (P) or grazing system (G) on pasture dry matter intake (PDMI) (Tables 8 and 9). There were neither interactions between protein supplementation and grazing system. In both experiments there was a significant effect ($P<0.001$) of measurement period (M) on PDMI. In addition to that there were significant interactions between G and M with $P<0.001$ and $P<0.023$ in 2016 and 2017, respectively (Annex 3). This implies that PDMI develops during the grazing season. In 2016, PDMI was higher ($P<0.05$) in C than in S during M1, but PDMI was numerical lower in C than in S in M2 and M3. In 2017, PDMI was significantly higher ($P<0.05$) in S than in C during M3, and PDMI was numerical higher in S in M1 and M2.

3.3.2.2 Total dry matter intake

In both experiments, there was a significant measurement period effect on total DMI (TDMI) (See tables Annex 3). This is partly inherent to the effects of measurement period on PDMI. In Exp1 conducted in 2016 there was a significant effect of grazing system on TDMI, but not in Exp2 that was conducted in 2017.

In Exp1 there was a significant effect of the dietary protein treatment on TDMI. The cows on dietary protein treatment L had larger refusals of maize silage than cows on dietary protein treatment H. This suggests that cows indeed seem to balance their rumen degradable protein intake. However, not through increasing the intake of grass but due to a reduction of the voluntary intake of supplemental roughage (i.e. maize silage). Because the cows were supplemented with fixed amounts of maize silage it is not possible to draw firm statistically substantiated conclusions.

In Exp2 there was no effect of any dietary protein treatment on TDMI and PDMI. This implicates that there were no differences in the intake of supplemental roughage between the dietary protein treatments.

3.3.2.3 Milk yield and milk constituents yield and composition

In Exp1 there was a significant effect of measurement period (M) on all performance traits (Annex 3.1). There was no effects of grazing system (G) on milk performance. However, there were no significant grazing system by measurement period interactions (G×M, see Annex 3). Supplementation with high RDP treatment (H) resulted in an increased milk and milk protein yield and lactose yield. Milk fat yield was unaffected by the dietary protein treatment. The concentrations of milk fat and lactose were unaffected by the dietary protein treatment. The concentration of milk protein was lower for the high RDP treatment. There were significant measurement period by dietary protein treatment interaction (M×P) effects on milk fat and milk protein concentrations. The high RDP treatment resulted in significantly higher milk urea concentrations.

In Exp2 there was a significant effect of measurement period (M) on all performance traits (see Annex 3.2). However, there were no effects of dietary protein treatment or grazing treatment on milk yield, milk fat yield and lactose yield.

There was a significant effect of dietary protein treatment on milk protein yield. During all measurement periods, treatment HH resulted in significant higher milk protein yields than treatment L. However, there were no significant differences between treatment HH and H. There were significant measurement period by grazing system effects on protein yield. In measurement period 3, treatment H resulted in higher milk protein yields than treatment L.

There were significant effects of grazing system and dietary protein treatment milk urea concentration. Grazing treatment C resulted in significantly higher milk urea concentrations. Milk urea concentrations were lower for L than for H and HH.

3.3.2.4 Energy and protein supply

In Exp 1 the NEL coverage (NEL cov%, net energy intake (MJ NEL)/net energy requirements (MJ NEL)×100%) was close to 100% and not significantly different between grazing system (Table 8 continued a). However, there was a tendency for a lower NEL cov% ($P<0.08$) for the H treatment.

In Exp1 the DVE coverage (DVE cov%; DVE intake (g/d)/DVE requirements) was not different between the grazing treatments. The results show also that DVE cov% was below 100% for all grazing and dietary protein treatments, indicating that DVE supply was limiting. The DVE cov% was significantly lower for the H treatment than for the L treatment.
In Exp2 the NEL cov% was close to or above 100% (Table 9 continued a) except for measurement period 1. The NEL cov% was different between grazing system. The NEL cov% was lower for the H and HH treatment than for L. In Exp1 the DVE cov% was fairly above 100%, except for measurement period 1. The was no difference in DVE cov% between the grazing treatments. However, DVE cov% was significantly lower for the H and HH treatment. However, DVE cov% was fairly above 100% for all treatments, indicating that, despite differences in DVE cov%, DVE supply was not limiting.

3.3.2.5 Organic matter digestibility

In Exp1 there were no effects of grazing system and differences in faecal output of organic matter (FO; kg OM/d) and organic matter digestibility (OMD) (Table 8 continued b). However, there was a tendency ($P < 0.084$) for higher FO ($P < 0.084$) and higher OMD ($P < 0.086$) with the H treatment.
In Exp2 there were no effects of grazing system nor dietary protein treatment on FO and OMD (Table 9 continued b).

3.3.2.6 Nitrogen use efficiency

In both Exp1 (Table 8 continued b) and Exp2 (Table 9 continued b) there were no effects of grazing system on total nitrogen intake (TN; g/d), pasture nitrogen intake (PN; g/d) and nitrogen use efficiency (NUE; milk N output (g/d)/TNin (g/d)). However, in both Exp1 and Exp2 there was a significant effects of dietary protein treatment on TNin (g/d) and NUE. In Exp1, TNin and NUE were significantly higher for L than for H. In Exp 2 TNin and NUE were significantly higher for L than for H and HH. However, there were no differences in TNin and NUE between H and HH.

Table 8 *<u>Experiment 1:</u> Treatment means, main and combined effects of two grazing management treatments (G) and two rumen degradable dietary protein treatments (P). Grazing management treatments: strip-grazing (S); compartmented continuous grazing (C);Dietary protein treatments low rumen degradable protein (L); high rumen degradable protein (H). Grazing and dietary protein treatments in a 2×2 arrangement: low rumen degradable protein – strip-grazing (LS1), low rumen degradable protein – compartmented continuous grazing (LC1), high rumen degradable protein – strip-grazing (HS1), and high rumen degradable protein – compartmented continuous grazing (HC1), during 3 measurement periods (M).Pasture DMI (PDMI), total DMI (TDMI), milk and milk constituents yield.*

		Grazing Treatment G		Rumen degradable dietary protein treatment P			Combined effect G×P					*P*-values		
	M	C	S	L	H	lsd	LC1	HC1	LS1	HS1	lsd	G	P	G×P
PDMI kg/d	P1	6.8	5.7	6.3	6.2	0.57	6.5	7.0	6.0	5.5	0.81	0.919	0.469	0.424
	P2	4.1	4.8	4.5	4.4		4.2	4.1	4.9	4.6				
	P3	3.2	3.7	3.6	3.3		3.4	3.0	3.8	3.6				
TDMI kg/d	P1	21.3	19.9	20.0	21.2	0.75	20.6	22.0	19.4	20.4	1.06	0.023	<0.001	0.853
	P2	18.8	18.4	18.2	19.1		18.3	19.3	18.1	18.8				
	P3	18.5	18.2	17.8	19.0		18.0	19.1	17.5	18.9				
Milk kg/d	P1	32.1	31.1	29.1	34.1	1.68	29.7	34.4	28.5	33.7	2.37	0.823	<0.001	0.893
	P2	28.3	28.5	25.9	30.9		25.8	30.8	25.9	31.0				
	P3	27.2	27.5	25.4	29.3		25.2	29.1	25.5	29.4				
Fat	P1	1231	1176	1147	1261	75.4	1172	1291	1121	1231	106.6	0.399	0.180	0.722
g/d	P2	1092	1056	1039	1109		1059	1125	1020	1093				
	P3	942	953	975	920		987	897	963	942				
Protein	P1	1119	1067	1015	1171	53.8	1041	1196	989	1145	76.1	0.245	<0.001	0.823
g/d	P2	1015	987	932	1070		946	1084	918	1056				
	P3	940	941	911	970		917	963	904	978				
Lactose	P1	1476	1403	1336	1542	83.4	1390	1562	1283	1522	117.9	0.600	<0.001	0.660
g/d	P2	1291	1300	1186	1404		1185	1398	1188	1411				
	P3	1223	1235	1138	1320		1136	1310	1141	1329				
Urea	P1	9	7	6	11	1.7	7	12	4	9	2.5	0.227	<0.001	0.688
Mg/0.1L	P2	10	12	9	13		8	13	10	14				
	P3	9	8	7	11		8	11	6	11				

Table 8 continued a. *Experiment 1: Treatment means, main and combined effects of two grazing management treatments (G) and two rumen degradable dietary protein treatments (P). Grazing management treatments: strip-grazing (S); compartmented continuous grazing (C);Dietary protein treatments low rumen degradable protein (L); high rumen degradable protein (H). Grazing and dietary protein treatments in a 2×2 arrangement: low rumen degradable protein – strip-grazing (LS1), low rumen degradable protein – compartmented continuous grazing (LC1), high rumen degradable protein – strip-grazing (HS1), and high rumen degradable protein – compartmented continuous grazing (HC1), during 3 measurement periods (M).*

		Grazing Treatment G		Rumen degradable dietary protein treatment P			Combined effect G×P					*P*-values		
	M	C	S	L	H	lsd	LC1	HC1	LS1	HS1	lsd	G	P	G×P
Fat	P1	3.87	3.80	3.97	3.70	0.219	3.99	3.76	3.95	3.65	0.310	0.517	<0.001	0.797
%	P2	3.88	3.74	4.03	3.59		4.11	3.65	3.95	3.52				
	P3	3.50	3.50	3.86	3.13		3.91	3.08	3.81	3.19				
Protein	P1	3.51	3.45	3.51	3.45	0.074	3.54	3.49	3.49	3.41	0.105	0.278	0.017	0.912
%	P2	3.60	3.48	3.62	3.47		3.68	3.53	3.56	3.41				
	P3	3.48	3.45	3.61	3.32		3.65	3.32	3.57	3.32				
Lactose	P1	4.61	4.51	4.59	4.59	0.096	1390	1562	1283	1522	0.135	0.299	0.364	0.453
%	P2	4.57	4.57	4.55	4.59		1185	1398	1188	1411				
	P3	4.50	4.48	4.50	4.49		1136	1310	1141	1329				

Table 8 continued b. *Experiment 1: Treatment means, main and combined effects of two grazing management treatments (G) and two rumen degradable dietary protein treatments (P). Grazing management treatments: strip-grazing (S); compartmented continuous grazing (C);Dietary protein treatments low rumen degradable protein (L); high rumen degradable protein (H). Grazing and dietary protein treatments in a 2×2 arrangement: low rumen degradable protein – strip-grazing (LS1), low rumen degradable protein – compartmented continuous grazing (LC1), high rumen degradable protein – strip-grazing (HS1), and high rumen degradable protein – compartmented continuous grazing (HC1), during 3 measurement periods (M).NEL-intake/NELrequirements×100 (NELcov%), DVE-intake /DVErequirements×100 (DVE cov%), feacal output (FO) of organic matter, organic matter digestibility (OMD), total nitrogen intake (TNin), Pasture nitrogen intake (PNin), Nitrogen Use Efficiency (NU-milk = Nitrogen in milk/Nitrogen intake).*

		Grazing Treatment G		Rumen degradable dietary protein treatment P			Combined effects G×P					*P*-values		
	M	C	S	L	H	lsd	LC1	HC1	LS1	HS1	lsd	G	P	G×P
NEL cov%	P1	102	101	103	99	3.4	103	100	103	98	4.8	0.630	0.081	0.629
	P2	99	100	102	97		101	97	103	97				
	P3	104	103	102	105		103	105	102	104				
DVE cov%	P1	89	89	92	86	4.5	91	87	94	85	6.4	0.437	0.002	0.485
	P2	91	95	97	89		95	87	100	90				
	P3	97	97	98	96		99	96	98	95				
FO kg OM/d	P1	6.7	6.4	6.4	6.7	0.34	6.5	6.9	6.3	6.5	0.47	0.392	0.084	0.497
	P2	6.7	6.5	6.5	6.7		6.6	6.8	6.5	6.5				
	P3	6.4	6.5	6.2	6.6		6.1	6.6	6.4	6.6				
OMD	P1	0.66	0.65	0.65	0.66	0.012	0.66	0.66	0.65	0.66	0.018	0.198	0.086	0.320
	P2	0.62	0.62	0.61	0.63		0.61	0.62	0.61	0.63				
	P3	0.63	0.62	0.62	0.63		0.64	0.63	0.61	0.63				
TNin g/d	P1	467	431	384	514	18.3	391	543	378	485	25.9	0.260	<0.001	0.29
	P2	413	427	365	476		354	472	375	480				
	P3	406	403	355	453		360	452	351	454				
PNin g/d	P1	202	171	177	195	18.8	180	224	174	167	26.6	0.925	0.894	0.217
	P2	136	163	149	150		132	140	166	161				
	P3	129	135	140	123		139	118	141	128				
NUE-milk	P1	0.38	0.39	0.42	0.36	0.018	0.42	0.35	0.41	0.37	0.026	0.537	<0.001	0.248
	P2	0.39	0.36	0.40	0.35		0.42	0.36	0.39	0.34				
	P3	0.37	0.37	0.40	0.34		0.40	0.33	0.41	0.34				

