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The effect of grass silage harvesting strategy and concentrate level on feed intake, diet digestibility and milk production of dairy cows

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Two experiments were conducted under Northern European conditions to quantify dairy cow responses to variable grass silage quality and concentrate feed supplementation. Experiment 1 included 3 primary growth grass silages (early, intermediate and late maturity stage) supplemented with three concentrate levels (9, 12 and 15 kg d^{-1}). Experiment 2 included three consecutive harvests (first, second and third harvest from the same sward within the growing season) and three levels of concentrate supplementation (9, 11 and 13 kg d^{-1}). Dairy cows responded clearly to changes in the harvesting time of silage in primary growth (quadratic effect) and amount of concentrate (linear effect) in the diet in Experiment 1. Milk yield was the lowest with third harvest silage in Experiment 2, and responses to increasing concentrate allowance were linear. Interactions between silage quality and concentrate supplementation were detected in Experiment 1, where milk production responses to additional concentrate decreased with increasing silage digestibility. No interactions were found in Experiment 2, probably due to the small range of differences in nutritional quality between the silages prepared from different harvests. The results demonstrated that it is difficult to compensate for low silage digestibility by increasing the amount of concentrate. The variable ECM response (from –0.01 to 0.85 kg ECM per kg DM incremental concentrate in the diet) is explained by the concomitant decrease in silage intake and negative effect on diet neutral detergent fibre digestibility.

Key words: forage quality, growth stage, maturity, primary growth, regrowth, substitution rate

Introduction

In Northern Europe, dairy cow rations are typically based on grass silage due to the climatic conditions which favour grass production (Huhtanen et al. 2013, Virkajärvi et al. 2015). Forage-based feeding of dairy cows has many benefits as grass is natural, locally produced, does not compete directly with human edible foods and promotes carbon sequestration in low carbon content soils (Kätterer et al. 2013) so that grass dominated feeding has benefits both from ethical and environmental points of view. However, grass silage has typically been supplemented with substantial amounts of cereal-based concentrate feeds. This has been economically viable due to increased milk production of the cows, and the relative prices of forage and concentrate feeds (Ferris et al. 2001, Pang 2018, Huhtamäki 2022).

Grass silage digestibility is the main parameter affecting voluntary silage dry matter (DM) intake (Huhtanen et al. 2006) and subsequently milk production of dairy cows (Rinne et al. 1999, Randby et al. 2012, Pang et al. 2019). The stage of maturity of grass tillers at harvest is the main factor affecting forage digestibility, and the relationship is particularly clear in the primary growth of grass (Kuoppala et al. 2008, Alstrup et al. 2016, Hyrkäs et al. 2018). If ensiling is successfully conducted, it only marginally decreases forage digestibility during preservation, so that the raw material digestibility mainly dictates the nutritional quality of the subsequent conserved forages (Huhtanen et al. 2005).

Grass silage is harvested several times over the growing season and successive harvests differ from each other in terms of morphological and chemical composition (Rinne and Nykänen 2000, Kuoppala et al. 2008, Pakarinen et al. 2008). Under Northern European conditions, the dynamics of forage quality change are clearly slower in regrowth than in primary growth (Rinne and Nykänen 2000, Kuoppala et al. 2008, Alstrup et al. 2016). As forage quality varies within time over the whole growing season, the quality of various harvests depends on the timing of silage preparation within the harvest (Kuoppala et al. 2008). Early harvested primary growth has been considered to provide the highest amount of nutrients to dairy cows (Kuoppala et al. 2008, Pang et al. 2019), but Alstrup et al. (2016) observed highest milk yields when regrowth grass-clover was fed. That may have been

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accentuated by changes in the botanical composition of the sward as in the grass-clover mixture, the clover proportion was clearly higher in regrowth than in primary growth, as typically observed (Rinne and Nykänen 2000).

Based on the above discussion, the nutritional quality of the forage available for dairy cow feeding may vary substantially. Further, forage can be supplemented with various amounts and qualities of concentrate feeds (Huhtanen et al. 2008). In order to make economically sound decisions about optimal amount of concentrate in the diet, it is important to estimate quantitatively the biological responses to increasing amounts of concentrate. As the range in both forage and concentrate components in the diet is large, the potential interactions between forage quality and concentrate supplementation need to be elucidated. Thus, it is important to complement previous scientific literature with diets presenting a wide range of combinations of both forage quality and concentrate supplementation.

The main objective of the present study was to evaluate the interactions between silage quality and amount of concentrate supplementation on milk production of dairy cows, when silage quality was manipulated either by timing of the harvest in primary growth, or when different harvests within the growing season (first, second and third harvest) were used. We hypothesized that the milk production responses to concentrate supplementation depend on forage nutritional quality.

Materials and methods

Production of the experimental silages

The silages were produced at the experimental farm of Natural Resources Institute Finland (Luke; until 2015 MTT Agrifood Research Finland) at Maaninka, Finland (63°09'N, 27°18'E) during growing seasons 2010 and 2011. The swards were cut using a mower conditioner, tedded and allowed to wilt for 12–24 h, before being harvested with a self-propelled forage harvester. The herbage was treated with a formic acid-based additive (AIV2 Plus, Eastman, Oulu, Finland) at a target rate of 5 litres ton^{-1} fresh herbage, before being ensiled in bunker silos.

In Experiment 1 (Exp 1), two silages were prepared from the primary growth of a timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward on 14 June (early) and 5 July (late) of 2010. The sward was dominated by timothy, and it received 100 kg N per hectare in early May in the form of a commercial mineral fertilizer.

For Experiment 2 (Exp 2), the silages were prepared from a mixed sward of timothy and meadow fescue. The silages were prepared on 12 June, 26 July and 4 September 2011 representing first, second and third harvest of the same sward. The nitrogen fertilization used was 90, 90 and 40 kg N per hectare for first, second and third harvest, respectively. The amount of P was 14 kg ha^{-1} and K 64 kg ha^{-1} over the whole growing season within both experiments, and was given in the form of a commercial mineral fertilizer. The silage treatments of Exp 2 have previously been reported by Sairanen et al. (2021) so that in this article, the emphasis is on the concentrate treatments.

