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Effect of spring versus autumn grass/clover silage and rapeseed supplementation on milk production, composition and quality in Jersey cows

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The composition of grass/clover silage varies depending on time of harvest time. In particular silage from late regrowths is expected to contain lower fibre and higher linolenic acid concentrations compared to spring growth, thereby autumn silage is expected to increase linolenic acid content of milk fat. Rapeseed supplementation is expected to increase milk production and to increase all C18 fatty acids in milk fat. An interaction between rapeseed and silage type is expected, as hydrogenation of unsaturated fatty acids in rapeseed is expected to be less when low fibre silage is fed. Thirty-six Jersey cows were used in a 4 × 4 Latin square design, for 4 periods of 3 weeks and with a 2 × 2 factorial arrangement of treatments: spring grass/clover silage from primary growth or autumn grass/clover silage which was an equal mixture of 3rd regrowth and 4th regrowth, with or without rapeseed supplementation. Dry matter intake and milk production was higher for autumn than for spring silage. Rapeseed supplementation did not affect dry matter intake, but increased milk production. The concentrations of C18 : 1*cis*9, C18 : 2*n*6 and β-carotene and C18 : 3*n*3 in milk were increased whereas the concentrations of C16 : 0, riboflavin and α-tocopherol were decreased with autumn silage. The majority of C18 FAs in milk and α-tocopherol concentration increased with rapeseed whereas C11 : 0 to C16 : 0 FA were reduced. Autumn silage reduced biohydrogenation of C18 : 2*n*6, whereas rapeseed increased biohydrogenation of C18 : 2*n*6 and reduced biohydrogenation of C18 : 3*n*3. Apparent recovery of C18 : 2*n*6 was reduced with rapeseed. Minor interaction effects of silage type and rapeseed addition were observed for some milk fatty acids. Feeding silage from late regrowth increased linolenic acid concentration in milk fat. Rapeseed inclusion increased milk production, and increased C18 : 0 as well as C18 : 1 fatty acids, but not C18 : 2 and C18 : 3 in milk fat. Interactions between silage type and rapeseed supplementation were minimal.

Keywords: Milk, fatty acids, antioxidants, grass silage, rapeseed, jersey.

Composition of grass varies during the season and compared to spring growth, grass from autumn regrowth has a lower concentration of sugars and fibres, a higher concentration of protein and fat, in particular linolenic acid (C18 : 3*n3*), and a higher proportion of leaves compared to stems (Witkowska et al. 2008). The higher proportion of leaves could result in a higher content of carotenoids (Nozière et al. 2006). In Denmark, commonly three to four regrowths are harvested after the spring growth in grass/clover leys for silage. Silage produced from autumn regrowth grass is by many practical farmers in Denmark regarded to be of a poorer quality compared to silage produced from spring growth grass. The reason why autumn regrowth should be poorer is unclear, but might be associated with less optimal conditions for prewilting and higher contamination with soil and fungi. Also the lower sugar concentration in autumn regrowth grass compared to spring growth can affect the ensiling process. The lower fibre concentration in autumn regrowth compared with spring growth could decrease ruminal biohydrogenation (Chilliard et al. 2007). Further late regrowth grass silage is expected to have a higher concentration of linolenic acid

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than spring growth. Therefore milk from cows fed late regrowth grass silage is expected to have higher concentration of linolenic acid than milk from cows fed spring grass silage.

High fat feeds such as rapeseed are used as feed supplements to increase ration energy concentration and milk production. Supplementation of rapeseed could further increase the concentration of unsaturated fatty acids (FA) in the milk, especially in combination with the low fibre autumn grass. Rapeseed supplementation gives a higher concentration of total C18 FA in milk. In particular C18 : 1*cis*9 increases as this is the main FA of rapeseed, but also C18 : 2 and C18 : 3 are increased due to the content of these FA in rapeseed (Larsen et al. 2013). Milk is a significant source of riboflavin (vitamin B2) in human nutrition but little is known on how the concentration in milk is controlled. Basically there are two sources of riboflavin for ruminants: supply from feed and synthesis in the rumen, where a range of B-group vitamins are synthesised to supply the entire microbiota as well as the cow (NRC, 2001).