Table 9 *<u>Experiment 2:</u> Treatment means, main and combined effects of two grazing management treatments (G) and three dietary protein treatments (P). Grazing management treatments strip-grazing (S); compartmented continuous grazing (C). Dietary protein treatments: low rumen degradable protein (L); high rumen degradable protein (H); high rumen degradable protein and high intestinal digestible protein (HH). The experiment was performed in a 2×3 arrangement: low rumen degradable protein–strip-grazing (LS2), low rumen degradable protein–compartmented continuous grazing (LC2), high rumen degradable protein–strip-grazing (HS2), and high rumen degradable protein–compartmented continuous grazing (HC2), high rumen degradable protein plus high intestinal digestible protein–strip-grazing (HHS2), high rumen degradable protein plus high intestinal digestible protein–compartmented continuous grazing (HHC2)), during 3 measurement periods (M).Pasture DMI (PDMI), total DMI (TDMI), milk and milk constituents yield.*

	M	Grazing Treatment G			Rumen degradable dietary protein treatment P				Combined effects G×P							*P*-values		
		C	S	lsd	L	H	HH	lsd	LC2	HC2	HHC2	LS2	HS2	HHS2	lds	G	P	G×P
PDMI	P1	7.1	7.2	0.80	7.3	7.2	6.9	0.99	7.5	6.6	7.1	7.1	7.7	6.7	1.39	0.118	0.132	0.602
	P2	6.6	6.9		7.4	6.7	6.2		7.4	6.6	5.9	7.4	6.8	6.5				
	P3	4.6	5.8		5.6	5.3	4.7		5.3	4.5	4.0	5.9	6.0	5.5				
TDMI	P1	18.3	17.8	0.87	18.2	18.0	18.1	1.07	18.6	17.8	18.6	17.7	18.1	17.7	1.51	0.443	0.485	0.593
kg/d	P2	16.9	18.2		18.0	17.4	17.1		17.6	16.8	16.2	18.5	17.9	18.1				
	P3	17.7	17.7		17.9	17.9	17.4		18.3	17.7	17.2	17.6	18.1	17.5				
Milk	P1	32.7	30.7	1.87	30.4	31.3	33.3	2.29	32.3	31.7	34.0	28.5	31.0	32.7	3.23	0.994	0.144	0.584
kg/d	P2	23.6	25.7		24.0	24.7	25.2		23.3	24.3	23.1	24.6	25.1	27.2				
	P3	24.1	23.9		22.9	24.7	24.5		22.6	25.7	24.2	23.2	23.8	24.8				
Fat	P1	1149	1097	79.4	1122	1132	1116	97.2	1186	1155	1107	1057	1110	1125	137.5	0.550	0.648	0.215
g/d	P2	930	978		912	953	996		920	928	942	905	978	1050				
	P3	868	930		882	904	912		898	845	860	866	962	963				
Protein	P1	1067	992	61.4	971	1034	1084	75.2	1041	1044	1117	902	1023	1050	106.3	0.548	0.006	0.313
g/d	P2	798	857		777	832	872		775	817	802	779	848	942				
	P3	857	828		775	881	872		797	920	855	753	842	890				
Lactose	P1	1459	1372	89.0	1371	1390	1486	109.0	1458	1396	1522	1283	1384	1450	154.1	0.955	0.291	0.706
g/d	P2	1026	1117		1052	1072	1091		1024	1054	1001	1080	1090	1180				
	P3	1054	1044		999	1082	1065		981	1126	1055	1017	1039	1075				
Urea	P1	21	14	2.0	14	19	21	2.4	16	23	24	11	14	17	3.5	<0.001	<0.001	0.466
mg/0.1L	P2	23	20		15	24	26		18	25	27	12	23	25				
	P3	14	16		9	17	19		10	14	18	9	19	20				

Table 9 continued a. *Experiment 2: Treatment means, main and combined effects of of two grazing management treatments (G) and three dietary protein treatments (P). Grazing management treatments strip-grazing (S); compartmented continuous grazing (C). Dietary protein treatments: low rumen degradable protein (L); high rumen degradable protein (H); high rumen degradable protein and high intestinal digestible protein (HH). The experiment was performed in a 2×3 arrangement: low rumen degradable protein–strip-grazing (LS2), low rumen degradable protein–compartmented continuous grazing (LC2), high rumen degradable protein–strip-grazing (HS2), and high rumen degradable protein–compartmented continuous grazing (HC2), high rumen degradable protein plus high intestinal digestible protein–strip-grazing (HHS2), high rumen degradable protein plus high intestinal digestible protein–compartmented continuous grazing (HHC2)), during 3 measurement periods (M).*

	M	Grazing Treatment G			Rumen degradable dietary protein treatment P				Combined effects G×P							*P*-values		
		C	S	lsd	L	H	HH	lsd	LC2	HC2	HHC2	LS2	HS2	HHS2	lds	G	P	G×P
Fat	P1	3.56	3.31	0.259	3.72	3.65	3.38	0.317	3.72	3.69	3.26	3.71	3.60	3.50	0.448	0.600	0.692	0.208
%	P2	4.00	3.83		3.82	3.89	4.03		3.95	3.86	4.20	3.69	3.92	3.87				
	P3	3.61	3.89		3.88	3.67	3.70		4.02	3.28	3.54	3.75	4.07	3.86				
Protein	P1	3.26	3.23	0.117	3.19	3.29	3.26	0.143	3.23	3.27	3.29	3.16	3.31	3.23	0.202	0.180	0.012	0.300
%	P2	3.46	3.35		3.25	3.38	3.51		3.33	3.37	3.55	3.18	3.39	3.48				
	P3	3.56	3.47		3.39	3.58	3.58		3.54	3.61	3.54	3.25	3.55	3.61				
Lactose	P1	4.47	4.47	0.070	4.51	4.46	4.44	0.086	4.52	4.41	4.47	4.50	4.46	4.44	0.122	0.806	0.443	0.917
%	P2	4.34	4.36		4.39	4.32	4.34		4.38	4.33	4.30	4.39	4.35	4.34				
	P3	4.36	4.36		4.36	4.35	4.37		4.35	4.38	4.37	4.38	4.37	4.33				

Table 9 continued b. *Experiment 2:* Treatment means, main and combined effects of of two grazing management treatments (G) and three dietary protein treatments (P). Grazing management treatments strip-grazing (S); compartmented continuous grazing (C). Dietary protein treatments: low rumen degradable protein (L); high rumen degradable protein (H); high rumen degradable protein and high intestinal digestible protein (HH). The experiment was performed in a 2×3 arrangement: low rumen degradable protein–strip-grazing (LS2), low rumen degradable protein–compartmented continuous grazing (LC2), high rumen degradable protein–strip-grazing (HS2), and high rumen degradable protein–compartmented continuous grazing (HC2), high rumen degradable protein plus high intestinal digestible protein–strip-grazing (HHS2), high rumen degradable protein plus high intestinal digestible protein–compartmented continuous grazing (HHC2)), during 3 measurement periods (M).Pasture DMI (PDMI), total DMI (TDMI), milk and milk constituents yield,
NEL-intake/NELrequirements×100 (NELcov%), DVE-intake /DVErequirements×100 (DVE cov%), feacal output (FO) of organic matter, organic matter digestibility (OMD), total nitrogen intake (TNin), Pasture nitrogen intake (PNin), Nitrogen Use Efficiency (NU-milk = Nitrogen in milk/Nitrogen intake).

		Grazing Treatment G			Rumen degradable dietary protein treatment P				Combined effects G×P							*P*-values		
	M	C	S	lds	L	H	HH	lds	LC2	HC2	HHC2	LS2	HS2	HHS2	lds	G	P	G×P
NEL	P1	94	97	4.1	98	95	94	5.0	95	92	94	101	97	94	7.1	0.379	0.004	0.698
cov%	P2	101	104		108	101	98		106	100	97	110	102	99				
	P3	108	107		112	107	104		113	107	105	111	108	103				
DVE	P1	95	102	5.6	103	94	98	6.9	97	91	96	109	97	100	9.7	0.116	<0.001	0.334
cov%	P2	113	110		119	105	110		117	106	115	120	105	105				
	P3	109	117		121	105	113		119	97	112	123	113	114				
FO kg	P1	4.0	4.2	0.23	4.0	4.1	4.1	0.29	4.0	4.0	3.9	4.1	4.3	4.3	0.40	0.593	0.132	0.109
OM/d	P2	4.6	4.8		5.0	4.5	4.6		4.8	4.6	4.3	5.2	4.3	4.8				
	P3	5.2	4.9		5.2	5.0	4.9		5.3	5.3	5.0	5.0	4.8	4.9				
OMD	P1	0.77	0.75	0.011	0.76	0.75	0.75	0.013	0.77	0.76	0.77	0.75	0.75	0.74	0.018	0.487	0.346	0.002
	P2	0.70	0.71		0.70	0.72	0.71		0.70	0.70	0.71	0.70	0.74	0.71				
	P3	0.69	0.70		0.69	0.70	0.69		0.69	0.68	0.69	0.69	0.72	0.70				
TNin	P1	472	453	26.7	410	475	502	32.7	429	469	517	392	481	487	46.3	0.599	<0.001	0.404
g/d	P2	424	434		390	439	458		393	435	445	387	442	472				
	P3	412	438		381	444	450		381	426	429	380	462	470				
PNin	P1	244	227	27.0	241	236	228	33.1	258	228	245	224	245	212	46.8	0.793	0.129	0.501
g/d	P2	215	205		230	207	193		240	213	192	220	200	194				
	P3	171	207		204	193	172		198	170	149	210	215	197				
NUE	P1	0.36	0.35	0.016	0.37	0.34	0.34	0.020	0.38	0.35	0.34	0.37	0.34	0.34	0.028	0.292	0.033	0.414
	P2	0.29	0.31		0.31	0.30	0.30		0.31	0.29	0.28	0.32	0.30	0.31				
	P3	0.33	0.30		0.32	0.31	0.31		0.33	0.34	0.31	0.31	0.29	0.30				

3.3.2.8 Rumen fermentation

3.3.2.9 Rumen pH

In Exp1 rumen pH was measured in pasture intake measurement period 2 (July) and 3 (September). In Exp2 rumen pH was measured in all pasture intake measurements periods (May, July, September). In Exp1 both experiments, the rumen pH was not different between the treatments groups (Figure 3). In Exp2 rumen pH was measured in all pasture intake measurement periods 1, 2 and 3. In Exp2 rumen pH was measured in all pasture intake measurements periods. The rumen pH was not different between grazing treatments C and S and dietary protein treatments L, H and HH (Figure 4).