Diets and design of the experiments

Both experiments involved cyclical changeover designs comprising four periods and nine diets. Each period lasted for three weeks, the first two weeks being for adaptation, and the measurements were taken during the last week. In Exp 1, a third (intermediate) silage was produced by mixing early and late harvested silage at 1:1 on DM basis using a TMR mixer wagon prior to offering it to cows. The diets were arranged in a 3 × 3 factorial design with three levels of concentrate (9, 12 and 15 kg d^{-1} in Exp 1 and 9, 11 and 13 kg d^{-1} in Exp 2) and three types of silages. The pelleted concentrate feeds were designed to represent typical mixtures used on commercial dairy farms in the area (Table 1), and they were offered using automatic feeding stations (Pellon Group Ltd., Ylihärmä, Finland). The cows were blocked, and the treatments were randomized to the cows within a block. The cows were milked twice daily in a milking parlour at 06:00 and 15:00 h. They received silage *ad libitum* from Insentec feeders (Insentec BV, Marknesse, Netherlands).

Exp 1

The diets were fed to 38 cows (including 12 primiparous cows), 12 being Nordic Red and 26 Holstein by breed. In the beginning of the experiment, the cows were on average 83 ± 31.8 days in milk (DIM), produced 36 ± 7.0 kg milk per day and weighed 630 ± 53.0 kg. Cows were blocked into three groups according to initial milk yield (low, medium or high). Because of a risk for abnormal rumen function, the combination of early harvested silage and the highest concentrate level was excluded from primiparous cows. The cows received mineral supplement (Lypsy Namino, Hankkija Ltd., Hyvinkää, Finland) from a separate trough in a feeding station at 300 g day^{-1}, if they produced less than 40 kg milk per day and at 400 g day,$^{-1}$ if they produced over 40 kg milk per day.

Exp 2

The diets were fed to 37 cows (including eight primiparous cows) out of which 11 were Nordic Red and 26 Holstein. Cows were grouped into four blocks according to initial milk yield and parity, where primiparous cows formed one block. The other blocks comprised low, medium and high producing cows. In the beginning of the experiment, the cows were on average 105 ± 46.9 DIM, produced 33 ± 6.6 kg milk per day and weighed 610 ± 71.5 kg. The cows received 200 g day^{-1} mineral supplement (Lypsy Namino, Hankkija Ltd., Hyvinkää, Finland) from a separate trough in a feeding station only if they were assigned to the concentrate level of 9 kg day^{-1}.

Experimental procedures and chemical analyses

Silage was representatively sampled during the measurement week on five consecutive days and frozen immediately at –20 °C. Concentrate feeds were sampled once during the measurement week. Milk samples were taken during the measurement week on three (Exp 1) or two (Exp 2) consecutive days at morning and evening milking. The feeding stations were equipped with scales so that live weight was recorded every time the cows visited the feeding stations.

The *in vivo* diet apparent digestibility was measured using acid insoluble ash (AIA) as an internal marker. For digestibility measurements, faecal grab samples were collected on five consecutive days in the morning and afternoon (at 06:30–8:30 and 16:00–18:00 h) during the sampling week of each period. In both experiments, 9 animals (the highest yielding block) were included in the faecal sampling. The samples were immediately frozen at –20 °C. The composite samples were thawed, mixed thoroughly, subsampled and dried at 60 °C in a forced air oven until dry.

The DM content of feed and faecal samples was determined by drying the samples at +105 ˚C for 20 h, while samples for chemical analyses were dried at +60 ˚C until dry. The details of laboratory analyses at Luke laboratory are described by Sairanen et al. (2021) unless otherwise stated. All samples were analysed for ash, crude protein (CP), neutral detergent fibre (NDF) and AIA (Van Keulen and Young 1977), while silage samples were also analysed for *in vitro* cellulase solubility, acid detergent lignin (AOAC method 973.18; Association of Official Analytical Chemists, Gaithersburg, MD, USA) and indigestible NDF (Huhtanen et al. 1994). Milk composition was determined by NIR-method (Valio Ltd., Seinäjoki, Finland) and energy corrected milk (ECM) calculated according to Sjaunja et al. (1990). Fresh silage samples were analysed for fermentation quality (pH, ammonium N, water soluble carbohydrates, lactic, acetic, propionic and butyric acids, and ethanol). The energy and protein values of feeds were calculated as described in Luke (2022). The silage metabolizable energy content was calculated from the *in vitro* organic matter cellulase solubility by converting it into organic matter digestibility (OMD) using empirical equations based on *in vivo* digestibility measured using sheep at maintenance level of feed intake as described by Huhtanen et al. (2006). The silage DM intake index was calculated according to Huhtanen et al. (2007). The N use efficiency in milk production (NUE) was calculated as kg N excreted in milk / kg N intake. Metabolizable energy (ME) and metabolizable protein (MP) balance were calculated for each cow by subtracting the ME or MP required for milk production and maintenance (Luke 2022) from the total ME and MP intake, respectively.

Statistical analysis

The data from both experiments was analysed with SAS MIXED procedure (Release 9.4, SAS Institute Inc., Cary, NC, USA). The model in Exp 1 included period, block, stage of grass maturity, the amount of concentrate and the interaction between the amount of concentrate and stage of grass maturity as fixed-class variables, and cow was used as a random variable. The model in Exp 2 included period, block, grass silage harvest, the amount of concentrate and the interaction between the amount of concentrate and harvest as fixed-class variables, and cow was used as a random variable. Although two breeds of dairy cows were used, the breed was not included in the statistical model as breed × nutrition interactions have generally not been observed in feeding trials (Ferris et al. 2020). The breed effect was also evaluated in the current data. In Exp 1, ECM production and milk fat concentration were higher for Nordic Red than for Holstein cows, but in Exp 2, no breed effects could be found. In both experiments no breed × diet interactions were found, so that removing the breed effect from the statistical model was considered justified. The effect of stage of grass maturity (Exp 1) and the increasing amount of concentrate (Exp 1 and Exp 2) was further divided into linear and quadratic effects, and interactions between linear effect of maturity (Exp 1) or harvesting strategy (Exp 2) with the level of concentrate were examined using contrasts. The pairwise comparison between harvests in Exp 2 were conducted using the contrast statements in SAS.

