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Omasal sampling technique to examine ruminal biohydrogenation of fatty acids

Steven van der Weerd

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Omasal sampling technique to examine ruminal biohydrogenation of fatty acids

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Preface

With this report I would like to present the final result of my master thesis, which is part of my study Animal Sciences. About two years ago I started with this master thesis at the Animal Nutrition group of Wageningen University. The subject of biohydrogenation in combination with an interesting *in vivo* experiment convinced me to participate in this PhD project of Attje-Rieke Sterk, who is a PhD-student at the Animal Nutrition group. My thesis focused on the omasal sampling technique which was used to determine biohydrogenation in cows in this experiment.

Cooperating in this project was very interesting because I got the chance to follow the whole process. From the design of the experiment until analyzing of the results. Although the experiment contained many working hours in the stable and in the lab I am glad that I had the possibility to participate in this. After a long break caused by my internship and working at the farm back home, I can now finally finish this project by writing my report.

My supervisor for this thesis was Attje-Rieke Sterk, who helped me a lot during the experiment. And also with writing this report which was not always easy, because of the long break in between. I really learned a lot from Attje-Rieke during this thesis, for which I would like to thank her. I would also like to thank Jan Dijkstra for his contribution to this thesis. Ronald Zom taught me how to work with the omasal sampling technique and therefore I would also like to thank him. During the experiment I worked a lot with Ard van Veen in the stable as well as in the lab and I would like to thank him for the nice cooperation during this project.

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Abstract

An increasing number of protection technologies to prevent or control rumen biohydrogenation of unsaturated fatty acids (UFA) are investigated. Sampling via a duodenal canal is the most commonly used technique to determine biohydrogenation. Duodenal sampling however, requires invasive surgical intervention and is susceptible for contamination of abomasal secretions. The omasal sampling technique (OST) takes samples from the omasum via the rumen cannula. Only a few published papers are known which used OST to assess the ruminal biohydrogenation of fatty acids. However, no study is known to investigate the potential of OST for determining ruminal biohydrogenation of linseed. The present study examines if the OST is a useful method to examine differences in the flow of UFA through the omasal canal due to differences in ruminal biohydrogenation between different sources of unsaturated fatty acids.

In an experiment with four periods of 21 days, four lactating Holstein Friesian cows fitted with a ruminal cannula received 4 dietary treatments in a 4x4 Latin-square design. Treatments were four different technological treatments of linseed: crushed linseed (CL), extruded linseed (EL), formaldehyde treated linseed oil (SLO) and DHA in combination with linseed oil (MALO). In the basal diet of TMR containing grass silage (31%), maize silage (29%) and concentrates (40%), part of the concentrates were replaced by the different linseed products (6.5% CL, 9.9% EL, 3.3% SLO and 0.4% DHA + 2.1% linseed oil). The experimental diets were leveled on the proportion of C18:3n3 in the diet (16.5 ± 1.3 g/kg DM). Omasal canal flows were assessed using a triple marker system with CrEDTA, Yb-acetate and the internal marker ADL. The recovery of markers was determined by feaces collection. The reconstitution technique described by France and Siddons (1986) was used to calculate digesta flows. Results were analyzed using the PROC MIXED procedure of SAS with treatment and period as fixed effects and cow as the random effect. Treatment means were compared by the Tukey test ($P < 0.05$).

Treatment did not affect DM intake, rumen nutrient digestion or milk yield. Ruminal nutrient digestibilities were comparable to values found in other studies. Omasal fatty acid flows differed between treatments. Total FA flow in the omasum accounted for approximately 70% of FA intake, indicating an underestimation of total FA flow in the omasum. This underestimation was probably caused by an inadequate method of extraction or methylation when preparing the samples for FAME analysis. *Trans* C18:1 fatty acid flows in the omasum were higher for the MALO diet ($P < 0.01$) because of the inhibition of the last step of biohydrogenation under influence of the marine algae. C18:3n3 in the MALO diet was for a large part biohydrogenated. The SLO treatment was expected to show a clearly lower biohydrogenation, but results showed a low C18:3n3 flow through the omasal canal, indicating a high biohydrogenation. C18:3n3 production in the milk, however, was 288% of omasal flow, indicating an error is made in C18:3n3 flow in the omasum for the SLO treatment. This is probably caused by different behavior of SLO in the digesta. Rumen C18:3n3 concentrations showed the same trends as were seen in the FA omasum. Data from the rumen was not sufficient to compare rumen biohydrogenation with biohydrogenation calculated from omasal flows.