Figure 3 *Experiment 1: Rumen pH measurement periods 2 and 3*

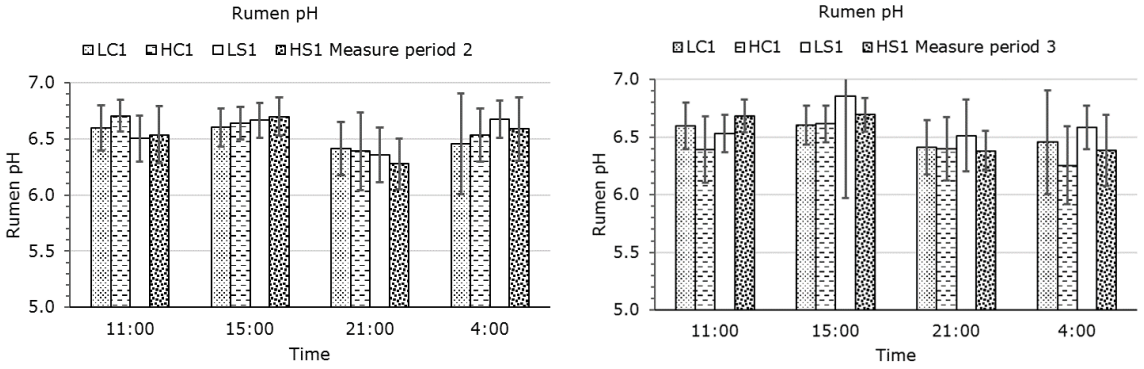
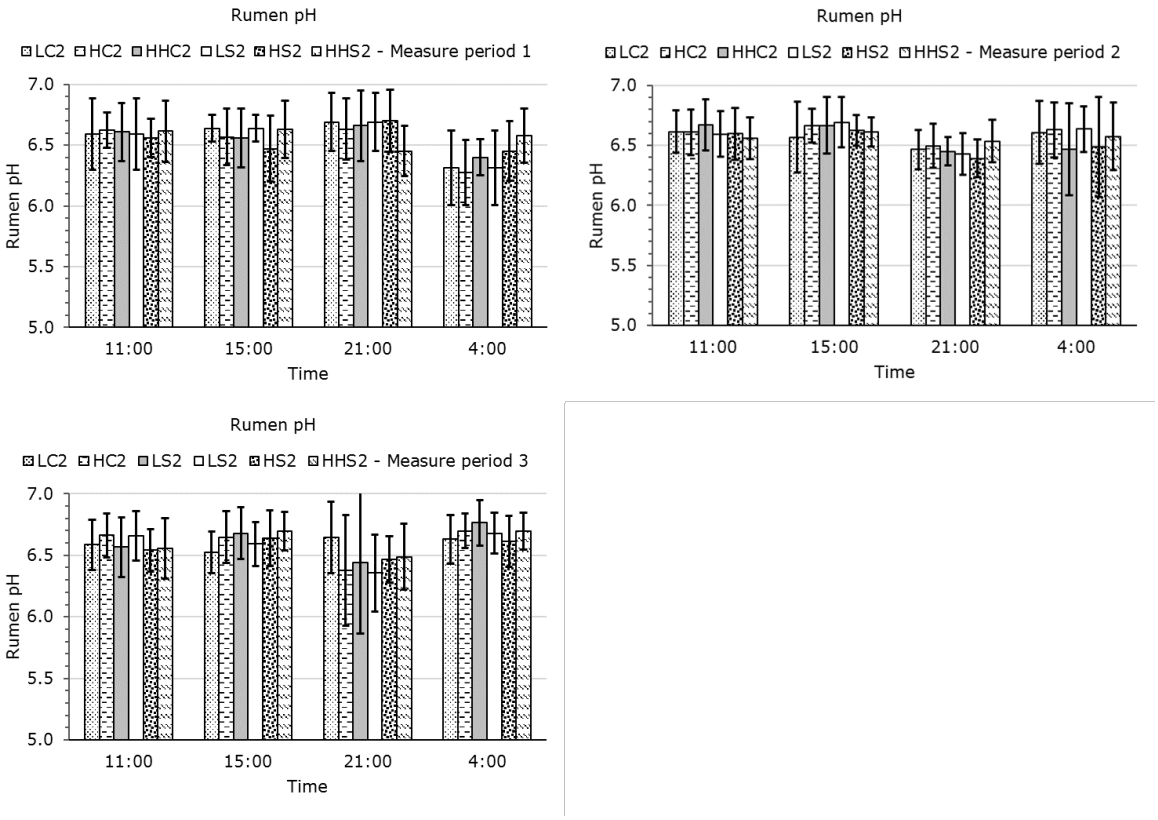


Figure 4 *Experiment 2: Rumen pH measurement periods 1, 2 and 3*



3.3.2.10 Rumen NH_3 concentrations

In Exp1 (2016) among all treatments during measurement period 2 and 3 low rumen NH_3 levels were observed (Figure 5). Within measurement periods and treatment groups there were no differences in rumen NH_3 between sampling time points. Despite the differences dietary RDP there were only numerical differences in rumen NH_3 concentration between the L and H treatment groups.

In Exp2, within measurement periods, the rumen NH_3 concentrations were lowest at 400 h (Figure 6). In measurement period 1 the NH_3 concentration were significantly lower at 400 h. During all treatment periods treatment the lowest NH_3 concentrations were observed with treatment LS2.

Figure 5 *<u>Experiment 1:</u> Rumen NH_3 concentrations*

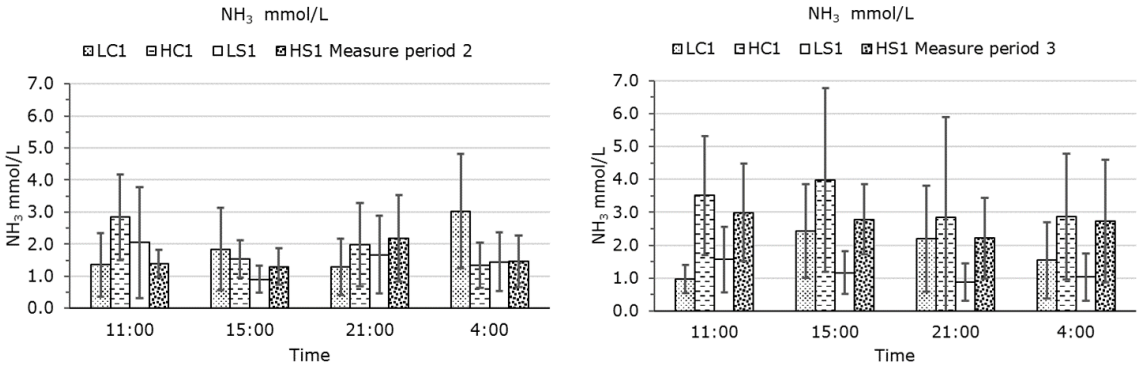
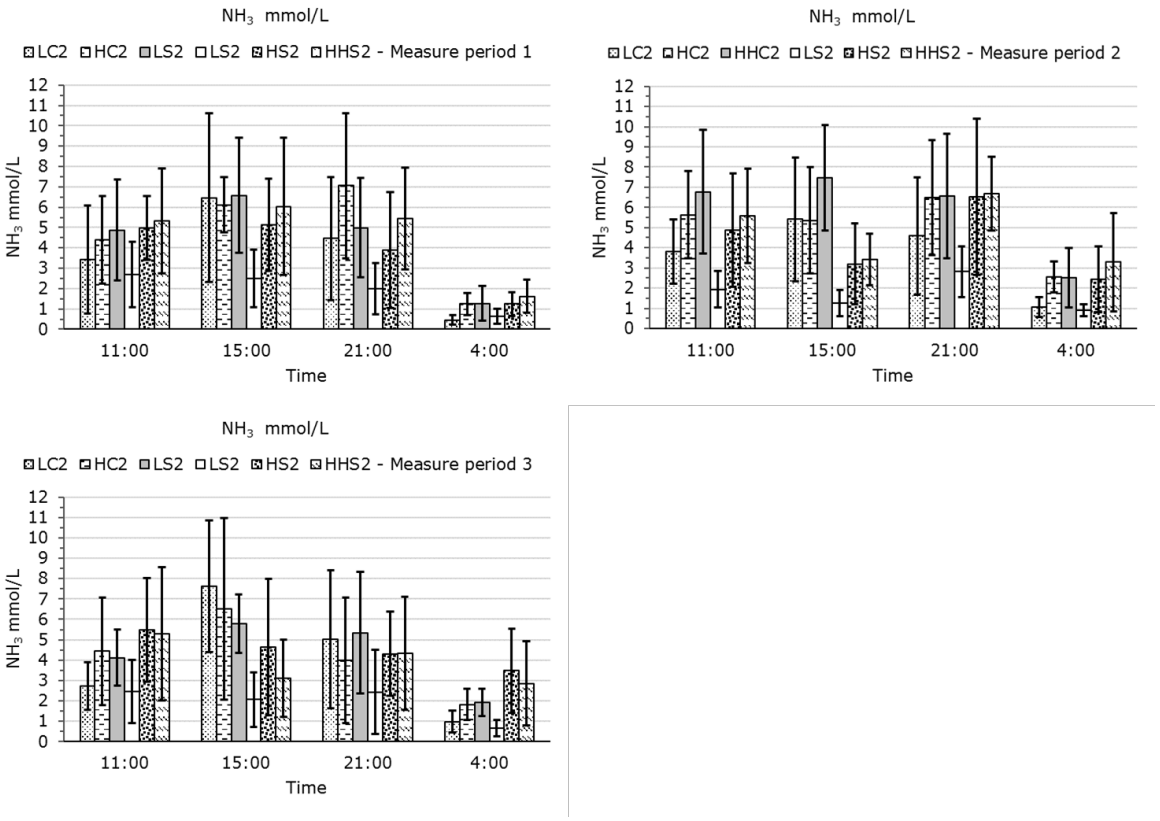


Figure 6. *<u>Experiment 2:</u> Rumen NH_3 concentrations*



3.3.2.11 Rumen volatile fatty acids concentrations

In both Exp 1 and Exp 2, within measurement period and time point, there were no differences in the concentrations of volatile fatty acids between the treatment groups (See Figure 7 for Exp 1; see Figure 8a and 8b for Exp2). However, in Exp 1, in both measurement period 2 and 3, the average NGR was lower for the HC and HS treatment groups than for LC and LS treatment groups.

Figure 7 *<u>Experiment 1:</u> Rumen volatile fatty acid (VFA) concentration and non-glucogenic to glucogenic VFA ratio's*

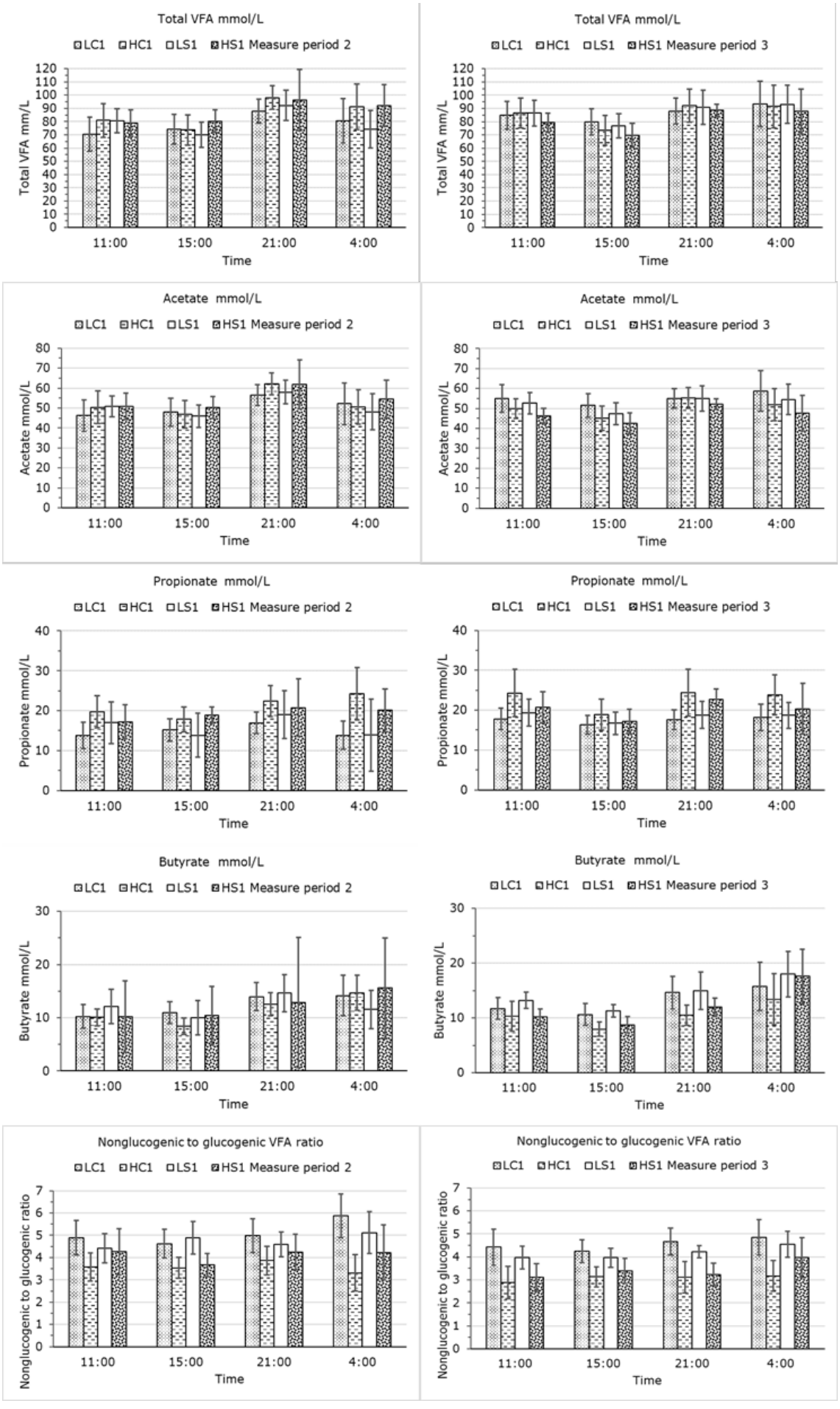


Figure 8a *<u>Experiment 2:</u> Concentrations of total volatile fatty acids (Total VFA), acetate, propionate*