Results

Silage characteristics

The chemical composition of the feeds offered is presented in Table 1. In Exp 1, the 20-day difference in the harvest resulted in a lower silage CP concentration and D-value, while the NDF and lignin concentrations increased. The late harvest was conducted in hot and dry weather conditions which resulted in unexpectedly high DM concentration and subsequently low concentrations of lactic acid and ammonium N in the silage. In Exp 2, the third harvest silage had the highest *in vitro* D-value and CP concentration while the concentrations of NDF and lignin were lower compared to silages from first and second harvest. The range in DM concentration of the three silages was small. Both pH and proportion of ammonium N in total N were slightly elevated in Exp 2 compared to Exp 1 but at the same level in all three silages.

Table 1. Composition of the experimental silages and concentrate supplements

	Experiment 1			Experiment 2			
	Harvest time of primary growth		Concentrate[1]	Harvest within season			Concentrate
	Early	Late		First	Second	Third	
Date of harvest	14 June	5 July		12 June	26 July	4 Sept	
Dry matter (DM), g kg^{-1}	260	478		226	251	242	870
Chemical composition, g kg^{-1} DM							
Ash	77	59	75	84	89	94	74
Acid insoluble ash	8.8	11.2	9.6	8.1	16.9	12.2	6.6
Crude protein	169	88	180	176	189	189	190
Neutral detergent fibre (NDF)	519	619	225	563	549	529	264
Acid detergent lignin	29	45		25	23	18	
Indigestible NDF	55	153	67	77	69	56	75
Lactic acid	34	8		43	48	48	
Acetic acid	11.6	6.6		15.6	23.7	19.2	
Propionic acid	0.22	0.15		0.79	0.65	0.46	
Butyric acid	0.28	0.12		0.62	0.24	0.18	
Ammonium N, g kg^{-1} N	75	26		108	109	96	
pH	4.44	4.65		4.59	4.61	4.67	
Organic matter solubility, g kg^{-1}	815	678		773	787	828	
Organic matter digestibility, g g^{-1}	0.778	0.660		0.742	0.727	0.773	
D-value[2], g kg^{-1} DM	718	621		679	663	700	
ME[3], MJ kg^{-1} DM	12.4	10.6	12.0	10.9	10.6	11.2	12.2
MP[4], g kg^{-1} DM	87	70	94	85	85	88	114
PBV[5], g kg^{-1} DM	38	-17	42	57	64	57	24
Silage DM intake index	113	93		98	90	99	

[1] The concentrate in both of the experiments comprised of barley, oats, rapeseed meal, molassed sugar beet pulp, molasses, minerals and vegetable oil in proportions of 25:25:25:17:4:3:1 on as fed basis. The mineral used was Lypsy Namino (Hankkija Ltd., Hyvinkää, Finland). [2] D-value = digestible organic matter in DM; [3] ME = metabolizable energy; [4] MP = metabolizable protein (amino acids absorbed from the small intestine); [5] PBV = protein balance in the rumen

Feed and nutrient intake and diet digestibility

The effects of experimental treatments on feed intake are presented in Table 2 for Exp 1 and in Table 3 for Exp 2. Delaying the harvest within primary growth in Exp 1 decreased silage (p< 0.01) and total (p< 0.05) DM intake in a quadratic manner as the daily silage DM intake declined 0.51 kg from early to intermediate maturity, while from intermediate to late the decline was 1.35 kg. In Exp 2 the silage DM intake was the lowest with third harvest silage (p< 0.001). The intakes were 13.4, 13.2 and 12.3 kg silage DM per day for the first, second and third harvest, respectively. In both experiments, total DM intake increased linearly with the concentrate level (p< 0.001), but there were no statistically significant interactions between the level of concentrate supplementation and harvesting strategies. In Exp 1, the energy and protein intakes decreased in response to delayed silage harvest, and the magnitude of the effect was even greater than for total feed intake as both intake and silage nutrient concentration decreased simultaneously. Also, the harvesting strategy × concentrate level interaction was significant (p< 0.05) for ME, CP and MP intakes. In Exp 2, interactions between harvesting strategy and concentrate level were not detected for nutrient intakes due to minor differences in feed composition between dietary treatments.

The coefficients for apparent total tract diet digestibilities are presented in Tables 2 and 3 for Exp 1 and Exp 2, respectively. A linear decrease in DM, organic matter, NDF and CP digestibility was detected with the delayed harvest of primary growth (Exp 1). In Exp 2, the third harvest was most digestible and first harvest least digestible. Increasing the level of concentrate tended to decrease NDF digestibility (p= 0.08) in Exp 1, and in Exp 2 the decrease was significant (p< 0.01).

Milk production

The effects of dietary treatments on milk production are presented in Table 4 for Exp 1 and in Table 5 for Exp 2, respectively. In Exp 1, ECM, fat, protein and lactose yields followed the same quadratically declining trend as feed intake with progressing maturity of grass ensiled. Also, milk fat and protein concentrations declined in response to decreased silage digestibility. For milk fat concentration, the greatest decline was between intermediate and late (quadratic effect p< 0.01), while for protein and urea concentration, the decline was linear (p< 0.001). In Exp 2, milk production responses to the differences between harvests were not as clear, but the third harvest was somewhat inferior compared to first harvest (p< 0.05 for protein yield) and second harvest (p< 0.05 for milk, ECM protein and lactose yields). Milk urea concentration was highest for second and lowest for first harvest (p< 0.001 for all pairwise comparisons). Otherwise, silage harvest effects on milk composition were minor except for a lower (p< 0.01) protein concentration when second rather than first harvest was fed.

Increased concentrate level increased linearly (p< 0.001) the yields of milk, ECM and milk components in both experiments, but in Exp 1 also a quadratic trend was detected for ECM and fat yields indicating that the responses declined with higher level of concentrate use. A significant harvest time × concentrate level interaction was also found for milk production in Exp 1 as response to concentrate was greater with the lowest digestibility silage (Fig. 1).