The purpose of the present study was to investigate how milk production and composition was affected by feeding Jersey cows on grass/clover silage from autumn regrowth compared to spring growth with or without supplementation of rapeseed. The main hypotheses were that silage from late regrowth would have a higher concentration of protein and fat, in particular C18 : 3, a lower concentration of fibre, and lower concentrations of fermentation products due to the lower sugar content compared to spring growth grass/clover silage. These differences were expected to result in similar milk production if digestibility of organic matter is similar, and to result in a lower ruminal biohydrogenation of polyunsaturated fatty acids (PUFA) and a higher concentration of C18 : 3*n*3 in milk fat from cows fed autumn compared to spring grass/clover silage. Supplementation of rapeseed was assumed to increase milk production, and to increase the concentration of all C18 FA in milk. An interaction between rapeseed supplementation and silage type was expected, as efficiency of hydrogenation of unsaturated rapeseed FA was expected to be less with the low fibre autumn silage.

Materials and methods

Cows, experimental design and treatments

The experiment complied with the guidelines of the Danish Ministry of Justice Law No. 726 (9th September 1993) concerning experiments with animals and care of experimental animals.

Fourty-four Jersey cows (mean ± SD; 473 ± 52 kg body weight; 134 ± 108 d in milk; 29·8 ± 5·9 kg energy corrected milk; 20 primiparous and 24 multiparous) were used in the experiment. The cows were blocked according to parity (first parity and older), calving date, and milk yield and assigned to one of four different treatments in a balanced 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments. Halfway through the experiment eight cows (one block of first parity and one older) in late lactation were exchanged with similar parity cows in early lactation, therefore only 36 cows were in experiment at a certain time. Cows were fed the experimental diets for periods of 3 weeks; 2 weeks adaptation and 1 week data collection. Cows were kept in a loose-housing system with slatted floors and cubicles with mattresses. The cows were milked in automatic milking unit (AMU) (DeLaval AB, Tumba, Sweden).

The four treatments were grass/clover silage made of spring grass/clover with or without rapeseed supplementation and grass/clover silage made of autumn grass/clover with or without rapeseed supplementation. The silages were mixtures of red (*Trifolium pratense*) and white clover (*Trifolium repens*), perennial ryegrass (*Lolium perenne*), and hybrid ryegrass (*Lolium* × *boucheanum Kunth*) harvested at the same field at AU-Foulum (56°N, 9°E). The spring silage was a first growth harvested May 23rd 2012 and contained 81, 9 and 10% of grass, white clover, and red clover on dry matter (DM) basis, respectively. The two autumn silages were a third and fourth regrowth and they were harvested September 8th and October 11th 2012, respectively. Grass, white clover and red clover concentrations were 77, 15 and 8% on DM basis for the third regrowth and 87, 8 and 6% on DM basis for the fourth regrowth. A mixture of equal amounts of 3rd and 4th regrowth was used in the feeding experiment to ensure sufficient amounts of silage during the experimental period. After prewilting, grass/clover was precision chopped with a forage harvester (Claas Jaguar 870, Claas KGaA mbH, Harsewinkel, Germany) and ensiled in bunker silos without any ensiling additives. The rapeseed was a double-00 variety and it was ground before inclusion in the rations.

The cows had *ad libitum* access to the four partial mixed rations (PMR; Table 1) which were mixed once daily in a vertical mixer wagon (JF-Stoll, Sønderborg, Denmark). The daily feed intake was automatically recorded by the Insentec RIC system (Insentec, Marknesse, The Netherlands). The cows were offered up to 3·0 kg of concentrate in the AMU daily. The actual intake (offer and left over) of AMU concentrate was registered. For more details see Bossen et al. (2009). Cows had free access to drinking water at all time.

