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Investigations on the influence of roughage/concentrate ratio and linseed oil supplementation on rumen fermentation and microbial protein yield in dairy cows

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ABSTRACT

The objective of the study was to investigate the influence of the roughage/concentrate ratio and linseed oil supplementation on rumen fermentation and microbial protein yield in late lactating dairy cows.

Seven late lactating double fistulated cows were randomly assigned to four experimental periods applying an incomplete 2 x 2 Latin square design. The rations consisted of meadow hay and a concentrate mixture given in a ratio of 70 : 30 (H 70) or 30 : 70 (H 30) on a dry matter basis. The basal mixtures fed were either supplemented with 200 g linseed oil daily (HLO 70 and HLO 30) or non-supplemented. After about three weeks of adaptation to the experimental rations, samples from the rumen and the duodenal chyme were taken to study parameters of rumen fermentation and the nutrient flow to the duodenum.

The pH-value, the NH_3-N concentration, and the content of short-chain fatty acids in the rumen as well as the apparent ruminal digestibility of organic matter (OM) and neutral detergent fibre (NDF) were not significantly affected by linseed oil supplements. However, low portions of hay in the ration significantly reduced the pH-value and increased the NH_3-N concentration.

An increased hay portion in the ration resulted in an increased NDF-degradation in the rumen as compared to the rations high in concentrate. Due to an increased flow of microbial OM after high concentrate feeding, the "apparent ruminal digestibility" of OM was calculated to decrease although the quantity of FOM (fermented organic matter) increased. Feeding the rations poor in hay increased the flow of non ammonia nitrogen (NAN) and utilisable crude protein (uCP) significantly due to the significantly increased microbial protein synthesis (g MP/d) and a improved efficiency of microbial protein synthesis (g MP/kg FOM) as well.

Keywords: dairy cows, roughage: concentrate, linseed oil, rumen fermentation, microbial protein yield

INTRODUCTION

The health and performance of dairy cows depends on the intake of digestible and metabolisable nutrients. Certain amounts of fibre from roughage are necessary for rumination and rumen processes (e.g. de Brabander et al., 1999; GfE, 2001; Meyer et al., 2001; 2002; Piatkowski, 1977). But normally roughage is less digestible than concentrate and can not provide enough energy for high yielding cows.

Fat can be added as so-called ruminally-protected or not protected fat. Unprotected fat supplements may affect rumen fermentation (Machmüller and Kreuzer, 1999; Palmquist and Kenkins, 1980; Sutton et al., 1983) and microbial protein synthesis (Doreau and Ferlay, 1995). Furthermore, added fats can influence fatty acids pattern of milk fat (e.g. Flachowsky et al., 1996; Griinari and Bauman, 2006; Jahreis et al., 1996; Kennely and Glimm, 1998; Wagner et al., 1998) depending on the fatty acid pattern of the supplemented fats.

Fewer studies are available investigating the effects of oils with high concentrations of unsaturated fatty acids, such as linseed oil, on parameters of rumen fermentation, and nutrient flow to the duodenum of cows fed with various kinds of diets. Therefore, the objective of the present study was to investigate the influence of two roughage/concentrate ratios (70 : 30 and 30 : 70) with and without linseed oil supplementation on parameters of rumen fermentation and nutrient flow to the duodenum in late lactating dairy cows.

This article is dedicated to our friend Prof. Dr. Alexander Sommer on the occasion of his 70th birthday

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MATERIAL AND METHODS

Seven multiparous lactating Friesian cows (initial body weight: 554 ± 45 kg) fitted with cannulae in the dorsal rumen, and simple T-shaped cannulae in the proximal duodenum, were randomly assigned to four experimental periods applying an incomplete 2 x 2 Latin square design. The cows were in late lactation (230 ± 25 days in milk), producing 21.8 ± 4.1 kg fat corrected milk per day. The animals were kept individually on rubber mats with slatted flooring without bedding material. Drinking water was freely available.

The cows were fed with four different diets varying in percentages of meadow hay (70 and 30 % of DM) and concentrate (30 and 70 % of DM) both supplemented with 200 g linseed oil per cow per day or unsupplemented (Table 1).