ECM production per kg DM increased with earlier silage harvest and increasing level of concentrate use, while NUE increased with later silage harvest and increasing level of concentrate use (Exp 1; p< 0.001). ECM production per kg DM was higher in third harvest silage compared to first harvest silage (Exp 2; p< 0.001) but no other differences were observed in converting feed DM into ECM. The NUE was highest in the first harvest and lowest in the third harvest, the second harvest being intermediate (Exp 2; p< 0.01). However, NUE was not affected by the concentrate level (p> 0.1) in Exp 2. Delaying silage preparation in primary growth decreased both ME and MP balance in Exp 1 (p< 0.01). In Exp 2, the harvest effect over the whole growing season was less clear, although the ME balance tended (p= 0.061) to be lower in second than in first harvest, and MP balance lower in first than in third harvest. Increasing concentrate level increased ME and MP balance in both experiments (p< 0.001).

Table 2. Feed intake and diet digestibility of dairy cows fed silages harvested at early or late stages of maturity in primary growth, or a mixture of them (intermediate), and offered different amounts of concentrate (Experiment 1)

Harvest (H):	Early			Intermediate			Late			SEM[1)]	*p*-value[2)]				
Concentrate (C):	9	12	15	9	12	15	9	12	15		H Lin	H Quad	C Lin	C Quad	H × C
Feed and nutrient intake, kg day^{-1}															
Silage dry matter (DM)	15.7	14.6	13.2	15.1	13.9	12.9	13.5	12.9	11.5	0.35	<0.001	0.007	<0.001	0.416	0.189
Concentrate DM	8.1	10.7	13.2	8.1	10.7	13.2	8.1	10.8	13.3	0.07	0.078	0.236	<0.001	0.011	0.268
Total DM	23.8	25.3	26.4	23.2	24.6	26.1	21.5	23.7	24.9	0.35	<0.001	0.017	<0.001	0.125	0.127
Organic matter	22.2	23.6	24.6	21.8	23.1	24.4	20.4	22.4	23.4	0.33	<0.001	0.016	<0.001	0.117	0.139
Neutral detergent fibre	9.9	10.0	9.8	10.4	10.3	10.3	10.1	10.4	10.2	0.2	<0.001	0.004	0.388	0.154	0.430
Crude protein	4.02	4.26	4.55	3.36	3.65	3.97	2.52	2.99	3.27	0.06	<0.001	0.053	<0.001	0.458	0.011
Metabolizable energy, MJ day^{-1}	257	273	286	242	257	274	215	239	253	3.4	<0.001	0.018	<0.001	0.162	0.044
Metabolizable protein	2.28	2.48	2.65	2.10	2.30	2.51	1.84	2.11	2.30	0.030	<.0001	0.049	<.0001	0.178	0.031
Protein balance in the rumen	0.788	0.797	0.844	0.391	0.434	0.484	-0.055	0.018	0.095	0.0203	<0.001	0.026	<0.001	0.380	0.020
Concentration in the diet, per kg DM															
Metabolizable energy, MJ	11.6	11.6	11.7	11.1	11.2	11.3	10.6	10.8	10.9	0.02	-	-	-	-	-
Crude protein, g	170	171	173	146	150	154	119	127	134	0.8	-	-	-	-	-
Metabolizable protein, g	91.4	92.5	93.7	86.0	87.8	89.3	80.4	82.9	85.4	0.24	-	-	-	-	-
Diet digestibility (g g^{-1})															
Dry matter	0.722	0.733	0.722	0.677	0.693	0.700	0.637	0.630	0.643	0.0165	<0.001	0.376	0.340	0.835	0.910
Organic matter	0.731	0.745	0.736	0.687	0.704	0.710	0.647	0.641	0.655	0.0163	<0.001	0.400	0.228	0.797	0.907
Neutral detergent fibre	0.631	0.609	0.593	0.542	0.550	0.541	0.494	0.460	0.441	0.0257	<0.001	0.676	0.080	0.955	0.694
Crude protein	0.697	0.710	0.699	0.672	0.680	0.691	0.620	0.610	0.624	0.0196	<0.001	0.068	0.481	0.972	0.945

[1)] SEM = Standard error of the mean; [2)] H Lin = linear effect of harvest time; H Quad = quadratic effect of harvest time; C Lin = linear effect of concentrate level; C Quad = quadratic effect of concentrate level; H × C = interaction of linear effects of harvest time and concentrate level

Table 3. Feed intake and diet digestibility of dairy cows fed silages prepared at subsequent harvests within the season and offered different amounts of concentrate (Experiment 2)