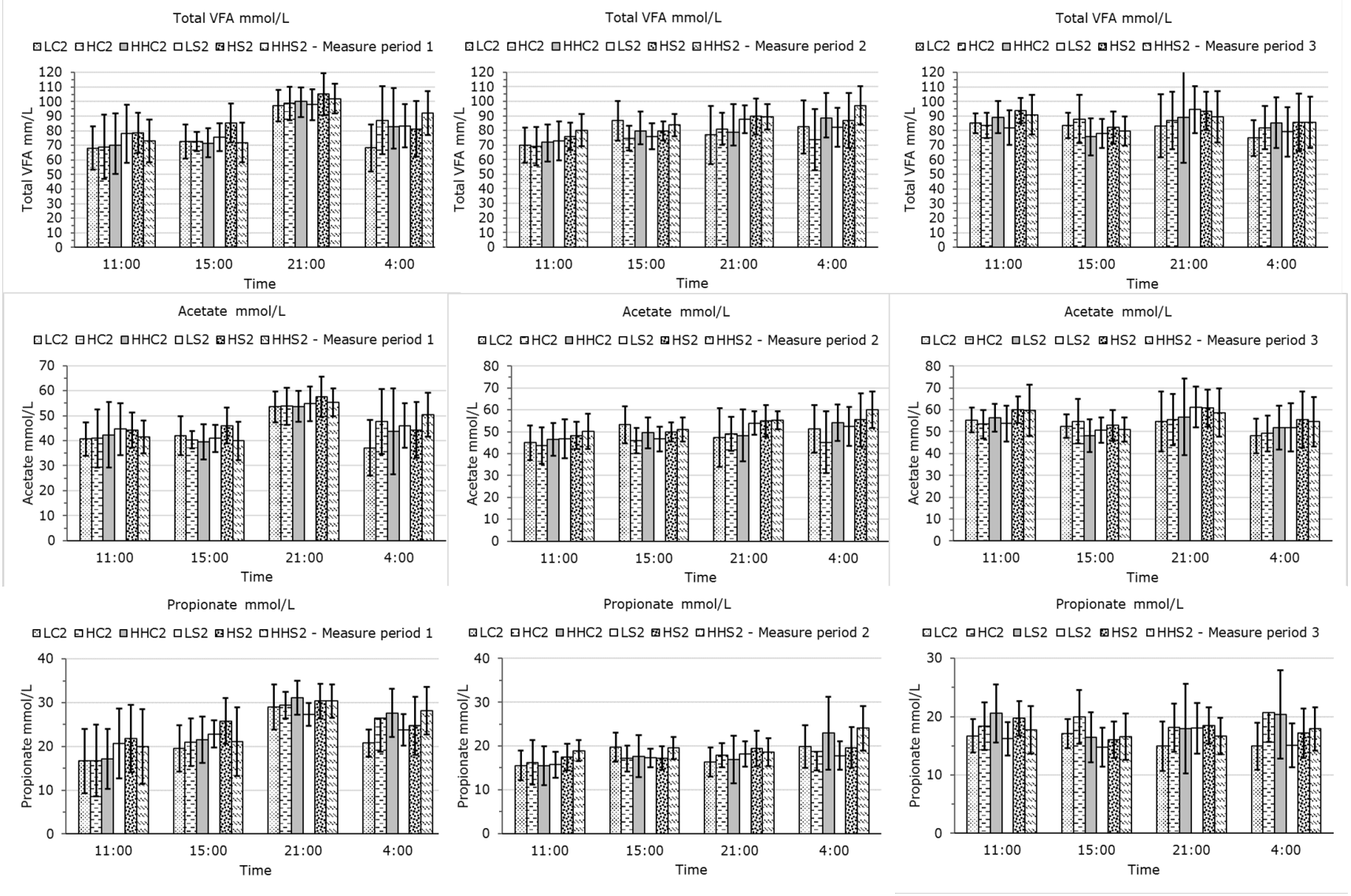
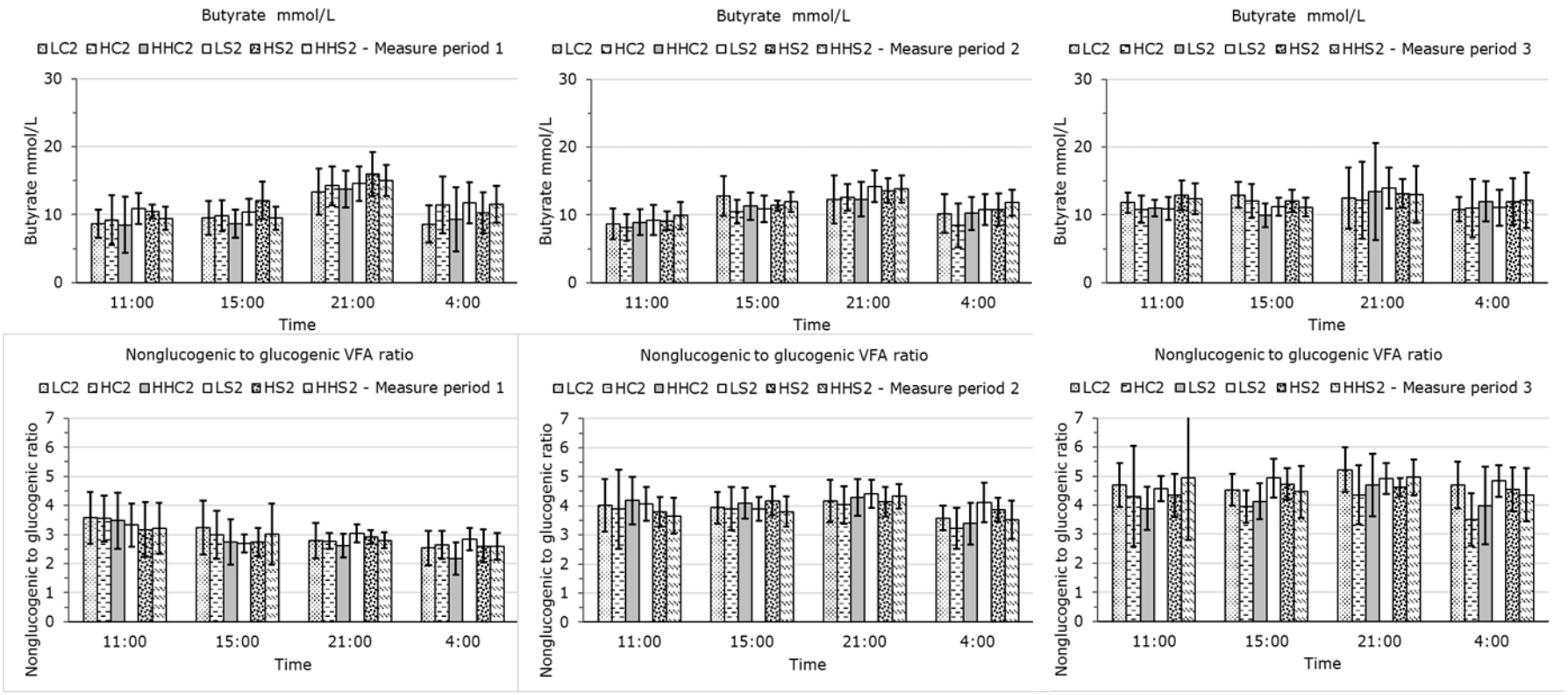


Figure 8b. *<u>Experiment 2:</u> Concentrations of butyrate and the non-glucogenic to glucogenic volatile fatty acid ratio's (NGR), NGR is calculated as (acetate+2×(butyrate+isobutyrate)+valerate+isovalerate)/(propionat+valerate+isovalerate)*



4 Discussion and conclusions

4.1 Experimental design

Both grazing experiments Exp1 and Exp2 were carried out within the framework of the project Amazing Grazing. The project Amazing Grazing is an initiative to promote grazing in the Netherlands and addresses the major constraints of grazing in intensive dairy farming in the Netherlands (Schils et al., 2018). Therefore, the grazing experiments were having multiple objectives. One of the objectives was a comparison of the effects of two contrasting grassland management systems, (i.e. compartmented continuous grazing (C) and strip grazing (S)) on pasture dry matter yield (kg DM/year) and pasture utilization (pasture intake (kg DM/ha)/pasture growth (kg DM/ha), and the relationships between intake behaviour (i.e. time spent grazing, ruminating, walking idling), pasture allowance (kg DM/cow/day), sward structure (i.e. sward height, leaf to stem ratio, dry matter yield) and herbage intake (i.e. the interaction of the animal and grass). The impact of grazing management systems cannot be studied during short periods of time. Grazing management studies must be carried out during the whole grazing season; the reason being that management decisions (e.g. pre- and post-grazing pasture mass, cutting strategy) which are implemented on a certain time point, will have inevitably an effect on sward structure (i.e. sward height, leaf to stem ratio, dry matter yields) later on in the grazing season (Holmes et al., 1992; Hoogendoorn et al., 1992). Therefore, a continuous experimental design is necessary to compare the effects of grazing systems on animal performance, pasture dry matter yield and pasture utilization.
However, with regard to animal production, a continuous design has the disadvantage that the stage of lactation is confounded with the progress of the grazing season and changes in sward structure, feeding value, available herbage during the grazing season and in conjunction with that, the level of supplemental roughage. Therefore, it should be beard in mind that animal performance is time related which is responsible for week or measurement period effects on pasture composition and animal performance parameters.

4.2 Pasture intake

4.2.1 Effect of grazing system

In both experiments there were significant measurement period (M) and grazing system by measurement period interactions (G×M). The effect of M and the G×M interactions are inherent to differences in grazing management of C and S. Factors such as pasture allowance, sward height and the contamination of the sward (i.e. net herbage allowance, amount of pasture rejected) have a strong effect on grazing intake (Peyraud and González-Rodríguez, 2000). In both grazing systems, pasture allowance pre-grazing sward height declined during the grazing season. There were differences in pre- and post-grazing sward heights and cutting rates between S and C (Holshof, 2019 in progress). In both Exp1 and Exp2, during the intake measurement periods, the mean pre-grazing sward heights were higher for S than for C, whereas the post-grazing sward heights were lower for S than for C (Holshof, 2019 in progress). Low pre-grazing sward heights improves herbage quality as this prevents the build-up of stem and dead material (Holmes et al., 1992; Hoogendoorn et al., 1992). Higher pre-grazing heights may result in reduction in herbage quality as it is associated with accumulation of stem and dead material at the base of the sward (Holmes et al., 1992; Hoogendoorn et al., 1992). The management of grazing systems S and C resulted in different in cutting rates (area cut for silage (ha) + area topped (ha))/total hectares×100% for grazing; (Holshof, 2019 in progress). The cutting rates were higher for S (359 and 206% in Exp1 and Exp2, respectively) than for C (265 and 198, respectively) (Holshof, 2019 in progress). Consequently, the area of clean aftermath pasture grazed by the cows on S was higher compared to C. Cutting and topping results in removal of old and tall herbage and a regrowth with and higher leaf to stem ratio. In addition, a higher cutting rate (i.e. alternating grazing and cutting) results also in a longer interval between two grazing events. The

decay of dung on pastures is time dependent (Castle and Macdaid, 1972; Macdiarmid and Watkins, 1972; Vadas et al., 2011). Therefore, longer intervals between two grazing events will result in a larger decay of dung spots and therefore in less rejection of grass by the cows and higher net herbage allowance per hectare. Despite difference in pre-grazing height, the composition and feeding values of the grazed herbage were similar for both grazing systems during all treatment periods. The period (M) and grazing system by measurement period interactions (G×M) are therefore mainly related to differences in available pasture and in conjunction with that the level of supplementary roughage.