Sampling

Representative samples of silages and concentrates were taken weekly and stored at −20 °C. Samples of silages and AMU concentrate were pooled for 6-week periods and all other ingredients were pooled for the whole experiment for chemical analysis. Milk yield was measured at every visit in the AMU using the DeLaval Free Flow meter MM25 (DeLaval AB, Tumba, Sweden) based on optical milk flow measurement. For determination of ECM yield representative milk samples were taken at each milking

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Table 1. Composition of partial mixed rations in g/kg dry matter for cows fed either silage made of spring or autumn grass/clover without or with rapeseed supplementation

	Treatments			
	Spring		Autumn	
Silage				
Rapeseed	–	+	–	+
Spring grass/clover silage	646	624		
Autumn grass/clover silage 3rd regrowth			339	326
Autumn grass/clover silage 4th regrowth			323	312
Barley	188	181	180	174
Rapeseed		70		67
Rapeseed meal	92	53	88	51
Dried sugar beet pulp	64	62	61	59
NaCl	2	2	2	2
Mineral mix	9	9	9	9

during a 48-h period in the last week of each period. These samples were sent directly to an external laboratory for analysis of protein, fat, and lactose concentrations. This was followed by another 24-h sampling period where samples for fatty acids, β-carotene, α-tocopherol, and riboflavin were taken. These samples were stored at at −20 °C until analysis.

Chemical analyses

Dry matter of ingredients and feed samples was determined at 60 °C for 48 h. Ash was determined by combustion at 525 °C for 6 h (AOAC, 1990). Nitrogen was determened by the Dumas principle (Hansen, 1989) using a Vario Max CN (Elementar Analysesysteme GmbH, Hanau, Germany). Crude protein concentration was calculated as $N \times 6{\cdot}25$. Crude fat was extraced with petroleum ether (Soxtec 2050, Foss analytical, Hillerød, Denmark) after hydrolysing with HCl (Stoldt, 1952). Total sugars were analysed by the Luff-Schoorl method (European Community, 2012, 71/250/EEC). Starch was analysed by an enzymatic calorimetric technique (Knudsen et al. 1987). Neutral detergent fibre (NDF) was analysed by neutral detergent extraction using heat stable amylase and reported as ashfree according to Mertens (2002) using a FibertecTM M6 system (Foss Analytical, Hillerød, Denmark). In vitro organic matter digestbility of silage followed Tilley & Terry (1963). The digestibility of concentrate was determined in vitro by enzymatic digestion. For further details and prediction of in vivo organic digestibility of silages and concentrate, see Åkerlind et al. (2011). Net energy for lactation was calculated from Weisbjerg & Hvelplund (1993). The concentration of acetate and lactate was analysed according to the method described by Canibe et al. (2007). Ammonium nitrogen was determined by alkalisation of the sample with KOH and the NH_3 was determined by titration after distillation using a Kjeltec 2400 (Foss Analytical, Hillerød, Denmark). The concentrations of fat, protein, and lactose in milk was determined on a Milkoscan 4000 infrared analyser at Eurofins Steins (Holstebro, Denmark).

Fatty acids

Fatty acid analysis in milk was performed based on Larsen et al. (2013), where fat was separated from milk by centrifugation, and fatty acids were methylated using sodium methylate. Fatty acid methyl esters (FAME) were quantified by the use of external standards (Supelco FAME mix C4–C24, Bellefonte, USA; PA and GLC 469 methyl ester standard from Nu-Chek Prep Inc. Elysian, MN, USA) and the concentrations were calculated in g/kg of identified milk fatty acids. Fatty acids from dried feed samples were analysed based on Jenkins (2010): 1 ml of heptane including internal standard (C12 : 1*cis*11 triglyceride, 0·4 mg/ml) was added to 0·2–0·3 g of silage or 30 mg of rapeseed or 0·1 g of the other feeds and mixed for 10 s. Then 0·2 ml of sodium-methylate (25%) was added to the solution and mixed for 10 sec and incubated at 50 °C for 10 min. After cooling on ice, 1·5 ml of methanolic hydrochloric acid (10%) was added and samples were incubated at 90 °C for 30 min. After cooling on ice, 1 ml of heptane and 3 ml of potassium carbonate (10%) were added. Samples were centrifuged and the heptane phase was used for GC analysis. Fatty acids were identified as above and based on the internal standard fatty acids in feed and were quantified and expressed as mg FA/g DM.