Harvest (H):	First (1)			Second (2)			Third (3)			SEM[1]	*p*-value[2]					
Concentrate (C):	9	11	13	9	11	13	9	11	13		1 vs 2	1 vs 3	2 vs 3	C Lin	C Quad	H × C
Feed and nutrient intake, kg day^{-1}																
Silage dry matter (DM)	14.1	13.3	12.7	14.0	13.3	12.4	13.2	12.4	11.3	0.27	0.334	<0.001	<0.001	<0.001	0.767	0.770
Concentrate DM	7.8	9.7	11.2	7.8	9.7	11.3	7.9	9.6	11.3	0.04	0.614	0.689	0.905	<0.001	0.000	0.400
Total DM	21.9	23.0	23.9	21.8	22.9	23.7	21.1	22.0	22.6	0.31	0.485	<0.001	<0.001	<0.001	0.273	0.660
Organic matter	20.1	21.1	22.0	20.0	21.0	21.7	19.3	20.2	20.6	0.28	0.277	<0.001	<0.001	<0.001	0.268	0.660
Neutral detergent fibre	9.82	9.83	9.87	9.59	9.65	9.54	8.90	8.90	8.72	0.171	0.010	<0.001	<0.001	0.545	0.499	0.880
Crude protein	3.84	4.04	4.16	4.15	4.37	4.48	4.00	4.14	4.24	0.052	<0.001	<0.001	<0.001	<0.001	0.128	0.700
Metabolizable energy, MJ day^{-1}	234	246	255	230	242	252	230	240	245	3.3	0.021	<0.001	0.057	<0.001	0.305	0.420
Metabolizable protein	2110	2263	2381	2110	2251	2364	2099	2223	2308	26.6	0.526	0.005	0.027	<0.001	0.163	0.550
Protein balance in the rumen	0.975	1.010	0.991	1.088	1.096	1.054	0.950	0.946	0.926	0.0494	<0.001	0.607	<0.001	<0.001	0.400	0.270
Concentration in the diet, per kg DM																
Metabolizable energy, MJ	11.4	11.4	11.5	11.2	11.3	11.4	11.6	11.7	11.7	0.01	-	-	-	-	-	-
Crude protein, g	180	180	180	188	188	187	187	187	187	0.7	-	-	-	-	-	-
Metabolizable protein, g	95.6	97.1	98.6	95.5	97.2	98.9	98.2	99.7	101.2	0.18	-	-	-	-	-	-
Diet digestibility (g g^{-1})																
Dry matter	0.706	0.723	0.717	0.732	0.722	0.727	0.738	0.745	0.745	0.0075	0.045	<0.001	0.018	0.415	0.638	0.330
Organic matter	0.716	0.732	0.727	0.746	0.735	0.743	0.757	0.763	0.763	0.0076	0.010	<0.001	0.005	0.406	0.720	0.360
Neutral detergent fibre	0.618	0.623	0.580	0.677	0.635	0.634	0.700	0.686	0.671	0.0139	0.010	<0.001	0.005	0.004	0.720	0.360
Crude protein	0.727	0.739	0.721	0.760	0.739	0.732	0.749	0.757	0.752	0.0071	0.006	<0.001	0.091	0.043	0.222	0.040

[1] SEM = Standard error of the mean; [2] C Lin = linear effect of concentrate level; C Quad = quadratic effect of concentrate level; H × C = interaction of harvesting strategy and concentrate level

Table 4. Milk production of dairy cows fed silages prepared at early or late stages of maturity in primary growth, or a mixture of them (intermediate), and offered different amounts of concentrate (Experiment 1)

Harvest (H):	Early			Intermediate			Late			SEM[1)]	*p*-value[2)]				
Concentrate (C):	9	12	15	9	12	15	9	12	15		H Lin	H Quad	C Lin	C Quad	H × C
Production per day															
Milk (kg)	36.1	37.7	38.3	34.4	36.2	37.0	31.1	33.5	35.6	0.69	<0.001	0.109	<0.001	0.123	0.006
ECM[3)] (kg)	37.4	39.2	39.0	35.8	37.2	37.6	30.5	33.2	34.9	0.71	<0.001	0.002	<0.001	0.044	0.010
Fat (g)	1564	1606	1550	1483	1516	1504	1223	1340	1360	26.7	<0.001	<0.001	0.019	0.016	0.005
Protein (g)	1214	1311	1347	1166	1240	1273	1004	1097	1205	17.0	<0.001	0.013	<0.001	0.183	0.051
Lactose (g)	1656	1757	1765	1610	1683	1729	1438	1550	1666	36.9	<0.001	0.026	<0.001	0.168	0.010
Milk composition															
Fat (g/kg)	43.5	42.7	40.8	43.4	42.1	40.8	39.6	40.2	38.5	0.94	<0.001	0.003	<0.001	0.117	0.165
Protein (g/kg)	33.9	35.0	35.5	33.9	34.3	34.5	32.4	33.1	34.0	0.41	<0.001	0.123	<0.001	0.538	0.871
Lactose (g/kg)	45.8	46.5	46.1	46.8	46.5	46.7	46.1	46.2	46.8	0.45	0.344	0.073	0.152	0.932	0.503
Urea (mg/100 ml)	23.9	24.3	24.1	20.0	21.9	22.4	16.0	18.3	18.7	0.93	<0.001	0.214	<0.001	0.095	0.041
Live weight (kg)	650	656	657	642	645	648	645	644	646	8.0	<0.001	0.214	<0.001	0.095	0.041
kg ECM / kg dry matter intake	1.57	1.55	1.47	1.54	1.51	1.44	1.41	1.40	1.40	0.021	<0.001	0.04	<0.001	0.230	0.013
NUE[4)]	0.30	0.30	0.29	0.34	0.33	0.31	0.39	0.36	0.36	0.006	<0.001	0.11	<0.001	0.757	0.007
Metabolizable energy balance (MJ/d)	-1.7	5.2	19.9	-8.4	-0.3	14.6	-7.7	2.4	8.7	2.74	0.002	0.148	<0.001	0.442	0.659
Metabolizable protein balance (g/d)	4	49	156	-95	-21	115	-118	-6	22	30.1	<0.001	0.211	<0.001	0.510	0.722

[1)] SEM = Standard error of the mean; [2)] H Lin = linear effect of harvest time; H Quad = quadratic effect of harvest time; C Lin = linear effect of concentrate level; C Quad = quadratic effect of concentrate level; H × C = interaction of linear effects of harvest time and concentrate level; [3)] ECM = energy corrected milk; 4) NUE = Nitrogen use efficiency (N output in milk [g] / N intake [g])

Table 5. Milk production of dairy cows fed silages harvested at subsequent harvests within the season and offered different amounts of concentrate (Experiment 2)