4.2.2 Effect of protein treatment

The main objective of this study was investigate whether creating a temporary shortage of RDP during night time could motivate cows to increase their voluntary intake of pasture in order to restore a shortage of RDP. This was tested with two contrasting grazing systems which cover the most common grazing practices in the Netherlands.
The treatments (concentrate composition and allowance) were imposed as intended and therefore the experimental design was implemented successfully.
In both Exp1 and Exp2 PMDI was numerically higher for protein treatment L, however these differences were not statistically significant. Neither there significant effects of additional DVE on PDMI (Exp2). From these results it was concluded that feeding low RDP supplements does not motivate cows to increase their grazing intake. However, these does not suggest that cows do not have the capability to optimize their diet. In Exp1 the cows on the low rumen degradable dietary protein treatment had a significantly lower TDMI than cows on the high rumen degradable dietary protein treatment. The lower TDMI was result of a reduced intake of maize silage of cows on the low RDP treatment compared to the cows on the high RDP treatment. However, because the cows were supplemented with fixed amounts of maize silage and therefore it is not possible to draw statistically substantiated conclusions. Nevertheless, the reduced intake of maize silage in cows fed low RDP supplements may that suggest cows on the low protein treatment have reduced the intake of maize silage which is low in RDP in Exp2. The TDMI was not affected by the protein treatment, neither there were indications for a reduced intake of maize silage or any other change in intake that could suggest any adaptation to a shortage of RDP.
The differences in the effect of protein treatment on TDMI between Exp1 and Exp2 may be related to the differences in rumen NH_3 concentration observed in Exp1 and in Exp2. In both treatment L and H of Exp1, the mean levels of ammonia in the rumen were below 1.8 mmol NH_3/L (0.8-3 mmol/L) and below 3 mmol/L (0.7-4.0 mmol/L), in measurement period 2 (July) and 3 (Sept) respectively. In addition, in Exp1 there were no distinct differences in rumen NH_3 concentration between sampling time points (400, 1100, 1500 and 2100 h). Russell and Strobel (1987) concluded that at least 50 mg NH_3 (2.94 mmol NH_3/L) is required for an unrestricted microbial protein synthesis. Broderick et al. (2010) concluded that N outflow from the rumen was equal to N intake at a diet CP concentration of 147 g/kg DM and a rumen NH_3 concentration of 71 mg NH_3-N/L (5 mmol NH_3/L) and milk urea concentration of 18 mg/dL (8.1 mg MUN/dL). This means that, in steady state, there is no net absorption of NH_3 from the rumen and that CP degradation in the rumen equals the microbial protein synthesis. This may suggest that in Exp1 rumen NH_3 levels were suboptimal for microbial protein synthesis. This is also confirmed by the low milk urea concentrations. However, this was not the case in Exp2, in which the rumen NH_3 levels were above the critical level of 2.94 mmol NH_3/L suggested by Russell and Strobel (1987), with an exception for treatment LS2. Kyriazakis et al. (1999) suggested that that there is a kind of 'requirement' for RDP. This because ruminants depend largely on RDP for the production of microbial protein. It could be possible that the cows receiving the low RDP diets in Exp1, which were sub-optimal for microbial protein synthesis, have maintained a desired minimum level of ammonia in the rumen by avoiding maize silage. Although, the rumen NH_3 levels in cows receiving the high RDP diets in Exp1, were higher than for the low RDP treatment. However these levels were still below the critical level for optimal microbial protein synthesis. The lack of response of rumen NH_3-levels to a higher RDP levels to higher protein intake has also observed elsewhere. Ahvenjärvi and Huhtanen (2018) found no response to ruminal urea-N infusions up to 49 N g/d in cows consuming a basal ration which delivered approximately 470 g N/d. The additional nitrogen was captured by the rumen microbes until a sufficient intracellular NH_3-N was reached (Ahvenjärvi and Huhtanen, 2018). These authors concluded that rumen bacteria are capable to deplete efficiently NH_3-

N from the rumen fluid when the rate of release of NH_3-N from protein degradation and microbial turn-over is lower than the microbial uptake. The results of Ahvenjärvi and Huhtanen (2018) indicate that rumen NH_3-N concentrations are a poor indicator of N deficiency in the rumen at low levels of diet CP. In Exp1 and Exp2, the H treatment resulted in improved milk protein yields. In Exp1, the improved milk protein yield is partly the result of an improved TDMI and net energy intake (Exp1) and an improved microbial protein synthesis (Exp1 and Exp2). Despite increased milk protein yield, the increased levels of RDP and DVE resulted in reduced nitrogen use efficiencies. Around 12 to 25 % of the supplementary dietary nitrogen was converted milk protein nitrogen. These levels compares with the results Ahvenjärvi and Huhtanen (2018). They found, at similar levels of N intake and milk yields as in our experiment, that 18% of supplementary N was converted in milk protein.
In Exp1 and Exp2 there were no effects of treatments on FO and OMD. The observed OMD was considerably lower in Exp1 than in Exp2. The levels of OMD in Exp1 were low compared with data from supplemented dairy cows published in literature e.g. Ouellet et al. (2004). However, in Exp2, the OMD ranged between 0.69 and 0.77 which is comparable to data from Ouellet et al. (2004). The reason for the low OMD in Exp 1. is unclear and cannot be related to dietary CP concentrations and the OMD derived from *in-vitro* feed analysis. Low rumen nitrogen levels and seem not to be the reason. Fibre digestion is reduced at dietary CP levels below 10% (Ahvenjärvi and Huhtanen, 2018). Whereas, the CP levels in our experiment were much higher. The low OMD levels can be related to an overestimation of faecal output. In this study, the faecal n-alkane concentrations were corrected using the n-alkane recovery rates from Mayes et al. (1986). Wright et al. (2019) found differences in recovery rates of alkanes between seasons. It cannot be ruled out that year-to-year differences alkane recovery may responsible for the differences between Exp1 and Exp2. It should also be noted that the choice of recovery rates may influence the calculated OMD. For example, van den Pol-Van Dasselaar et al. (2006) found in study with cows fed diet based on grass supplemented with maize silage, higher recovery rates of n-alkanes than those reported by Mayes et al. (1986). However, under of overestimation of the recovery rates would only introduce a systematic error, but will not influence difference between the treatments.
In both Exp 1 and Exp2, rumen pH was not different between grazing and protein treatments and above pH 6. Neither, the concentration of VFA's were influenced by grazing and protein treatment. In Exp2, overall, the non-glucogenic to glucogenic VFA ratio (NGR) ratio was lower in measurement period 1, than in measurement period 2 and 3. This is probably related to lower NDF, higher WSC and higher OMD in measurement period 1 compared to 2 and 3.

4.3 Milk performance during the grazing season

4.3.1 Grazing system

Grazing system influenced the shapes of the curves of milk and milk constituent yield during the whole grazing period. However, the accumulated milk, fat, protein yields were not different for strip-grazing and compartmented continuous grazing. From the invention of strip-grazing up to now, many studies have been carried out to compare strip-grazing with any other grazing system. In general, the impact of grazing system on animal performance expressed as yield/cow (e.g. milk, body weight gain), is small. Many studies, reported either no advantageous or disadvantageous effects of grazing system on animal performance (de Geus, 1946; Holmes et al., 1950; Brundage and Petersen, 1952; Arnold and Holmes, 1958; Hood, 1971; Ernst et al., 1980; Remmelink, 1981; Walton et al., 1981; Volesky et al., 1994; Kuusela and Khalili, 2002; Palladino et al., 2009).

4.3.2 Dietary protein treatments

In both Exp1 and Exp2 supplementing cows with concentrate high in RDP resulted in a more persistent milk and milk constituent yield during the whole grazing period than with the low RDP treatment. In Exp1 treatment H resulted in higher cumulative milk, milk protein, and FPCM yield during 185 days of the grazing season. In Exp2 treatment H and HH (High RDP and additional DVE) resulted in higher cumulative milk and milk protein yield during the 132 days of the grazing season. However, cumulative milk, fat, protein and FPCM yields were not significantly different between H and HH. The positive response of additional RDP and DVE are in agreement with many studies which show a

positive response to additional dietary protein and milk and milk protein production (Vérité and Delaby, 2000; Ipharraguerre and Clark, 2005; Huhtanen et al., 2011; Ahvenjärvi and Huhtanen, 2018).

4.4 Differences between years

Despite similar nitrogen intake, and diet composition, we observed within treatment L and H large differences in rumen NH_3 concentration, nitrogen utilisation and OMD between Exp1 conducted in 2016 and Exp2 conducted in 2017. The reason for this is unclear. Differences in growth conditions between 2016 and 2017 may have induced differences plant composition. It is known that grassland management, growth rate, defoliation, fertilisation, duration of regrowth can influence the partitioning of nitrogen between different plant fractions (Delagarde et al., 2000; Duru, 2003; Bryant et al., 2012). These changes in partitioning may cause a shift between more and less soluble nitrogen components (Bryant et al., 2012). Bryant et al. (2012) concluded that although the observed changes in the relative proportion of different nitrogen fractions, the impact on total nitrogen solubility was small. However, the large differences in rumen NH_3 concentration, nitrogen utilisation and OMD between years cannot be neglected. In Exp1, the calculated OEB values of the diets of the L treatment (low RDP) were in the range of -430 to -100 OEB. Whereas the OEB values of the diets of the H treatment (high RDP) were in the range of +250 to +350 OEB. Based on the milk urea prediction model of Schepers and Meijer (1998) for the H treatment (with observed DVE and OEB balances around zero), a milk urea concentration of 18 mg/dL could be expected. However, the observed milk urea concentrations were much lower. This indicates that the N supply in rumen was inadequate and may suggest that the DVE and OEB values of the diet were overestimated.

4.5 Conclusions

The cows were not motivated to increase their pasture intake when fed low protein supplements and hence this is not a viable strategy to improve pasture intake. Nevertheless, the results of the 2016 study showed that cows seem to balance their rumen degradable protein intake; not through a higher intake of grass but through reducing the voluntary intake of maize silage. The study also showed considerable differences in organic matter and protein digestibility between years along with low rumen ammonia levels indicating a shortage of rumen degradable protein. The results of the 2016 study suggest that DVE and OEB values of the grass pasture were probably overestimated. Additional protein resulted in higher milk and milk protein yields, resulting in a reduced nitrogen use efficiency however.

Summarized conclusions

1. There were no significant effects of grazing system on pasture dry matter intake.
2. Supplementation with low protein feed does not stimulate pasture dry matter intake.
3. Dietary protein treatment influenced the lactation curves of milk, fat, protein and lactose
4. Despite relatively small differences in pasture and diet composition between experiments, there were large differences in rumen NH_3 and milk urea.
5. The results suggests that, despite similar pasture and diet composition, there are large year to year differences in (rumen) digestibility of pasture and feeds which are not reflected in the feeding values.

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Annex 1 Experimental feeds

A1.1a Concentrates: ingredient composition (% fresh feed)

Experiment 1 and 2

L = low rumen degradable protein (OEB, OEB1991)
H = high rumen degradable protein
HH = high rumen degradable protein and high intestinal digestible protein (DVE, DVE1991)
C32 = alkane (dotriacontane) labelled concentrate

	Experimental concentrates				C32 concentrates	
Dietary protein treatment	L	H	HH		Experiment 1	Experiment 2
Barley					6.2	
Corn	37.6	37.6	19.0		18.2	44.5
Rapeseed meal		30.3	25.0			20.0
Rapeseed meal formaldehyde treated	8.4					
Citrus pulp	19.0	19.0	19.0			10.0
Sugar beet molasses	7.3	5.0	5.0			
Sugar beet pulp (20-25% sugar)	24.6	3.0	3.0		16.4	10.0
Palm oil	1.5	2.3	2.3			
Soybean meal			23.9		12.7	10.0
Soybean meal formaldehyde treated						1.5
Soy hulls >31% crude fiber					15.7	
Palmkernel expeller					12.0	
Wheat midlings					4.0	
Wheat					5.0	
Sun flower meal					4.4	
Candy syrup					4.0	4.0
Chalk					1.4	
Urea		1.40				
Salt	0.56	0.59	0.50			
Magnesium Oxide (90%)	0.63	0.51	0.38			
Monocalcium phosphate	0.12	0				
Trace mineral premix	0.30	0.30	0.30			

A1.1b Concentrates: chemical composition and feeding value

Experiment 1 and 2

L = low rumen degradable protein (OEB, OEB1991)
H = high rumen degradable protein
HH = high rumen degradable protein and high intestinal digestible protein (DVE, DVE1991)
C32 = alkane (dotriacontane) labelled concentrate

	Concentrates			Concentrates				C32 concentrates	
	Experiment 1			Experiment 2				Exp1	Exp2
Dietary protein treatment	L	H		L	H	HH			
Chemical composition (g/kg DM, except were indicated else)									
Dry matter (g/kg)	895	898		897	881	884		892	888
Ash	73	72		60	61	74		64	51
Crude protein	110	221		112	228	246		179	198
Crude fiber	97	86		84	71	74		145	67
Ether extract	41	53		41	57	54		32	33
Fat (hydrolysis)	46	57		46	57	64		31	31
Sugar	127	106		129	119	136		111	111
Starch (Amyloglucosidase)	268	267		265	250	149		225	270
NDF	211	197		201	167	189		318	187
ADF	120	112		115	113	112		187	100
ADL	18	25		28	36	37		23	30
Nitrogen	17.5	35.3		17.9	36.5	39.3		28.7	31.7
Phosphorus	2.79	5.01		2.61	4.50	5.02		3.99	4.05
Organic Matter Digestibility % [1]	87.0	84.0		90.3	88.5	88.9		83.2	88.5
Feeding values [2] (g/kg DM, except were indicated else)									
VEM (van Es, 1977)/kg DM	1137	1128		1131	1129	1137		1171	1133
NEL (van Es, 1977) MJ/kg DM	7.85	7.79		7.80	7.79	7.84		8.08	7.82
DVE	112	117		117	115	147		125	125
OEB	-57	56		-60	54	59		-2	6
OEB-2h	-25	39		-25	41	14		-7	-7
FOM_R	567	595		595	585	590		587	598
FOM_R-2h	281	295		295	297	292		278	263
DVE1991	101	107		110	109	146		124	167
OEB1991	-45	57		-47	64	63		0	24
FOM1991	573	543		614	584	596		635	556

[1] In Exp1, chemical composition and organic matter digestibility were obtained from the manufacturer. In Exp2, chemical composition and organic matter digestibility were analysed using wet chemical analysis and in vitro analysis (Tilley & Terry, 1963), respectively.
[2] Feeding values: VEM, NEL (1VEM = 6.9 kJ NEL; Van Es, 1978), DVE, OEB, rumen degradable protein 2h after ingestion (OEB-2h), rumen fermentable organic matter (FOM_R), rumen fermentable organic matter 2h after ingestion (FOM_R-2h; Van Duinkerken et al., 2011), and DVE1991, OEB11991, fermentable organic matter FOM1991 (Tamminga et al., 1994) were obtained from the feed manufacturers.