Analysis of β-carotene and α-tocopherol

Milk or dried feed samples were saponified prior to extraction of β-carotene or α-tocopherol for HPLC analysis (Slots et al. 2009; Larsen et al. 2013). External standards of β-carotene and α-tocopherol were used for quantification.

Riboflavin analysis

Milk proteins were precipitated and serum was used for HPLC analysis of riboflavin (Poulsen et al. 2015).

Calculations and statistical analysis

Energy corrected milk (ECM, 3·14 MJ/kg) was calculated according to Sjaunja et al. (1991).

Apparent recovery of C18 : 2*n*6 and C18 : 3*n*3 was calculated as the ratio between total amount excreted in milk and total amount ingested from feed. The degree of biohydrogenation of C18 : 2*n*6 and C18 : 3*n*3 was estimated using the following formula (where C18 : a is C18 : 2*n*6 or C18 : 3*n*3) (Larsen et al. 2012):

$$\mathrm{BH(C18:}a) = 1 - \frac{(\mathrm{C18:}a/\mathrm{Total\ C18})\ \mathrm{in\ milk}}{(\mathrm{C18:}a/\mathrm{Total\ C18})\ \mathrm{in\ feed}}$$

Data were analysed using PROC MIXED in SAS (SAS® version 9.2, Cary, NC, USA) with the cow as experimental unit using the following model:

$$X_{ijklm} = \mu + \alpha_i + \beta_j + \delta_\kappa + \gamma_l + \alpha_i\beta_j + \beta_j\delta_\kappa + \alpha_i\delta_k + E_{ijklm} + e_{ijklm}$$

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Where X_{ijklm} was the dependent variable, μ was the overall mean, α_i was the fixed effect of grass/clover silage type i (spring, autumn), β_j was the fixed effect of the rapeseed j (−, + rapeseed), δ_k was the fixed effect of parity k (first parity or older), γ_l was the fixed effect of period l (1–4), E_{ijklm} was the random effect of the cow, e_{ijkm} was the random residual error. Degree of freedom was estimated by the Satterthwaite procedure. Results were reported as least square means and standard error of mean (SEM). Results were considered to differ significantly if the P-value of the model was less than 0·05. Pairwise comparisons of LS means for significant effects were performed using the PDIFF option adjusted with Turkey-Kramer. Four cows were considered as outliers and omitted from the analyses because either the cows were sick or cows had dubious feed intake registrations.

Results

Composition of the three silages is shown in Table 2. The crude protein, crude fat, and C18 : 3 concentrations were higher in autumn silage compared to spring whereas the NDF concentration was lower in autumn silage compared to spring (Table 2). The sugar concentration was high in the fourth regrowth of the autumn silage compared to spring as well as third regrowth autumn silage. The acetic acid, lactic acid, α-tocopherol and β-carotene concentrations were lower in the 4th regrowth compared to spring and 3rd regrowth.

The intake of PMR, total DM, net energy for lactation (NE_L), crude protein, NDF, crude fat, total FA as well as C18 : 2 and C18 : 3 were higher ($P < 0·001$) for treatments with autumn silage compared to spring silage (Table 3). The DM intake was similar between treatments with and without rapeseed. Inclusion of rapeseed increased the intake of NE_L ($P < 0·05$), crude fat ($P < 0·001$) and total FA ($P < 0·001$) including C18 : 2 ($P < 0·001$) and C18 : 3 ($P < 0·001$).

Data for milk yield and production efficiency are presented in Table 4. The milk and ECM yield were higher ($P < 0·01$) for cows fed the treatments with autumn silage compared to spring silage. The fat concentrations and the utilisation of NE_L for ECM were lower ($P < 0·05$) for cows fed the treatments with autumn silage compared to spring. Supplementation of the ration with rapeseed increased ($P < 0·001$) milk and ECM yield, whereas the protein concentration was decreased ($P < 0·001$). The number of daily visits in the milking robot was similar for all four treatments (Table 4).