Harvest (H):	First (1)			Second (2)			Third (3)			SEM[1)]	*p*-value[2)]					
Concentrate (C):	9	11	13	9	11	13	9	11	13		1 vs 2	1 vs 3	2 vs 3	C Lin	C Quad	H × C
Production per day																
Milk (kg)	32.8	34.3	35.3	33.8	34.3	35.6	32.3	33.3	34.9	0.70	0.265	0.065	0.003	<0.001	0.612	0.750
ECM[3)] (kg)	32.3	34.0	34.7	33.3	33.4	35.3	31.8	33.4	34.5	0.80	0.434	0.241	0.041	<0.001	0.915	0.460
Fat (g)	1286	1352	1368	1348	1321	1403	1284	1354	1363	40.5	0.330	0.924	0.246	<0.001	0.967	0.300
Protein (g)	1118	1189	1216	1118	1157	1218	1088	1134	1198	24.6	0.410	0.004	0.032	<0.001	0.938	0.630
Lactose (g)	1483	1549	1594	1528	1547	1613	1458	1516	1585	39.0	0.287	0.228	0.018	<0.001	0.684	0.810
Milk composition																
Fat (g/kg)	39.4	39.6	38.9	40.0	39.0	39.8	40.0	40.8	39.0	0.88	0.603	0.221	0.486	0.280	0.551	0.300
Protein (g/kg)	34.3	34.8	34.4	33.3	34.2	34.5	33.8	34.3	34.6	0.41	0.004	0.101	0.129	<0.001	0.030	0.050
Lactose (g/kg)	45.2	45.0	44.8	45.2	45.0	45.3	45.1	45.4	45.3	0.23	0.357	0.086	0.437	0.927	0.959	0.200
Urea (mg/100 ml)	32.8	32.5	32.1	36.8	38.5	36.6	35.2	35.0	34.1	1.19	<0.001	0.000	<0.001	0.279	0.152	0.650
Live weight (kg)	659	665	650	649	643	652	642	644	644	12.0	0.038	0.002	0.297	0.778	0.736	0.350
kg ECM / kg dry matter intake	1.46	1.46	1.45	1.51	1.44	1.47	1.49	1.50	1.51	0.030	0.328	0.011	0.112	0.529	0.309	0.279
NUE[4)]	0.28	0.28	0.28	0.26	0.26	0.27	0.27	0.27	0.28	0.276	<0.001	0.001	0.001	0.162	0.228	0.382
Metabolizable energy balance (MJ/d)	2.7	6.4	13.9	-4.1	7.4	7.8	3.7	5.0	6.1	2.98	0.061	0.172	0.513	<0.001	0.453	0.115
Metabolizable protein balance (g/d)	-18.0	22.0	94.7	-15.0	62.0	85.5	29.9	78.0	69.8	28.18	0.493	0.092	0.320	<0.001	0.326	0.220

[1)] SEM = Standard error of the mean; [2)] C Lin = linear effect of concentrate level; C Quad = quadratic effect of concentrate level; H × C = interaction of linear effects of harvest time and concentrate level; [3)] ECM = energy corrected milk; [4)] NUE = Nitrogen use efficiency (N output in milk [g] / N intake [g])

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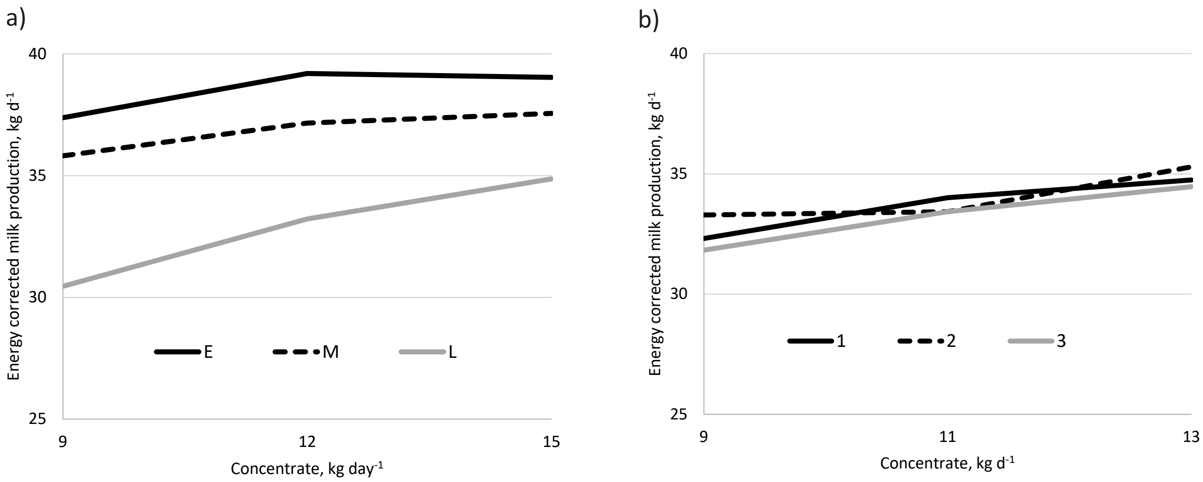

Fig. 1. Interactions of forage type (early (E), late (L) or mixture (M) of them in Exp 1; first (1), second (2) or third (3) harvest within the growing season in Exp 2) and amount of concentrate fed on energy corrected milk production in Exp 1 (a) and Exp 2 (b)

Discussion

Silage characteristics

The rapid development of primary growth grass is well established under Northern climatic conditions (Hyrkäs et al. 2018). The daily decline of D-value with progressing growth in Exp 1 was 4.9 g kg^{-1} DM which is in good agreement with earlier reports (Rinne et al. 1999, Kuoppala et al. 2008). The D-value of third harvest towards the autumn with decreasing temperature and day length has typically been over 680 g kg^{-1} DM (Hyrkäs et al. 2016) as found also here. Higher D-values of third harvest compared to second harvest were also observed in two later series at the same site with similar comparisons between harvests (Sairanen et al. 2021).

The clear decline in diet digestibility with progressing growth of silage is well established (e.g., Rinne et al. 1999, Kuoppala et al. 2008, Alstrup et al. 2016). The digestibility of regrowth grass silages under Finnish conditions is typically lower compared with early harvested primary growth (Huhtanen et al. 2006, Kuoppala et al. 2010). In Exp 2, *in vivo* OMD and NDF digestibilities were highest for third, intermediate for second and lowest for first harvest while the ratio of indigestible NDF and lignin to NDF showed an opposite trend. The lignification is a consequence of maturation which is advanced by high temperature and progressing time (Wilson et al. 1991, Sairanen and Hyrkäs 2015). The effective temperate sum (average daily temperature in °C minus 5 °C summed since the onset of growing season) was not exceptionally high (321 °Cd) when first harvest silage was prepared for Exp 2 and harvesting was conducted slightly late but still within the recommended timing for commercial farms in the area.