A1.2 Supplemental roughage (maize silage): chemical composition and feeding value

Experiment 1 and 2: Chemical composition and feeding value of the maize silages during pasture measurement periods P1, P2 and P3. Chemical composition (g/kg DM), unless indicated else. n.a. = not analysed

	Experiment 1			Experiment 2		
Dietary protein treatment	P1	P2	P3	P1	P2	P3
Dry matter (g/kg)	314	329	336	358	393	391
Ash	44	44	44	51	45	51
Organic matter	956	956	956	949	955	949
Crude protein	60	60	59	58	57	55
NH3-N (% of N)	11	12	12	5	5	5
Total crude protein	67	68	67	61	60	57
Crude fat	28	32	34	33	35	35
Crude fiber	214	198	197	189	183	196
Sugar	12	12	12	n.a.	n.a.	n.a.
Starch	304	341	337	342	388	388
NDF	426	409	387	189	183	196
ADF	252	241	234	211	211	201
ADL	17	19	18	32	23	22
Phosphorus	2.1	2.0	2.0	2.5	2.4	2.6
Organic Matter Digestibility (%)	73.7	72.8	74.0	80.1	79.8	79.9
Digestible organic matter	705	696	707	760	762	758
Intestinal degradable protein (DVE)	44	45	46	48	48	49
Rumen degradable protein balance (OEB)	-35	-36	-37	-39	-41	-45
Rumen degradable protein balance 2-h (OEB 2h)	2	1	0	0	-2	-6
Rumen fermentable organic matter (FOM)	527	530	528	514	509	529
Rumen fermentable organic matter 2-h (FOM 2h)	263	269	265	244	242	264
Feed unit milk (van Es, 1977) (/kg DM)	932	918	937	1026	1028	1023
NEL (MJ/kg DM)	6.43	6.34	6.47	7.08	7.09	7.06
DVE1991	45	42	44	52	51	49
OEB1991	-36	-32	-34	-47	-46	-46
FOM1991	507	484	497	555	547	533

Feeding values: VEM, NEL (1VEM = 6.9 kJ NEL; Van Es, 1978), DVE, OEB, rumen degradable protein 2h after ingestion (OEB-2h), rumen fermentable organic matter (FOMR), rumen fermentable organic matter 2h after ingestion (FOMR-2h; Van Duinkerken et al., 2011), and DVE1991, OEB11991, fermentable organic matter FOM1991 (Tamminga et al., 1994) were obtained from the feed manufacturers

A1.3 Pasture grass: chemical composition and feeding value

Experiment 1: Chemical composition and feeding values of the grazed grass by treatment HS1, LS1, HC1 and LC1 in pasture during measurement periods P1, P2, P3. All values in g/kg DM, except when indicated otherwise.

Pasture measurement period	P1				P2				P3			
Grazing treatment	Strip-grazing		Continuous		Strip-grazing		Continuous		Strip-grazing		Continuous	
Dietary Rumen degradable protein	HS1	LS1	HC1	LC1	HS1	LS1	HC1	LC1	HS1	LS1	LC1	HC1
Crude protein	190	182	200	172	218	211	212	199	226	234	250	259
Ash	103	106	98	98	113	106	114	111	112	103	108	110
Organic Matter	897	894	902	902	887	894	886	889	888	897	892	890
OMD (%)	81.0	80.8	79.2	78.1	82.1	82.1	80.9	81.5	82.5	82.8	81.8	82.5
Crude fat	38	38	36	34	42	42	42	41	43	45	41	45
Crude fiber	235	228	247	252	230	232	234	235	216	227	225	217
Sugars	118	121	84	102	91	96	85	100	117	103	77	80
NDF	527	517	567	553	532	534	538	524	492	523	538	511
Phosphorus	4.5	4.4	4.4	4.3	5.3	4.9	5.4	4.7	5.1	5.3	5.0	5.3
Intestinal degradable protein (DVE)	89	86	88	78	97	95	93	90	100	104	106	109
Rumen degradable protein balance (OEB)	35	30	42	22	58	52	53	44	62	67	79	88
Rumen degradable protein balance (2-h) (OEB-2h)	6	4	13	4	16	14	15	11	14	18	25	27
Rumen Fermentable Organic Matter (FOM)	556	548	554	537	561	560	552	547	571	582	582	581
Rumen Fermentable Organic Matter (2 h) (FOM 2h)	187	186	162	166	174	176	166	173	198	191	175	180
VEM (van Es, 1977) (/kg DM)	960	954	935	913	970	979	953	963	979	995	969	985
NEL (MJ/kg DM)	6.62	6.58	6.45	6.30	6.69	6.75	6.57	6.64	6.76	6.87	6.69	6.79
DVE 1991	94	92	95	86	101	100	98	96	104	107	109	112
OEB 1991	30	25	38	20	50	44	47	37	55	59	72	78
FOS 1991	630	627	616	617	618	626	609	621	619	625	611	609

Feeding values: VEM, NEL (1VEM = 6.9 kJ NEL; Van Es, 1978), DVE, OEB, rumen degradable protein 2h after ingestion (OEB-2h), rumen fermentable organic matter (FOM_R), rumen fermentable organic matter 2h after ingestion (FOM_R-2h; Van Duinkerken et al., 2011), and DVE1991, OEB11991, fermentable organic matter FOM1991 (Tamminga et al., 1994) were obtained from the feed manufacturers

A1.3

Experiment 2*:* Chemical composition and feeding values of the grazed pasture in by treatment HS2, LS2, HHS2, LC2, HC2 and HHC2 in pasture measurement periods P1, P2, P3. All values in g/kg DM, except when indicated otherwise

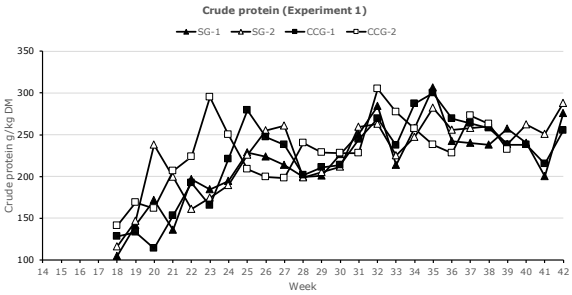
	P1			P2			P3			P1			P2			P3		
	LC2	HC2	HHC2	LC2	HC2	HHC2	LC2	HC2	HHC2	LS2	HS2	HHS2	LS2	HS2	HHS2	LS2	HS2	HHS2
Crude Protein	214	215	214	203	203	204	234	234	232	198	198	197	187	188	187	224	224	224
Ash	91	92	91	122	122	119	116	116	115	87	87	87	124	125	124	110	110	110
Organic Matter	908	909	909	878	878	881	884	884	885	913	913	913	876	875	876	890	890	889
Ether Extract	40	40	40	48	48	47	54	54	54	42	42	42	47	48	47	53	53	52
Crude fiber	202	201	202	235	234	232	234	234	234	200	200	200	250	251	251	234	234	234
Sugar	129	128	129	77	77	80	64	65	65	134	134	133	87	86	87	67	67	66
NDF	202	201	202	235	234	232	234	234	234	200	200	200	250	251	251	234	234	234
DOM	757	757	757	687	688	691	715	714	715	786	788	788	690	689	690	728	728	728
DVE	100	100	100	85	85	86	99	98	98	100	100	100	81	81	81	97	97	97
OEB	53	53	52	46	46	47	68	68	67	43	43	43	35	36	36	63	63	63
OEB2h	10	10	10	14	14	14	23	23	22	5	5	5	10	10	10	21	21	21
FOSp	581	581	580	529	529	533	561	561	560	571	570	570	524	524	524	557	557	557
FOSp2	206	206	206	153	153	157	157	157	157	204	204	204	156	156	156	155	155	155
VEM/kg DM	1012	1012	1012	920	921	924	972	970	971	1062	1064	1065	922	920	921	989	989	989
NEL MJ/kg DM	6.98	6.98	6.98	6.35	6.35	6.38	6.70	6.69	6.70	7.32	7.34	7.35	6.36	6.35	6.36	6.82	6.82	6.82
DVE1991	106	106	105	91	91	92	103	103	102	106	106	106	88	88	88	102	102	102
OEB1991	44	44	44	48	48	48	66	66	65	28	28	28	36	36	36	57	57	57
FOS1991	652	652	652	578	578	581	590	589	591	684	686	686	586	585	586	607	607	607

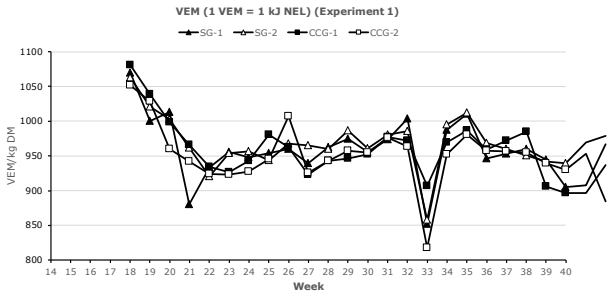
continued Pasture grass: chemical composition and feeding value

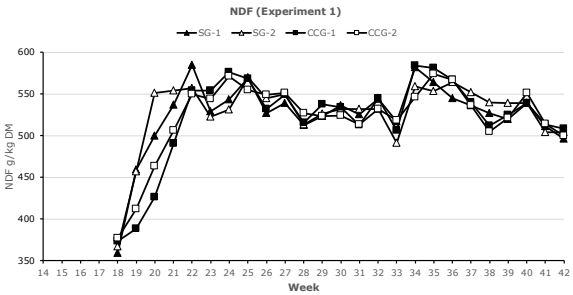
Annex 2 Development of composition and feeding value of pasture during the grazing season

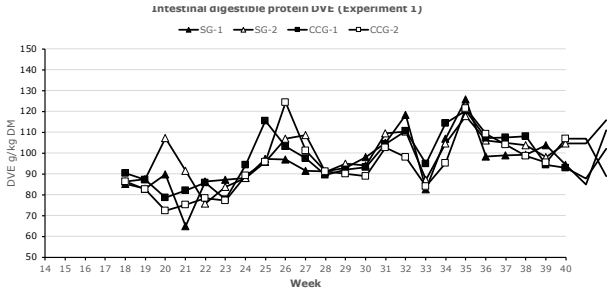
A2.1

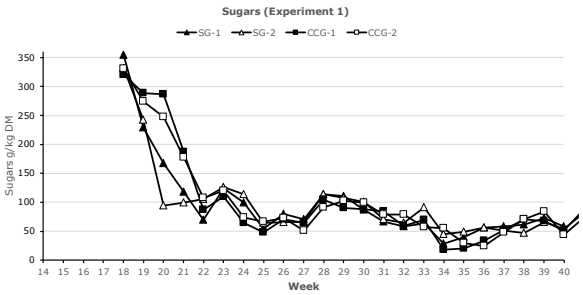
Experiment 1: Chemical composition and feeding values of the pastures used for strip-grazing (S1 and S 2) and compartmented continuous grazing (C1 and C2) during the grazing season; concentrations of crude protein, neural detergent fiber (NDF), VEM (VEM = feed unit milk 1 VEM = 6.9 kJ net energy for lactation; Van Es, 1978), DVE (intestinal digestible protein), OEB (rumen degradable protein balance) according to the DVE/OEB protein evaluation system (Tamminga et al., 1994, revised van Duinkerken et al, 2011).