Data for milk fatty acids and vitamins/antioxidants are presented in Table 5. Rapeseed inclusion affected milk FA proportions of all FA except for C18 : 3*n*3 and CLA*cis*9*trans*11. Proportions of C4 : 0 to C14 : 0, C11 : 0 to C17 : 0, C16 : 0, C14 : 1*cis*9, C16 : 1*cis*9, and C18 : 2*n*6 decreased ($P < 0·05$) with rapeseed supplementation, and C18 : 0, C18 : 1*trans*ALL, and C18 : 1*cis*9 increased ($P < 0·001$) with rapeseed supplementation. Spring silage increased ($P < 0·001$) the proportions of C11 : 0 to C17 : 0, and C16 : 0, whereas autumn silage resulted in higher proportions of C18 : 1*cis*9, C18 : 2*n*6 and C18 : 3*n*3 in milk fat ($P < 0·05$). Apparent recovery of C18 : 3*n*3 was not influenced by treatments, whereas rapeseed supplementation decreased recovery of C18 : 2*n*6 ($P < 0·001$). The estimated biohydrogenation of C18 : 2*n*6 was increased ($P < 0·001$) by feeding rapeseed or spring silage and the estimated biohydrogenation of C18 : 3*n*3 was decreased ($P < 0·001$) when rapeseed were included in the diet. Interaction between silage type and rapeseed supplementation was observed ($P < 0·05$) for C11 : 0 to C17 : 0, C16 : 0, C18 : 0, and C18 : 1*trans*ALL, and in all cases the effect of rapeseed inclusion was most pronounced in combination with spring silage. The concentration of α-tocopherol in milk was higher ($P < 0·01$) when spring silage was included in the diets whereas β-carotene concentrations were higher ($P < 0·05$) when autumn silage was in the diets (Table 5). Rapeseed inclusion increased ($P < 0·01$) α-tocopherol concentrations and decreased ($P < 0·05$) β-carotene concentrations (Table 5). Riboflavin content in milk was higher ($P < 0·001$) when spring silage was fed, and rapeseed inclusion did not affect riboflavin concentration (Table 5).

Discussion

Effect of harvest season on silage composition

The crude protein and crude fat concentrations were higher and NDF was lower as expected in autumn silages compared to spring silage. The sugar concentration was higher than expected in the fourth regrowth of the autumn silage (Table 2). This silage also had a higher DM concentration and lower concentrations of lactic and acetic acids compared to the two other silages, which indicates that the high DM concentration had restricted the fermentation process. Silage from the fourth regrowth had much lower concentrations of β-carotene and α-tocopherol; this lower concentration of β-carotene could also be a result of higher pH and less anaerobic conditions due to a different fermentation process (Nozière et al. 2006), and α-tocopherol was most likely affected in the same way.

Concentration of FA, in particular C18 : 3 (Table 2) were higher in autumn silage compared to spring silage. This is due to grass harvested from May to June has a larger proportion of stems with lower concentration of fatty acids, whereas leaf proportion, with a higher concentration of fatty acids increases in the end of the growing season. Also VanRanst et al. (2009) have reported seasonal variation in FA concentration in grass and clover with the highest values in the autumn. However, they also describe losses between 0 and 15% during wilting and ensiling so differences between FA content of grass and corresponding silage should be expected (VanRanst et al. 2009).

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Table 2. Chemical composition, ensiling characteristics, fatty acid composition and antioxidants in grass/clover silages in g/kg dry matter if nothing else is stated

	Spring (first growth)	Autumn (third regrowth)	Autumn (fourth regrowth)
Dry matter (g/kg)	289	315	437
Ash	116	138	149
Crude protein	189	216	229
Crude fat	36	43	44
Sugar	2·2	3·0	53
Neutral detergent fiber	356	319	301
Organic matter digestibility (g/kg organic matter)†	806	790	813
Net energy for lactation (MJ/kg dry matter)‡	7·3	7·3	7·1
Ammonia-Nitrogen (g/1000 g N)	83	68	64
Acetic acid	24	17	9
Lactic acid	118	105	41
C16 : 0	4·1	4·6	4·7
C18 : 0	0·4	0·6	0·5
C18 : 1	0·4	0·5	0·5
C18 : 2	3·8	4·6	4·2
C18 : 3	18·7	23·2	25·6
Total FA§	27·5	33·4	35·5
α-tocopherol (mg/kg DM)	91·2	85·2	24·2
β-carotene (mg/kg DM)	268·2	326·3	172·1

†Estimated from in vitro rumen fluid digestibility according to Åkerlind et al. (2011).
‡Net energy estimated according to Weisbjerg & Hvelplund (1993) as 7·89 MJ per feed unit.
§Total FA: C16 : 0, C18 : 0, C18 : 1*cis*9, C18 : 2*n*6, C18 : 3*n*3.