The OMD of regrowth silage stays at high level if the length of the growing interval is short (Kuoppala et al. 2010, Pang et al. 2021) but in Exp 2 the regrowth interval between first and second harvest as well as D-values obtained can be considered typical for the harvesting strategy used. The growth rate of grass is slow in autumn and the digestibility of new vegetative tillers is high resulting in high digestibility of the third harvest (Pakarinen et al. 2008, Pang et al. 2021). Regrowth grass contains more leaves relative to stems compared with primary growth (Rinne and Nykänen 2000, Kuoppala et al. 2008, Alstrup et al. 2016), and leaves contain less structural fibre which maintains higher digestibility with progressing regrowth.

The silages were prewilted and formic acid was used as an additive to minimize the differences caused by variable preservation conditions as large difference in the fermentation quality of the silages could affect intake and milk production and mask the sward related effects. The DM concentration of the primary growth typically increases as the plants mature (Rinne et al. 1999), but unfortunately the weather conditions exaggerated this difference so that the higher DM concentration of late harvested silage in Exp 1 resulted in more restricted silage fermentation compared to the early harvested silage. High DM content together with restricted fermentation has positive effects on intake (Huhtanen et al. 2007), which has likely decreased the expected difference in voluntary DM intake between silages in Exp 1. All three silages of Exp 2 were rather similar regarding DM content and fermentation quality so that these factors have probably had limited effects on the comparison of the voluntary feed intake potential of the silages. As clearly evidenced by Exp 1, silage quality during progressing growth is highly variable, and conducting comparisons between harvests is greatly affected by the choice of silage preparation time within each harvest. In Exp 2, the choices of cutting time within first, second and third harvest were according to the typical farming practises in the area and prevailing weather conditions.

Silage effects on feed intake and production

Exp 1

A decrease of 10 g kg^{-1} in silage D value decreased silage DM intake by 0.162 kg day^{-1}, which is in line with earlier reports (Rinne et al. 1999, Kuoppala et al. 2008). In earlier data sets, the feed intake response to progressing grass primary growth have shown quadratic or even cubic trends (Rinne et al. 1999). This could mainly be attributed to the nonlinear changes in grass digestibility, which is greatly influenced by variable weather conditions, particularly cumulative temperature, during the growing period (Hyrkäs et al. 2018). Indeed, when feed intake responses have been presented in relation to silage D-value, the effect has been linear (Huhtanen et al. 2007). In the current data set, the intermediate silage was a 1:1 mixture of early and late harvested silages so that the quadratic effects of grass maturity on feed intake cannot be explained by the non-linear development of grass but should be due to feed intake capacity of the cows, balance of nutrients or some other cow related factor(s).

The quadratic trend in intake to progressing maturity of grass ensiled may be explained by the lower digestibility of the late harvested silage compared to earlier reports. It has been shown that both restricted fermentation and increased DM concentration *per se* increase the voluntary silage intake by dairy cows (Huhtanen et al. 2007), and this should have benefitted the late harvested silage, so the actual quadratic effect could have been even greater if silage characteristics would have been similar in those respects. On the other hand, the low CP concentration of the late harvested grass silage has probably also contributed to the decline in milk production. Based on the Finnish feed evaluation system (Luke 2022), only the diet with lowest concentrate allowance with late harvested silage resulted in negative rumen protein balance. On that diet, also milk urea concentration was low suggesting a suboptimal rumen degradable CP intake.

Earlier harvest in Exp 1 increased milk production by 0.56 kg day^{-1} per an increase of 10 g kg^{-1} in silage D value and it is explained by the increased energy supply of the cows due to increased feed intake and higher digestible energy concentration of the diet similarly as e.g., in Rinne et al. (1999) and Kuoppala et al. (2008). The numerical effect was slightly higher compared with an average of 0.45 kg d^{-1} reported in the review by Huhtanen et al. (2011). The response was particularly high between late and intermediate silages (0.85 kg d^{-1}), which contributes to the overall difference. In fact, review studies include only limited amount of very low digestibility silages, which may be the reason that a quadratic milk production trend to varying silage digestibility has not always been detected.

Exp 2

Comparisons of different harvests from the same swards over 3 growing seasons, including the same data as used in the present study, have been presented in detail by Sairanen et al. (2021). In brief, it seems that the intake and subsequently milk production potential of autumn-harvested silages is not as high as that from those made of previous harvests, even though the feed analyses would indicate equal or even superior quality. This phenomenon was evident in the current experiment, where harvest 2 had a clearly lower D-value than harvest 3 (663 vs. 700 g kg^{-1} DM) and still ECM yield was lower when harvest 3 rather than harvest 2 was used.

Sairanen et al. (2021) speculated that the lower than expected intake potential of autumn harvested silages may be related to the palatability or feed texture, which may be related to the long regrowth period under humid conditions of autumn. Another hypothesis could be a higher incidence of high mycotoxin concentrations in autumn silages, although evidence of that is not very conclusive (Manni et al. 2022). The recommendation by Sairanen et al. (2021) under Northern European conditions was to prioritize grass silage from first harvest to high yielding dairy cows when possible.

Concentrate effects on feed intake and production

It could be argued that the concentrate composition should have been tailored for changing silage quality, but to prevent confounding effects, the same concentrate composition was used within experiments. The amount of concentrate in dairy cow diets is a major decision in ration formulation. On top of biological production responses, it depends on several other factors such as availability and price of both concentrate and forage feeds, and sustainability issues (environmental emissions, strive to decrease the human edible dietary components in livestock production). The concentrate used in the current experiment represents a typical composition under Finnish conditions including locally available components. The average concentrate proportions in diet DM for the low, medium and high concentrate levels were 0.35, 0.44 and 0.51 in Exp 1, and the corresponding values for Exp 2 were 0.36, 0.43 and 0.48 being within the range of Finnish national average, which was 0.47 in 2021 (Huhtamäki 2022).