Table 1: Feeding of cows in various periods

Feedstuffs	Ration			
	H 70	HLO 70	H 30	HLO 30
Meadow hay[1)] (% of DMI) (kg DM day^{-1})	70 9.1	70 9.1	30 4.0	30 4.0
Concentrate [2)] (% of DMI) (kg DM day^{-1})	30 3.9	30 3.9	70 8.9	70 8.9
Linseed oil (g d^{-1})	-	200	-	200

[1)] 98.5 g crude protein, 625 g NDF per kg DM,
[2)] Ingredients of concentrate: 38% barley, 35% wheat, 13% rye, 11.5% extr. soybeanmeal, 2.5% vit/min premix; 177.0 g crude protein, 214.0 g NDF per kg DM

To avoid feed refusals, all cows consumed adequate amounts of feed based on the dry matter intake (DMI) of the cow with the lowest DMI.. Concentrate was given in four (equally-sized) portions at 5:30 and 7:30 a.m. and 4:30 and 6:30 p.m. Meadow hay was offered at 5:30 a.m. and 4:30 p.m. At 5:30 a.m. and 4;30 p.m., 100 g of linseed oil (Protein- und Ölwerk Neuss GmbH & Co KG, Neuss, Germany) were administered directly onto the feedstuffs with a syringe.

Diets were given to the cows for four weeks, three weeks were planned for adaptation, chymus collection was carried out in the fourth week. Cows were milked at 5:00 a.m. and 3:00 p.m. and milk yield was recorded at each milking.

Ruminal fluid for the determination of pH, ammonia and short chain fatty acids (SCFA) was sampled during the third week for two days via the rumen cannulae using a hand vacuum pump at 5:30, 6:00, 6:30, 7:00, 7:30, 8:30 and 10:30 a.m. Rumen pH was measured immediately after sampling using a digital pH meter (pH 525, WTW, Weilheim, Germany). The ammonia content of rumen fluid was determined according to DIN 38406-E5-2 (1998).

The concentrations of SCFA were analysed by gas chromatography (Hewlett Packard 5580; Hewlett Packard, Avondale, PA, USA) with a flame ionization detector after sample-preparation according to Geisler et al. (1976). Duodenal flow was measured during the last week of each experimental period. To estimate the digesta flow, Cr_2O_3 was mixed with wheat flour, weighed into filter paper and given in two portions per day into the rumen beginning ten days before the start of the duodenal digesta collection, and in four portions per day during the collection period (Rohr et al., 1984). Spot samples of duodenal chyme were taken in 2-h intervals from Monday to Saturday as described by Rohr et al. (1979). After nitrogen (N; Kjeldahl method) determination in the fresh duodenal digesta, the digesta was freeze-dried and ground to pass through a 1mm-sieve. Cr_2O_3 in marker and digesta samples was analysed applying atomic absorption spectrometry according to Williams et al. (1962). The microbial portion of non-ammonia-N (NAN) in the freeze-dried duodenal samples was estimated using near infra-red spectroscopy (NIRS) according to Lebzien and Paul (1997). The chemical composition of the feedstuffs, as well as the NDF content of the digesta samples were determined according to the VDLUFA methods (Naumann and Bassler, 1993).

RESULTS AND DISCUSSION

The dry matter intake (DMI) of cows fed with diets high and low in roughage or concentrate and supplemented with 200 g linseed oil per day or non-supplemented was nearly the same (Table 2).

Table 2: Dry matter, nutrients and energy intake of cows during experimental periods

Parameters	Ration			
	H 70	HLO 70	H 30	HLO 30
	kg day^{-1}			
DM	12.82 ± 0.08	12.94 ±0.35	12.83 ± 0.03	13.04 ± 0.18
OM	12.05^{a} ± 0.03	12.17^{ab} ± 0.28	12.19^{ab} ± 0.03	12.38^{b} ± 0.07
CP	1.52^{a} ± 0.06	1.61^{a} ± 0.12	2.01^{b} ± 0.13	1.98^{b} ± 0.13
Ether extract	0.26^{a} ± 0.02	0.42^{b} ± 0.04	0.29^{a} ± 0.10	0.47^{b} ± 0.04
NDF	6.45^{b} ± 0.07	6.39^{b} ± 0.19	4.36^{a} ± 0.08	4.39^{a} ± 0.10
	MJ day^{-1}			
ME	126.5^{a} ± 0.4	131.3^{b} ± 3.3	148.5^{c} ± 0.4	154.4^{d} ± 0.8
NEL	76.3^{a} ± 0.3	79.3^{b} ± 2.1	93.8^{c} ± 0.2	97.6^{d} ± 0.4