Table 3. Intake of concentrate, partial mixed ration in dry matter, net energy for lactation and selected nutrients for cows fed either silage made of spring or autumn grass/clover without or with rapeseed supplementation

	Treatments					*P*-values	
Silage	Spring		Autumn				
Rapeseed	–	+	–	+	SEM	Growth	Rapeseed
Concentrate (kg DM/d)	2·5	2·4	2·5	2·4	0·03	0·72	0·28
PMR (kg DM/d)	13·6	13·5	15·1	14·9	0·4	<0·001	0·51
Total (kg DM/d)	16·1	16·0	17·5	17·3	0·4	<0·001	0·44
Net energy for lactation (MJ/d)	125	130	136	140	2·9	<0·001	0·02
Nutrients							
Crude protein	3·0	2·9	3·6	3·4	0·08	<0·001	0·01
Sugar (kg/d)	0·5	0·5	0·7	0·7	0·01	<0·001	0·03
Starch (kg/d)	2·1	2·0	2·2	2·1	0·04	0·003	0·006
Neutral detergent fiber (kg/d)	4·9	4·8	5·0	4·8	0·11	0·79	0·02
Crude fat (kg/d)	0·5	0·9	0·6	1·1	0·02	<0·001	<0·001
Total FA (kg/d)	0·45	0·85	0·56	0·98	0·018	<0·001	<0·001
C18 : 2 (kg/d)	0·12	0·21	0·14	0·23	0·004	<0·001	<0·001
C18 : 3 (kg/d)	0·18	0·23	0·26	0·30	0·007	<0·001	<0·001
α-tocopherol (g/d)	0·93	0·94	0·66	0·68	0·02	<0·001	0·18
β-carotene (g/d)	2·4	2·3	2·5	2·4	0·006	0·14	0·01

No significant interactions between Growth and Rapeseed.

Dry matter intake and milk production

The DM intake (DMI) was higher ($P < 0·001$) for cows fed the diet with autumn silage compared to spring silage, although the digestibility of organic matter was similar. Huthanen et al. (2007) concluded from a meta-analysis that intake of regrowth silage (all regrowths) was lower than primary growth, increasing digestibility of organic matter increased silage intake, increasing concentration of total acids decreased intake and effect of dry matter concentration of the silage was curvilinear with a maximum intake at 419 g/kg DM. In the present study the average DM concentration was higher, and average concentration of acetate and lactate was lower for the autumn silage

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Table 4. Number daily visits in milking robot, yield in kg, composition of milk in g/kg and efficiency as energy corrected milk (ECM)/net energy for lactation (NE_L) in kg ECM/MJ NE_L for cows fed either silage made of spring or autumn grass/clover without or with rapeseed supplementation

	Treatments					*P*-values	
Silage	Spring		Autumn		SEM	Growth	Rapeseed
Rapeseed	–	+	–	+			
Visits in milking robot	2·7	2·8	2·7	2·8	0·09	0·82	0·09
Milk yield	20·1	21·7	21·4	22·5	0·71	<0·001	<0·001
Fat	63·5	63·5	62·2	62·0	1·3	0·03	0·83
Protein	42·9	41·1	42·7	41·6	0·5	0·62	<0·001
Lactose	47·7	48·5	48·1	48·6	0·2	0·02	<0·001
ECM	26·8	28·7	28·2	29·3	0·8	0·001	<0·001
ECM/NE_L	0·214	0·220	0·208	0·210	0·005	0·004	0·21

No significant interactions between Growth and Rapeseed.