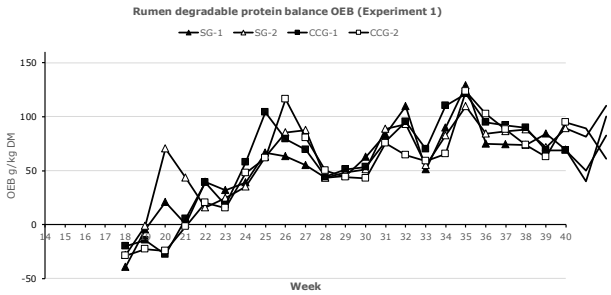


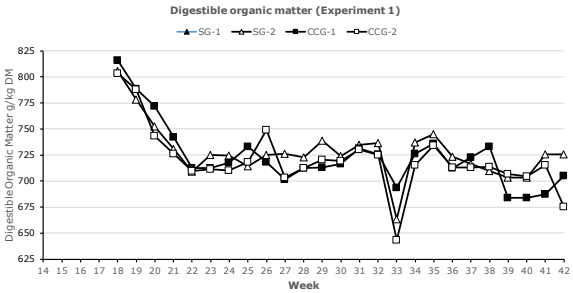






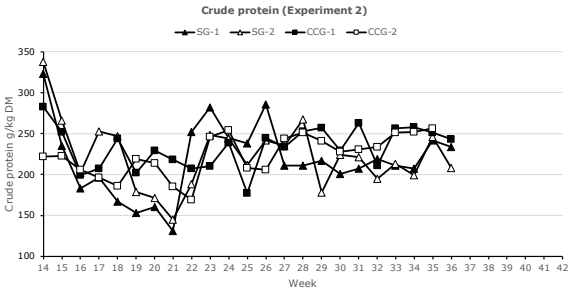


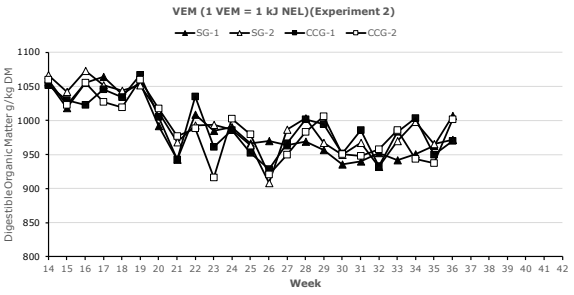


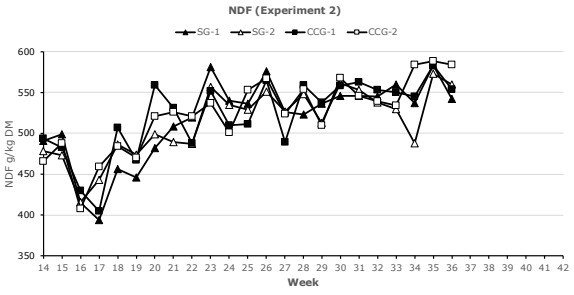


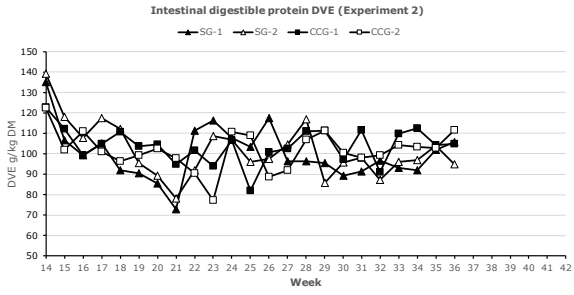
A2.2

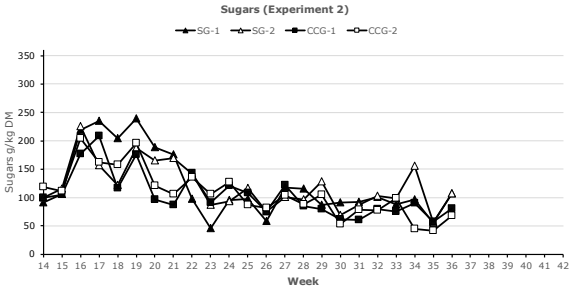
Experiment 2: Chemical composition and feeding values of the pastures used for strip-grazing (S1 and S 2) and compartmented continuous grazing (C1 and C2) during the grazing season concentrations of crude protein, neural detergent fiber (NDF), VEM (VEM = feed unit milk 1 VEM = 6.9 kJ net energy for lactation van Es, 1978), DVE (intestinal digestible protein), OEB (rumen degradable protein balance) according to the DVE/OEB protein evaluation system (Tamminga et al., 1994, revised van Duinkerken et al, 2011).

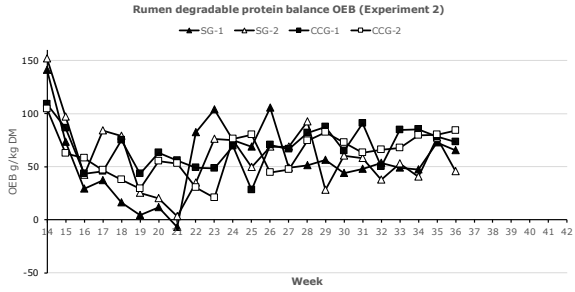


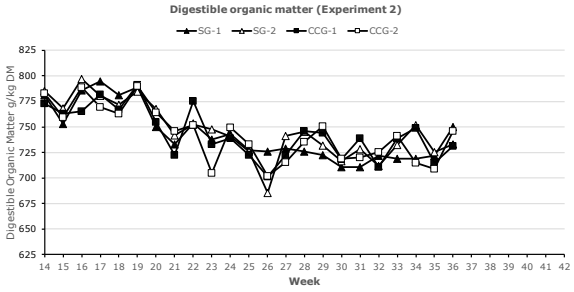






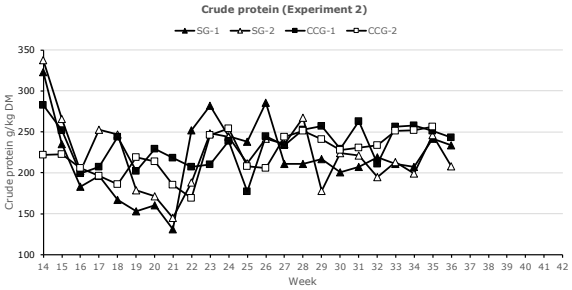


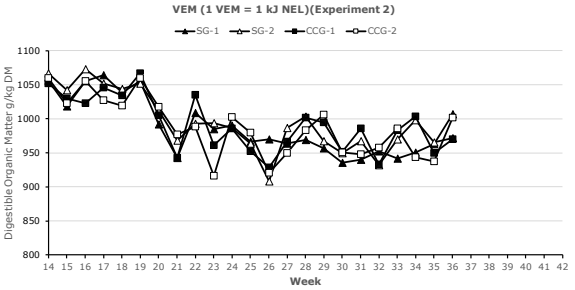


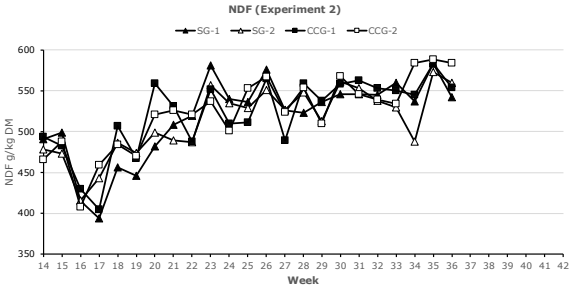


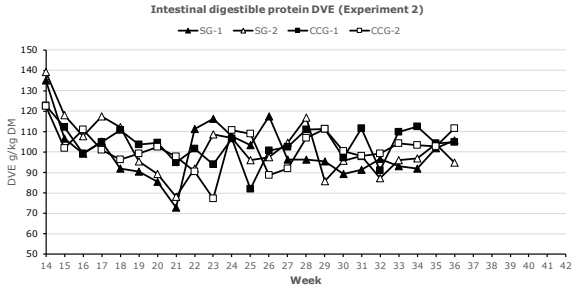
A2.2 continued

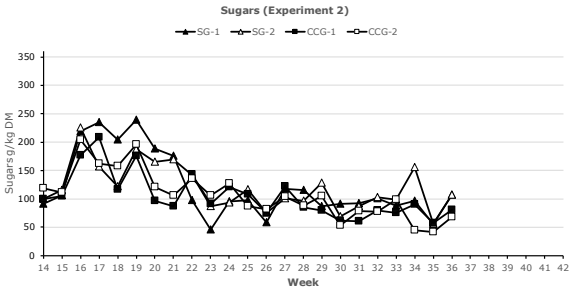
Experiment 2: Chemical composition and feeding values of the pastures used for strip-grazing 1 and 2 (S1 and S2) and compartmented continuous grazing plots 1 and 2 (C1 and C2) during the grazing season concentrations of crude protein, neural detergent fiber (NDF), VEM (VEM = feed unit milk 1 VEM = 6.9 kJ net energy for lactation van Es, 1978), DVE (intestinal digestible protein), OEB (rumen degradable protein balance) according to the DVE/OEB protein evaluation system (Tamminga et al., 1994, revised van Duinkerken et al, 2011).

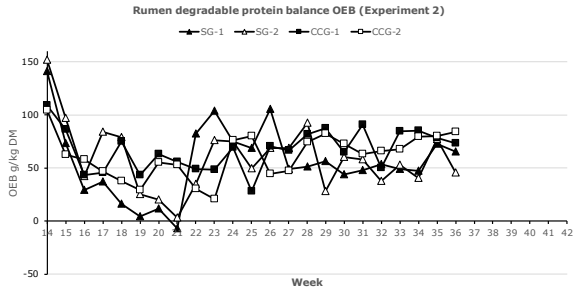


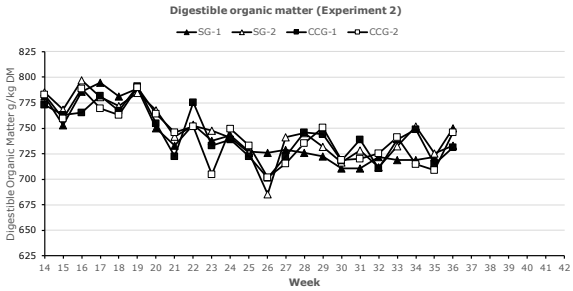












Annex 3 Effects on feed intake

A3.1

Experiment 1. Treatment means of main effects of grazing management treatments (S: strip grazing and C: compartmented continuous grazing) and three diet treatments (L, H, HH) in a 2×3 arrangement: low rumen degradable protein – strip grazing (LS1), low rumen degradable protein – compartmented continuous grazing (LC1), high rumen degradable protein – strip grazing (HS1), and high rumen degradable protein – compartmented continuous grazing (HC1) during 3 measurement periods (M). Pasture DMI (PDMI), total DMI (TDMI), milk and milk constituents yield, NEL-intake/NELrequirements×100 (NELcov%), DVE-intake /DVErequirements×100 (DVE cov%), feacal output (FO) of organic matter, organic matter digestibility (OMD), total nitrogen intake (TNin), Pasture nitrogen intake (PNin), Nitrogen Use Efficiency (NUE-milk = Nitrogen in milk/Nitrogen intake)

		Grazing Treatment (G)		Protein Treatment (P)			Combined effect (G×P)					*P*-Values						
	M	CCG	SG	L	H	lds	LC1	HC1	LS1	HS1	lds	G	P	G×P	M	M×G	M×P	M×G×P
PDMI	P1	6.8	5.7	6.3	6.2	0.57	6.5	7.0	6.0	5.5	0.81	0.919	0.469	0.424	<0.001	<0.001	0.69	0.236
	P2	4.1	4.8	4.5	4.4		4.2	4.1	4.9	4.6								
	P3	3.2	3.7	3.6	3.3		3.4	3.0	3.8	3.6								
TDMI kg/d	P1	21.3	19.9	20.0	21.2	0.75	20.6	22.0	19.4	20.4	1.06	0.023	<0.001	0.853	<0.001	0.007	0.577	0.578
	P2	18.8	18.4	18.2	19.1		18.3	19.3	18.1	18.8								
	P3	18.5	18.2	17.8	19.0		18.0	19.1	17.5	18.9								
Milk kg/d	P1	32.1	31.1	29.1	34.1	1.68	29.7	34.4	28.5	33.7	2.37	0.823	<0.001	0.893	<0.001	0.242	0.339	0.945
	P2	28.3	28.5	25.9	30.9		25.8	30.8	25.9	31.0								
	P3	27.2	27.5	25.4	29.3		25.2	29.1	25.5	29.4								
Fat g/d	P1	1231	1176	1147	1261	75.4	1172	1291	1121	1231	106.6	0.399	0.18	0.722	<0.001	0.182	<0.001	0.538
	P2	1092	1056	1039	1109		1059	1125	1020	1093								
	P3	942	953	975	920		987	897	963	942								
Protein g/d	P1	1119	1067	1015	1171	53.8	1041	1196	989	1145	76.1	0.245	<0.001	0.823	<0.001	0.159	0.001	0.852
	P2	1015	987	932	1070		946	1084	918	1056								
	P3	940	941	911	970		917	963	904	978								
Lactose g/d	P1	1476	1403	1336	1542	83.4	1390	1562	1283	1522	117.9	0.6	<0.001	0.66	<0.001	0.087	0.687	0.767
	P2	1291	1300	1186	1404		1185	1398	1188	1411								
	P3	1223	1235	1138	1320		1136	1310	1141	1329								
Urea Mg/0.1L	P1	9	7	6	11	1.7	7	12	4	9	2.5	0.227	<0.001	0.688	<0.001	0.003	0.698	0.545
	P2	10	12	9	13		8	13	10	14								
	P3	9	8	7	11		8	11	6	11								