$^{a, b, c, d}$ = values in the rows with different letters differ significantly ($p \leq 0.05$)

Cows fed with low hay (H 30 and HLO 30) consumed less fibre, but more protein and energy. Oil supplementation (HLO 70 and HLO 30) increased the intake of ether extract and energy in comparison to the non-supplemented groups (Table 2).

Rumen fermentation

The pH-value in the rumen liquid was significantly influenced by ration composition, but mostly not influenced ($p>0.05$) by the supplementation of linseed oil (Table 3). value (Table 3). The data are in agreement with references, where no significant effects of linseed oil supplementation on rumen pH were described (e.g. Doreau and Ferlay, 1995; Ueda et al., 2003). Doreau et al. (1993) added 0.99 kg rapeseed oil per day to rations of dairy cows and did not observe any influence on rumen pH-value.

The ammonia-concentration in the rumen increased up to 90 – 120 min after feeding (Table 4) and later decreased.

Both 30 and 60 minutes after feeding, the NH_3-N-concentration was significantly higher in animals fed

Table 3: Effects of feeding on the pH-value in rumen liquid before and after morning feeding

Time after feeding (min)	Ration					Effects[1)]		
	H 70	HLO 70	H 30	HLO 30	PSEM	Hay	Linseed oil	HxLO
Before	6.55	6.49	6.88	6.81	0.07	<0.001	0.424	0.938
30	6.47	6.37	6.69	6.57	0.08	0.014	0.177	0.902
60	6.37	6.22	6.55	6.31	0.08	0.094	0.021	0.603
90	6.18	6.09	6.32	6.08	0.10	0.477	0.113	0.487
120	6.20	5.96	5.96	5.75	0.11	0.049	0.052	0.853
180	5.98	5.89	5.72	5.62	0.13	0.058	0.476	0.980
300	6.03	5.90	5.70	5.71	0.83	0.009	0.479	0.434

1) $p<0.05$: sign. differences between treatments

Table 4: Effects of feeding on the NH_3-N-concentration (mmol/l) in rumen liquid before and after morning feeding

Time after feeding (min)	Ration					Effects[1)]		
	H 70	HLO 70	H 30	HLO 30	PSEM	Hay	Linseed oil	HxLO
Before	3.1	3.8	6.5	5.5	1.3	0.036	0.771	0.407
30	3.8	4.9	8.1	7.1	1.8	0.050	0.993	0.511
60	7.4	6.2	11.5	9.7	1.7	0.019	0.300	0.851
90	8.9	7.9	12.1	10.1	1.6	0.105	0.392	0.553
120	8.8	8.2	12.2	10.7	2.5	0.167	0.621	0.830
180	6.9	7.7	4.9	7.2	2.0	0.485	0.380	0.664
300	2.8	3.2	1.2	1.7	0.8	0.056	0.504	0.929

[1)] see Table 3

Low portions of hay in the ration (H 30 and HLO 30) reduced the rumen pH significantly at different times after feeding. These results are caused by a lower rumination and a more intensive fermentation of easily fermentable carbohydrates from concentrates, and are in agreement with some references (Flachowsky et al., 1993; Griinari et al., 1998; Kalscheur et al., 1997).

Except for the time 60 min. after the start of feeding, linseed oil did not significantly influence the rumen pH- with concentrate rich rations (H 30, HLO 30). Later the NH_3-N-concentration in rumen liquid was lower in the H 30 and HLO 30-rations in comparison with the rations rich in hay, probably caused by the high microbial activity and the N-demand during this time. Linseed oil did not significantly influence the NH_3-N-concentration in rumen liquid (Table 4), which is in agreement with papers reviewed by Doreau and Ferlay (1995).