OST provides a promising sampling method to determine differences in FA outflows from the rumen when feeding different linseed sources. Therefore, OST can provide a good alternative for duodenal sampling as it requires less surgical intervention and it avoids contamination from abomasal secretions. However, quantification of FA flows in the omasum with OST needs further evaluation

1. Introduction

Dairy products form an important source of fatty acids in the Western society. These products have high concentrations of saturated fatty acids (SFA). SFA are known to increase the risk of cardiovascular disease and cause also increased plasma cholesterol levels in humans. Unsaturated fatty acids (UFA) however, decrease plasma cholesterol and low density lipo-cholesterol levels in the blood (Noakes et al., 1996). Therefore, UFA are often regarded to be healthier for humans (Givens, 2005, Lawson et al., 2001). To improve fatty acid composition of dairy products there is an increasing interest to alter fatty acid composition of milk fat to a higher concentration of UFA.

Although UFA are abundant in grass and certain other ruminant feedstuffs, their concentration in milk fat is low (Jenkins et al., 2008). In ruminants, the dietary UFA are subject to extensive biohydrogenation in the rumen, where the UFA are transferred into SFA under the influence of microbes (Harfoot and Hazlewood, 1997). Therefore a large part of the consumed UFA does not become available for milk and meat production (Chilliard et al., 2000, Shingfield et al., 2003).

1.1 Biohydrogenation

Consumed lipids entering the rumen undergo extensive transformations under the influence of rumen microbes (Chilliard et al., 2000). Two major processes can be distinguished: lipolysis and biohydrogenation. Lipolysis causes the release of free fatty acids (FFA) from esterified lipids (Jenkins, 1993). Microbial lipases extensively hydrolyse dietary galactolipids, phospholipids and triglycerides shortly after entering the rumen, producing FFA and glycerol as main end-products (Chilliard et al., 2000, Jenkins, 1993, Jenkins et al., 2008). The second major process is biohydrogenation. After lipolysis the UFA are hydrogenated to a large extent, but before being hydrogenated UFA first have to be isomerized (Chilliard et al., 2000, Jenkins, 1993). For example the cis-12 double bond of linoleic acid (cis-9, cis-12 C18:2) will be isomerized to a trans-11 intermediate. Once the fatty acid (FA) is isomerized, hydrogenation of first the cis-9 bond and then the trans-11 bond occurs under influence of microbial reductase, producing a SFA (Chilliard et al., 2000, Jenkins, 1993). In figure 1.1 a schematic overview is shown of the lipolysis and biohydrogenation of linoleic acid. This figure shows the main pathway of the biohydrogenation of linoleic acid, but in fact there are many biochemical pathways. As a result, many different intermediate FA are produced which is mostly dependent on the microbial ecosystem (Chilliard et al., 2000). But not all UFA are hydrogenated completely. This leads to a considerable amount of *trans*-FA intermediates in duodenal contents and consequently in the milk (Chilliard et al., 2000, Jenkins et al., 2008).

1.1.1 Factors which influence the extent of biohydrogenation

The extent of lipolysis and biohydrogenation in the rumen is dependent on several factors. Beam et al. (2000) showed that both the type and the amount of fat added to the diet had an effect on

biohydrogenation rates. An increasing amount of double bonds increased biohydrogenation rates and an increased linoleic acid supplementation decreased biohydrogenation rates. Another important factor is ruminal pH. Van Nevel and Demeyer (1996) reported that a drop in rumen pH resulted in a reduced lipolysis and thus lower biohydrogenation rates. Jenkins et al. (2008) reported the importance of the microbial population. Different bacteria species play their own specific role in the biohydrogenation process. Eliminating a certain species can influence the extent of biohydrogenation by inhibiting a step in the biohydrogenation process.