Table 5. Concentration of fatty acids in milk (g/kg fatty acids), recovery and biohydrogenation of fatty acids and concentration of vitamins/antioxidants for cows fed either spring or atumn grass/clover silage without or with rapeseed supplementation

	Treatments					*P*-values		
Silage	Spring		Autumn		SEM	Growth	Rapeseed	G*R
Rapeseed	–	+	–	+				
Milk fatty acids								
C4 : 0 to C14 : 0	289·0	282·8	295·3	282·6	4·3	0·41	0·015	0·39
C14 : 1*cis*9	10·1	8·1	10·0	8·5	0·3	0·41	<0·001	0·12
C11 : 0 to C17 : 0	24·9	19·9	21·3	18·3	0·5	<0·001	<0·001	0·011
C16 : 0	342·2	280·9	315·9	274·8	4·7	<0·001	<0·001	0·028
C16 : 1*cis*9	17·9	13·1	17·4	13·8	0·5	0·77	<0·001	0·13
C18 : 0	91·7	136·6	99·4	133·6	2·1	0·25	<0·001	0·009
C18 : 1*trans* ALL	15·4	20·7	17·9	21·0	0·44	<0·001	<0·001	0·005
C18 : 1*cis*9	152·3	185·6	168·2	194·7	4·3	0·001	<0·001	0·42
C18 : 2*n*6	14·2	13·4	14·5	14·1	0·26	0·018	0·002	0·13
C18 : 3*n*3	7·8	7·9	9·6	9·4	0·19	<0·001	0·66	0·40
CLA*cis*9*trans*11	5·5	6·0	5·2	5·1	0·37	0·12	0·60	0·66
Fatty acid recovery†								
C18 : 2*n*6	0·147	0·089	0·139	0·086	0·004	0·07	<0·001	0·42
C18 : 3*n*3	0·054	0·048	0·050	0·049	0·003	0·48	0·16	0·33
Biohydrogenation‡								
C18 : 2*n6*	0·841	0·861	0·835	0·850	0·002	<0·001	<0·001	0·23
C18 : 3*n3*	0·941	0·924	0·941	0·913	0·005	0·27	<0·001	0·29
Vitamins/antioxidants								
Riboflavin (mg/l)	2·1	2·1	1·8	1·9	0·07	<0·001	0·90	0·26
α-tocopherol (mg/l)	1·75	2·01	1·59	1·76	0·10	0·008	0·006	0·55
β-carotene (mg/l)	1·37	1·24	1·50	1·35	0·09	0·044	0·021	0·81

†Fatty acid recovery from feed to milk [output in milk per day (g)/intake in feed per day (g)].

‡Degree of biohydrogenation calculated as $BH = (C18 : a) = 1 - \frac{(C18 : a/\text{Total } C18)\text{in milk}}{(C18 : a/\text{Total } C18)\text{in feed}}$.

compared with spring which might be the reason for the higher intake of autumn silage compared to spring silage. This further indicate that for grass/clover silages under Danish conditions there is no specific negative late summer or autumn cut effect of on feed intake, as also indicated by Alstrup et al. (2016).

The higher intake of the PMR for cows fed autumn silage compared to spring caused an increase in milk production ($P < 0·001$). However, the efficiency of NE_L for milk was lower for autumn silage compared to spring. Decreased efficiency is commonly seen at increased DM intake (Jensen et al. 2015).

The DMI was not affected by the supplementation of rapeseed, probably because the resulting fat concentration in fat supplemented rations was moderate (59–62 g/kg DM in total diet). A positive response in milk yield to

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supplementation with rapeseed was found in the present experiment, in concordance with the general positive milk response to moderate fat supplementation of dairy cow (Weisbjerg et al. 2008). The milk protein concentration decreased for cows feed the silage diets supplemented with rapeseed, which is a common response to fat supplementation (Wu & Huber, 1994; Weisbjerg et al. 2008).