Apart from the valeric acid portion, the concentration

and pattern of short-chain fatty acids (SCFA) and the acetate: propionate ratio in the rumen liquid were not significantly influenced by ration composition and 200 g linseed oil supplementation (Table 5). Higher concentrations of acetic acid and lower values for propionic acid after feeding rations rich in roughages are described in many papers (e.g. Griinari et al., 1998; Ueda et al., 2003), but could be observed only in tendency between individual measurements.

Nutrient flow and protein yield at the duodenum

The ration composition significantly influenced the apparent ruminal digestibility and the flow of organic matter (OM) and NDF to the duodenum (Table 6). The lower apparent ruminal digestibility of the OM of the

Table 5: Effect of feeding on the concentration of volatile fatty acids (VFA) in rumen liquid 180 min after morning feeding

VFA	Ration					Effects[1)]		
	H 70	HLO 70	H 30	HLO 30	PSEM	Hay	Linseed oil	HxLO
Total VFA (mmol/l)	144.6	140.4	136.5	149.6	13.1	0.967	0.738	0.519
				Molar proportions (%)				
Acetic acid	64.3	64.2	58.9	61.3	2.4	0.100	0.632	0.598
Propionic acid	19.9	19.5	20.2	20.4	1.6	0.724	0.923	0.896
Butyric acid	12.5	12.7	15.3	13.7	1.1	0.087	0.516	0.387
Valeric acid	1.7	1.8	3.6	3.0	0.3	<0.001	0.426	0.363
Acetic acid: Propionic acid	3.3	3.4	3.2	3.3	0.4	0.801	0.857	0.967

[1)] see Table 3

Table 6: Nutrient flow to the duodenum

Nutrients	Ration					Effects[1)]		
	H 70	HLO 70	H 30	HLO 30	PSEM	Hay	Linseed oil	HxLO
				Flow (kg day $^{-1}$)				
OM	5.50	5.70	5.85	6.05	0.14	0.019	0.167	0.986
NDF	2.22	2.32	1.99	1.83	0.10	0.001	0.749	0.199
				Ruminal digestibility (%)				
OM	55.0	53.0	52.0	50.6	1.0	0.042	0.335	0.888
NDF	65.6	63.7	54.3	58.3	1.8	<0.001	0.582	0.132

[1)] see Table 3

($p>0.05$, Table 5). Reasons for this could be the feeding frequency and the low level of DMI (see Table 2).

Some authors described a decrease in the acetic acid concentration in rumen liquid after supplementation of oil or other so-called rumen-unprotected fats (e.g. Doreau et al., 1993; Jenkins, 1993) because of the lower microbial fibre degradation. The present data did not show any significant influence of linseed oil on SCFA, probably because of the low amount of oil supplementation. Additionally some parameters of rumen fermentation failed to reach significance due to large differences concentrate rich rations (H 30, HLO 30) was caused by more microbial OM at the duodenum (Table 8).

The amount of fermented organic matter was significantly higher with H 30 and HLO 30 as compared to H 70 and HLO 70 (Table 8).

The increased hay portion (H 70, HLO 70) in the ration and higher fibre intake (Table 2) resulted in an increased NDF-degradation in the rumen because of more stable pH but a higher NDF-flow to the duodenum as compared to the rations high in concentrate (H 30, HLO 30; Table 6).

The low amounts of supplemented linseed oil did not significantly influence ruminal digestibility and the duodenal flow of OM and NDF. The feeding of rations rich in concentrates (H 30, HLO 30) significantly increased the N-intake of cows, the N-flow to the duodenum, and the NAN at the duodenum (Table 7). The N-flow was significantly higher than the N-intake in all groups due to the microbial protein synthesis (see Table 8). Linseed oil supplementation showed no significant influence on parameters of N-flow to the duodenum (Table 7).

Higher amounts of unsaturated fatty acids have been under discussion for their ability to affect microbial protein synthesis and efficiency of microbial protein synthesis, because of their depressant effects on protozoal populations (Doreau and Ferlay, 1995). Accordingly, the efficiency of microbial protein synthesis (g MP/kg FOM) tended ($p = 0.07$) to be higher with oil supplementation (Table 8).