Experiment 1. Continued Treatment means of main effects of grazing management treatments (S: strip grazing and C: compartmented continuous grazing) and three diet treatments (L, H, HH) in a 2×3 arrangement: low rumen degradable protein – strip grazing (LS1), low rumen degradable protein – compartmented continuous grazing (LC1), high rumen degradable protein – strip grazing (HS1), and high rumen degradable protein – compartmented continuous grazing (HC1) during 3 measurement periods (M). Pasture DMI (PDMI), total DMI (TDMI), milk and milk constituents yield, NEL-intake/NELrequirements×100 (NELcov%), DVE-intake /DVErequirements×100 (DVE cov%), feacal output (FO) of organic matter, organic matter digestibility (OMD), total nitrogen intake (TNin), Pasture nitrogen intake (PNin), Nitrogen Use Efficiency (NUE-milk = Nitrogen in milk/Nitrogen intake)

		Grazing System (G)		Protein Treatment (P)			Combined effects (G×P)					*P*-values							
	M	C	S	L	H	lds	LC1	HC1	LS1	HS1	lds	G	P	G×P	M	M×G	M×P	M×G×P
NEL cov%	P1	102	101	103	99	3.4	103	100	103	98	4.8	0.63	0.081	0.629	<0.001	0.422	<0.001	0.904
	P2	99	100	102	97		101	97	103	97								
	P3	104	103	102	105		103	105	102	104								
DVE cov%	P1	89	89	92	86	4.5	91	87	94	85	6.4	0.437	0.002	0.485	<0.001	0.118	0.065	0.429
	P2	91	95	97	89		95	87	100	90								
	P3	97	97	98	96		99	96	98	95								
FO kg OM/d	P1	6.7	6.4	6.4	6.7	0.34	6.5	6.9	6.3	6.5	0.47	0.392	0.084	0.497	0.125	0.029	0.458	0.96
	P2	6.7	6.5	6.5	6.7		6.6	6.8	6.5	6.5								
	P3	6.4	6.5	6.2	6.6		6.1	6.6	6.4	6.6								
OMD	P1	0.66	0.65	0.65	0.66	0.012	0.66	0.66	0.65	0.66	0.018	0.198	0.086	0.32	<0.001	0.05	0.719	0.431
	P2	0.62	0.62	0.61	0.63		0.61	0.62	0.61	0.63								
	P3	0.63	0.62	0.62	0.63		0.64	0.63	0.61	0.63								
N intake	P1	467	431	384	514	18.3	391	543	378	485	25.9	0.26	<0.001	0.29	<0.001	<0.001	0.007	0.027
	P2	413	427	365	476		354	472	375	480								
	P3	406	403	355	453		360	452	351	454								
PNin	P1	202	171	177	195	18.8	180	224	174	167	26.6	0.925	0.894	0.217	<0.001	<0.001	0.004	0.016
	P2	136	163	149	150		132	140	166	161								
	P3	129	135	140	123		139	118	141	128								
NUE-milk	P1	0.38	0.39	0.42	0.36	0.018	0.42	0.35	0.41	0.37	0.026	0.537	<0.001	0.248	<0.001	0.002	0.319	0.196
	P2	0.39	0.36	0.40	0.35		0.42	0.36	0.39	0.34								
	P3	0.37	0.37	0.40	0.34		0.40	0.33	0.41	0.34								

A3.2

Experiment 2 Treatment means of main effects of grazing management treatments (S: strip grazing and C: compartmented continuous grazing) and three diet treatments (L, H, HH) in a 2×3 arrangement: low rumen degradable protein – strip grazing (LS2), low rumen degradable protein – compartmented continuous grazing (LC2), high rumen degradable protein – strip grazing (HS2), and high rumen degradable protein – compartmented continuous grazing (HC2), high rumen degradable protein plus high intestinal digestible protein – strip grazing (HHS), and high rumen degradable protein plus high intestinal digestible protein – compartmented continuous grazing (HHC)) during 3 measurement periods (M).Pasture DMI (PDMI), total DMI (TDMI), milk and milk constituents yield, NEL-intake/NELrequirements×100 (NELcov%), DVE-intake /DVErequirements×100 (DVE cov%), feacal output (FO) of organic matter, organic matter digestibility (OMD), total nitrogen intake (TNin), Pasture nitrogen intake (PNin), Nitrogen Use Efficiency (NUE = Nitrogen in milk/Nitrogen intake).

		Grazing Treatm. (G)			Protein Treatment (P)				Combined effects (G×P)							*P*-values						
	M	C	S	lds	L	H	HH	lds	LC2	HC2	HHC	LS2	HS2	HHSC	lds	G	P	G×P	M	M×G	M×P	M×G×P
PDMI	P1	7.1	7.2	0.80	7.3	7.2	6.9	0.99	7.5	6.6	7.1	7.1	7.7	6.7	1.39	0.118	0.132	0.602	<0.001	0.023	0.516	0.401
	P2	6.6	6.9		7.4	6.7	6.2		7.4	6.6	5.9	7.4	6.8	6.5								
	P3	4.6	5.8		5.6	5.3	4.7		5.3	4.5	4.0	5.9	6.0	5.5								
TDMI	P1	18.3	17.8	0.87	18.2	18	18.1	1.07	18.6	17.8	18.6	17.7	18.1	17.7	1.51	0.443	0.485	0.593	0.051	<0.001	0.561	0.517
kg/d	P2	16.9	18.2		18	17.4	17.1		17.6	16.8	16.2	18.5	17.9	18.1								
	P3	17.7	17.7		17.9	17.9	17.4		18.3	17.7	17.2	17.6	18.1	17.5								
Milk	P1	32.7	30.7	1.87	30.4	31.3	33.3	2.29	32.3	31.7	34.0	28.5	31.0	32.7	3.23	0.994	0.144	0.584	<0.001	<0.001	0.26	0.099
kg/d	P2	23.6	25.7		24.0	24.7	25.2		23.3	24.3	23.1	24.6	25.1	27.2								
	P3	24.1	23.9		22.9	24.7	24.5		22.6	25.7	24.2	23.2	23.8	24.8								
Fat	P1	1149	1097	79.4	1122	1132	1116	97.2	1186	1155	1107	1057	1110	1125	137.5	0.55	0.648	0.215	<0.001	0.014	0.525	0.907
g/d	P2	930	978		912	953	996		920	928	942	905	978	1050								
	P3	868	930		882	904	912		898	845	860	866	962	963								
Protein	P1	1067	992	61.4	971	1034	1084	75.2	1041	1044	1117	902	1023	1050	106.3	0.548	0.006	0.313	<0.001	<0.001	0.488	0.128
g/d	P2	798	857		777	832	872		775	817	802	779	848	942								
	P3	857	828		775	881	872		797	920	855	753	842	890								
Lactose	P1	1459	1372	89	1371	1390	1486	109	1458	1396	1522	1283	1384	1450	154.1	0.955	0.291	0.706	<0.001	<0.001	0.185	0.053
g/d	P2	1026	1117		1052	1072	1091		1024	1054	1001	1080	1090	1180								
	P3	1054	1044		999	1082	1065		981	1126	1055	1017	1039	1075								
Urea	P1	21	14	2.0	14	19	21	2.4	16	23	24	11	14	17	3.5	<0.001	<0.001	0.466	<0.001	<0.001	0.101	0.059
mg/0.1L	P2	23	20		15	24	26		18	25	27	12	23	25								
	P3	14	16		9	17	19		10	14	18	9	19	20								

Experiment 2 continued Treatment means of main effects of grazing management treatments (S: strip grazing and C: compartmented continuous grazing) and three diet treatments (L, H, HH) in a 2×3 arrangement: low rumen degradable protein – strip grazing (LS2), low rumen degradable protein – compartmented continuous grazing (LC2), high rumen degradable protein – strip grazing (HS2), and high rumen degradable protein – compartmented continuous grazing (HC2), high rumen degradable protein plus high intestinal digestible protein – strip grazing (HHS), and high rumen degradable protein plus high intestinal digestible protein – compartmented continuous grazing (HHC)) during 3 measurement periods (M).Pasture DMI (PDMI), total DMI (TDMI), milk and milk constituents yield, NEL-intake/NELrequirements×100 (NELcov%), DVE-intake /DVErequirements×100 (DVE cov%), feacal output (FO) of organic matter, organic matter digestibility (OMD), total nitrogen intake (TNin), Pasture nitrogen intake (PNin), Nitrogen Use Efficiency (NUE = Nitrogen in milk/Nitrogen intake).

		Grazing Treatm. (G)			Protein Treatment (P)				Combined effects (G×P)							*P*-values						
	M	C	S	lds	L	H	HH	lds	LC2	HC2	HHC	LS2	HS2	HHSC	lds	G	P	G×P	M	M×G	M×P	M×G×P
NEL	P1	94	97	4.1	98	95	94	5	95	92	94	101	97	94	7.1	0.379	0.004	0.698	<0.001	<0.001	0.056	0.507
cov%	P2	101	104		108	101	98		106	100	97	110	102	99								
	P3	108	107		112	107	104		113	107	105	111	108	103								
DVE	P1	95	102	5.6	103	94	98	6.9	97	91	96	109	97	100	9.7	0.116	<0.001	0.334	<0.001	<0.001	0.18	0.042
cov%	P2	113	110		119	105	110		117	106	115	120	105	105								
	P3	109	117		121	105	113		119	97	112	123	113	114								
FO	P1	4.0	4.2	0.23	4.0	4.1	4.1	0.29	4.0	4.0	3.9	4.1	4.3	4.3	0.4	0.593	0.132	0.109	<0.001	<0.001	0.005	0.171
kg OM/d	P2	4.6	4.8		5.0	4.5	4.6		4.8	4.6	4.3	5.2	4.3	4.8								
	P3	5.2	4.9		5.2	5.0	4.9		5.3	5.3	5.0	5.0	4.8	4.9								
OMD	P1	0.77	0.75	0.011	0.76	0.75	0.75	0.013	0.77	0.76	0.77	0.75	0.75	0.74	0.018	0.487	0.346	0.002	<0.001	<0.001	0.02	0.286
	P2	0.70	0.71		0.70	0.72	0.71		0.70	0.70	0.71	0.70	0.74	0.71								
	P3	0.69	0.70		0.69	0.70	0.69		0.69	0.68	0.69	0.69	0.72	0.70								
TNin	P1	472	453	26.7	410	475	502	32.7	429	469	517	392	481	487	46.3	0.599	<0.001	0.404	<0.001	0.012	0.556	0.471
g/d	P2	424	434		390	439	458		393	435	445	387	442	472								
	P3	412	438		381	444	450		381	426	429	380	462	470								
PNin	P1	244	227	27.03	241	236	228	33.11	258	228	245	224	245	212	46.8	0.793	0.129	0.501	<0.001	<0.001	0.64	0.381
g/d	P2	215	205		230	207	193		240	213	192	220	200	194								
	P3	171	207		204	193	172		198	170	147	210	215	197								
NUE	P1	0.36	0.35	0.016	0.37	0.34	0.34	0.020	0.38	0.35	0.34	0.37	0.34	0.34	0.028	0.292	0.033	0.414	<0.001	<0.001	0.13	0.33
	P2	0.29	0.31		0.31	0.30	0.30		0.31	0.29	0.28	0.32	0.30	0.31								
	P3	0.33	0.30		0.32	0.31	0.31		0.33	0.34	0.31	0.31	0.29	0.30								

To explore the potential of nature to improve the quality of life

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