In general, substitution rate (SR; kg decrease in silage DM intake per one kg increase in concentrate DM intake) depends on concentrate level and silage quality (Huhtanen et al. 2008). Randby et al. (2012) reported increasing SR from 0.31 to 0.95 when the amount of concentrate supplementation increased from 4 kgd^{-1} to 16 kg d^{-1} on a diet with “normal stage of plant maturity at harvest” as the authors described the silage. The average SR increased from 0.36 to 0.50 and from 0.43 to 0.60 for Exp 1 and Exp 2, respectively, when supplementation increased from low to intermediate, and from intermediate to high level, showing a typical increasing trend with increasing level of concentrate supplementation.

Concentrate supplementation did not improve total diet OMD because NDF digestibility decreased linearly as concentrate intake increased despite of a higher inherent digestibility of concentrate foods compared to forages (Luke 2022). The results are consistent with the meta-analysis by Nousiainen et al. (2009). Reasons for low NDF digestibility can be associated with a lower rumen pH and decreases in particle-associated enzyme activities (Stensig and Robinson 1997). The effects of increasing amounts of concentrate feeds on increasing SR and decreasing NDF digestibility results in clear underestimation of dairy cow energy intake to increasing concentrate level, if not taken into account (Luke 2022).

In studies where there have only been small effects of concentrate supplementation on total DM intake, also ECM responses have been negligible (Alstrup et al. 2016). On the other hand, moderate use of concentrate may lead to higher milk production responses as noted by Randby et al. (2012), who reported a 1.6 kg milk response per kg concentrate DM when the amount of concentrate increased from 0 to 4 kg per day with early maturity silage while the response was even negative between 8 and 12 kg supplementation. The average ECM production responses per kg incremental concentrate DM were 0.52 in Exp 1 and 0.48 in Exp 2 being intermediate compared to the previously cited studies, which emphasizes the need to take into account the variable conditions in different experiments. In Exp 1 the average supplementation level was higher, but the average silage D-value was lower compared with Exp 2 resulting in comparable concentrate responses to supplementation between experiments.

Increasing amount of concentrate led to a higher milk protein in both experiments and a lower milk fat concentration in Exp 1 confirming previous results (Huhtanen and Rinne 2007). Milk protein concentration typically increases with increasing energy balance of cows. The highest energy balance (+19.9 MJ ME d^{-1}) was observed with early harvested silage and 15 kg concentrate supplementation. Milk fat concentration may be quite resistant to increased concentrate supplementation at least moderate levels are used (Huhtanen and Rinne 2007). When very high levels of concentrate are used, decreased milk fat concentration may be related to changes in rumen pH, fermentation pattern and fatty acid biohydrogenation resulting in increased flow of trans-10 isomers of C18:1 to the small intestine (Griinari et al. 1998). The quadratic effect of concentrate level was not significant in the current experiments so that milk fat depression was not detected, and rumen function can be assumed to have remained normal within the range of diets used.

Interactions between silage quality and concentrate supplementation

The main aim of the current study was to evaluate the potential interactions between silage quality and amount of concentrate. The daily ECM yields of cows fed different silages at different concentrate level are presented in Figure 1. The interaction between the two dietary factors is clearly visible for Exp 1 (Fig. 1a). The ECM response was even slightly negative with early harvested silage between 12 and 15 kg supplementation whereas the response was 0.66 kg kg^{-1} DM with late harvested silage. The highest ECM yield with early harvested silage and 12 kg concentrate level indicates that poor nutritional quality of silage can only partially be compensated by increasing the level of concentrate feeding.

It appears that ME and MP intakes have been limiting factors with the diet based on late harvested silage supplemented with the lowest level (9 kg) of concentrate. The MP balance was lowest with this diet, whereas the balance was positive when early harvested silage was supplemented with 9 kg of concentrate. High intake, as with early harvested silage, maintains high microbial protein synthesis independent of the protein supplementation. In Exp 2, interactions between dietary treatments could not be detected (Fig. 1b) due the small differences in nutritive values between experimental silages.

The results regarding simultaneous changes in forage nutritional quality and concentrate allowance are variable as interactions have been detected by e.g., Ferris et al. (2001), Aston et al. (1994) and Randby et al. (2012) while no interaction was observed by Steen and Gordon (1980), Phipps et al. (1987) and Rinne et al. (1999). The lack of interaction can in some cases be explained by smaller range of differences between treatments as the effect seems to be the stronger the lower the silage digestibility (Fig. 1a). Álvarez et al. (2020) compiled data from 9 published studies and observed curvilinear milk production responses to concentrate allowance and a linear response to silage digestibility. They also detected an interaction so that the response to concentrate feeding was lower with increasing digestibility of silage. According to their study, the highest milk yield was observed with a concentrate level of 10 kg DM per day combined with the highly digestible silage.

The general trends of higher SR with higher digestibility of silage (e.g., Huhtanen et al. 2008, Randby et al. 2012) where detectable in Exp 1 (0.49, 0.43 and 0.38 for early, intermediate and late harvested silages, respectively). The lowest SR was observed with late harvested silage between 9 and 12 kg concentrate (0.19). Surprisingly the highest SR (0.56) was observed simultaneously with early and late harvested silage when supplementation increased from 12 kg d^{-1} to 15 kg d^{-1}. It is possible that rumen fill was a limiting factor for late harvested silage whereas metabolic regulation limited intake with early harvested silage. The SR for first, second and third harvest silages in Exp 2 were 0.41, 0.46 and 0.56, respectively. The highest D-value of third harvest silage could be linked with the somewhat higher SR, but comparison between first and second harvest silage is not consistent.

Conclusions

Delaying the harvest of primary growth decreased milk production significantly and concentrate supplementation compensated the difference only partially. Exp 1 supported our hypothesis about higher response to concentrate supplementation with low digestibility silage, but within the recommended range of feed quality, the interaction is likely to be small. No systematic responses to first, second and third harvest silages could be detected with the range of feeds of the current experiment and the response to concentrate supplementation did not depend on the season of silage harvest. The digestibility of third harvest silage was the highest but low voluntary silage intake of the dairy cows resulted in the lowest milk production. Increasing the level of concentrate supplementation resulted in decreased silage intake and neutral detergent fibre digestibility, and these negative effects on dairy cow energy supply should be considered in ration formulation.

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