The roughage/concentrate ratio significantly influenced the amounts of fermented organic matter (FOM) in the rumen, the microbial protein (MP) per day and per kg FOM, and the amounts of utilisable crude protein (nCP, Table 8).

The higher flow of NAN and nCP at the duodenum in animals fed with rations rich in concentrates

Table 7: Flow of Nitrogen to the duodenum

Parameters	Ration					Effects[1]		
	H 70	HLO 70	H 30	HLO 30	PSEM	Hay	Linseed oil	HxLO
N-intake (g day^{-1})	243.9	256.9	321.3	316.0	7.3	<0.001	0.594	0.227
N-Flow to the duodenum (g day^{-1})	343.6	371.1	394.5	409.5	15.2	<0.001	0.060	0.566
(% of N-intake)	141.1	145.0	123.2	125.9	5.2	0.005	0.312	0.785
NAN[2] at the duodenum (g day^{-1})	325.6	351.8	373.9	389.3	10.3	<0.001	0.056	0.604
NAN/ME (g MJ ME^{-1})	2.6	2.7	2.5	2.5	0.01	0.163	0.460	0.470

1) see Table 3
2) Non Ammonia-N

Table 8: Parameters of rumen fermentation and microbial protein yield

Parameters	Ration					Effects[1]		
	H 70	HLO 70	H 30	HLO 30	PSEM	Hay	Linseed oil	HxLO
FOM[2]								
(kg d^{-1})	9.0	9.1	9.4	9.5	0.1	0.03	0.297	0.932
(% OM intake)	74.4	74.7	76.9	76.9	1.1	0.047	0.874	0.893
MN[3]								
(% of NAN)	62.8	63.1	68.7	69.3	0.8	<0.001	0.522	0.868
MP[4]								
(g d^{-1})	1278.8	1387.5	1605.6	1688.1	45.8	<0.001	0.048	0.782
(g MJ ME^{-1})	10.1	10.6	10.8	10.9	0.3	0.104	0.369	0.607
(g kg FOM^{-1})	142.2	152.6	171.3	177.7	4.4	<0.001	0.071	0.654
Endogenes protein[5]								
(g d^{-1})	157.4	165.3	160.6	166.5	3.6	0.551	0.072	0.784
UDP[6]								
(g d^{-1})	599.1	646.4	571.2	578.4	28.6	0.108	0.351	0.491
(% of CP-intake)	27.9	27.8	23.2	22.6	0.8	<0.001	0.659	0.801
uCP								
(gd^{-1})	1877.6	2033.6	2176.6	2266.7	62.8	<0.001	0.062	0.605

[1] see Table 3
[2] Fermented organic matter in the rumen = OM intake – (OM in the duodenum – OM of microorganisms); OM of microbes = 11.8 % microbial N
[3] Microbial N
[4] Microbial protein
[5] EP = 3.6 g/kg DM at the duodenum x 6.25
[6] UDP = uCP – MP [6] uCP = (NAN x 6.25) – endogenes protein (EP)

bases on the increase of microbial protein synthesis (Table 8). The microbial protein synthesis per kg FOM varied between 142.2 and 177.7 g. The average of the H 70-rations amounted to 197.4, those of the H 30 – groups to 174.5 g per kg FOM. Kalscheur et al. (1997b) reduced the roughage portion from 60 to 25 % and measured an increase of MP from 195.0 to 316.3 g/kg FOM based on the reduction of FOM from 52 to 39 % of OM intake. The MP synthesis per kg FOM of the present study is in the range of measurements by Stern et al. (1994; $\bar{x}$: 181; range: 69 – 266 g MP/kg FOM) and Lebzien and Voigt (1999; $\bar{x}$: 188; range: 63 – 313 g MP/kg FOM). NRC (2001) considered an average of 186 g (75 – 338 g MP/kg FOM). There was no significant influence of linseed oil supplementation on parameters of N-flow and protein yield at the duodenum (Tables 7 and 8) in agreement with some references (Doreau and Ferlay, 1995; Kalscher et al., 1997a; Oldich and Firkins, 2000; Ueda et al., 2003).

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