Milk fatty acid composition

The overall main effects of type of silage were as expected as higher concentrations of C18 : 3*n*3 in the silage gave higher concentrations of C18 : 3*n*3 as well as most other C18 fatty acids in the milk and lower concentrations of de-novo synthesised fatty acids (C4 : 0 to C16 : 0). In contrast, for rapeseed supplementation a higher concentration of C18 : 2*n*6 and C18 : 3*n*3 in the PMR was accompanied by a decrease in C18 : 2*n*6 and no effect on C18 : 3*n*3 in milk fat. This is in disagreement with Larsen et al. (2013), but similar to findings by Collomb et al. (2004). CLA*cis*9*trans*11 concentration in milk fat was not affected by treatments, which was unexpected as this fatty acid normally increases when the content of PUFA in feed is increased (Chilliard et al. 2007; Larsen et al. 2012, 2013). Supplementing a hay diet with 1 kg rapeseed resulted in lower proportions of C10 to C16 FA in milk and increased concentrations of C18 : 0 and C18 : 1*cis*9 fatty acids (Collomb et al. 2004). These results are in agreement with the current study as well as Chilliard et al. (2007).

The main affects of both autumn silage and rapeseed inclusion were similar on the reduction of C11 : 0 to C17 : 0 and C16 : 0 as well as the increase of C18 : 0 and C18 : 1*trans* concentrations in milk fat, and the interaction showed that the effects of rapeseed supplementation were more pronounced in combination with spring silage compared to autumn silage. Most likely the same biological processes were affected by rapeseed and silage type, but the effects were not additive, and showed highest increase due to rapeseed supplementation for the spring silage, which was opposite to the hypothesis.

Recovery of fatty acids

The apparent recovery is a measure of the share of an ingested FA excreted into milk. The remaining part of this FA is either biohydrogenated or used by the animal for other metabolic purposes. The estimated degree of biohydrogenation is based on the assumption that C18 : 2*n*6 and C18 : 3*n*3 are transferred to milk in the same ratio as the total pool of C18 FA. When rapeseed was fed, the biohydrogenation of C18 : 2*n*6 increased and the biohydrogenation of C18 : 3*n*3 decreased. This could be due to a higher share of the PUFA being C18 : 2*n*6. Similarly, the biohydrogenation of C18 : 2*n*6 decreased when autumn silage was fed, this could be due to a higher share of the PUFA being C18 : 3*n*3. The lack of difference in apparent recovery of C18 : 2*n*6 and C18 : 3*n*3 in combination with the reduced biohydrogenation when autumn silage or rapeseed, respectively, was fed indicates that the higher available amount of PUFA was used for other purposes than milk fat. For rapeseed feeding increased biohydrogenation of C18 : 2*n*6 was in line with a lower recovery and a lower concentration in milk fat. However, the biohydrogenation increased 0·02 relative units whereas the recovery decreased 0·05–0·06 relative units, and this difference can be interpreted as a higher share of C18 : 2*n*6 was used for other purposes than milk fat when rapeseed was fed. Differences in fibre content of the diets were assumed to affect the biohydrogenation, but these differences were probably too small to affect biohydrogenation systematically.

Vitamins

The higher concentration of riboflavin in milk after feeding spring silage shows a potential for controlling the riboflavin concentration of milk by feeding. However, further investigations are needed to establish to which extent the riboflavin concentration in milk is controlled mainly by ruminal synthesis or by differences in riboflavin concentration of feed. The differences in content of α-tocopherol and β-carotene in milk varied moderately between treatments, and were in accordance with moderate variations in the PMR.

In conclusion, this study has shown that autumn silage can be used as a sole forage source. Compared to spring silage autumn silage has a higher content of protein and fat, in particular linolenic acid. Opposed to the hypothesis the fibre content was almost the same of the silages and silage from the third regrowth had similar concentration of fermentation products as spring silage. Silage from fourth regrowth showed an atypical fermentation pattern, but this was supposed to be an effect of high DM rather than an effect of grass/clover composition itself. The higher linolenic acid content of autumn silage compared to spring silage has resulted in higher content of linolenic acid in milk fat, but the expected effects on ruminal biohydrogenation were not observed because there was no difference in the fibre content of the silages. Rapeseed supplementation resulted in expected effects on milk production and composition, but there were barely any interaction effects between silage type and rapeseed supplementation.

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