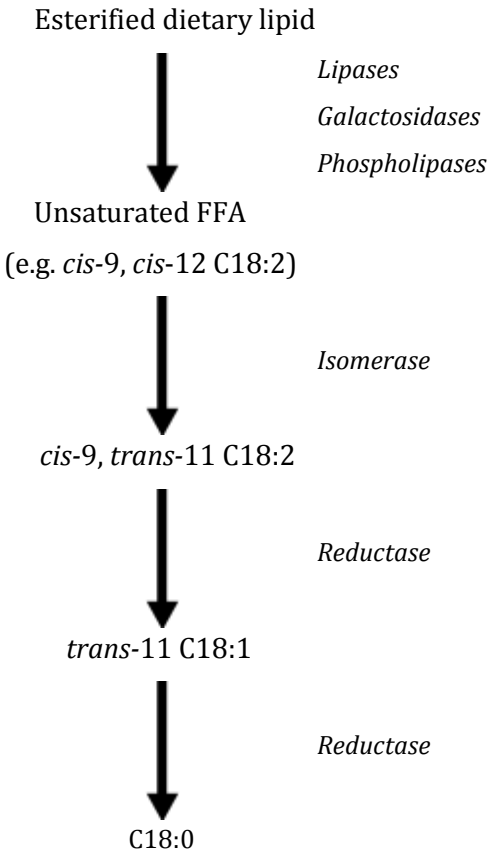

Figure 1.1 Overview of the conversion of an esterified lipid to an unsaturated fatty acid.

1.1.2 Protection of fat against ruminal biohydrogenation

To protect UFA against ruminal biohydrogenation and increase the postruminal UFA flow, three different protection technologies can be distinguished (Fievez et al., 2007). The first is chemical protection. With chemical protection a chemical layer is formed around the lipids preventing lipolysis and thus biohydrogenation (Scott et al., 1971). Examples are protection by lipid encapsulation in a protein matrix by aldehyde treatment, direct formaldehyde treatment of oilseeds or formation of a whey gel complex. The second protection technology is the formation of calcium salts and amides of fatty acids to alter FA structures. With this method the FA structure is changed in such a way that the carboxyl group is blocked. A free carboxyl group is needed for the action of the *isomerase,* so

isomerization of UFA cannot occur (Jenkins and Bridges, 2007). The last method protects the lipids by technological treatment of oilseeds such as extrusion, roasting, cracking, etc. One of the mechanisms involved is the chemical changes to protein by heating, which prevent the FA being available for digestion (Chouinard et al., 1997).

1.2 Motive for the current experiment

Several *in vitro* and *in vivo* experiments have been carried out to assess the potential of different protection technologies. Sterk et al. (2010) evaluated several chemically and technologically treated linseed products *in vitro* under identical experimental conditions. Linseed is known to be rich in linolenic acid (C18:3n3). A total of twelve different linseed products were tested and three proved to have potential in inhibiting rumen biohydrogenation.

Chemical protection applied as formaldehyde proved to be effective in protecting biohydrogenation (Sterk et al., 2010). Sinclair et al. (2005) reported that linseed needs a pre-treatment to induce the permeability of the seed coat. Sterk et al. (2010) compared chemical pre-treatment by means of sodium hydroxide with a technical pre-treatment by crushing the linseed and found crushing to be more effective in protecting C18:3 against biohydrogenation.

Technical protection proved to be effective by means of extrusion of whole linseed (Sterk et al., 2010). Extrusion of oilseeds might influence the production of intermediates and end products of biohydrogenation by increasing the availability of UFA by rupturing the seed coat (Doreau et al., 2009, Neves et al., 2007). Extruded crushed linseed was biohydrogenated to a larger extent then extruded whole linseed (Sterk et al., 2010). This might be caused by leaving a larger part of the seed coat intact. Hence, the post ruminal availability of C18:3 might be limited because the seed coat will also limit linseed digestion in the intestines (Chilliard et al., 2000; Jenkins, 2006).

Linseed oil in combination with DHA did not reduce the biohydrogenation of C18:3. Compared with the other treatments however, the last biohydrogenation step from *trans*10+11-C18:1 to C18:0 seemed to be inhibited (Sterk et al., 2010). This result was in line with the results by Vlaeminck et al. (2008) and Boeckaert et al. (2008). Both attributed this to changes in the microbial population caused by the addition of DHA.

In continuation of the *in vitro* experiment carried out by Sterk et al. (2010) an *in vivo* experiment was carried out to determine the effect of the three most promising treatments. Within this *in vivo* experiment a formaldehyde treatment of linseed oil treated with casein, extruded whole linseed and linseed oil in addition with DHA were tested on ruminal biohydrogenation, milk FA composition and production performance in lactating dairy cows. Crushed linseed was included in the experiment as fourth technological treatment. The crushed linseed was considered to be biohydrogenated to a large

extent (Doreau et al., 2009, Sterk et al., 2010). Comparison of the other treatments with the crushed linseed makes it possible to determine the UFA protection properties of the different treatments.

1.3 Experimental techniques to assess biohydrogenation properties

To test the potential of the different treatments information is needed about the process of biohydrogenation using different treatments. Information is needed on the disappearance of polyunsaturated fatty acids (PUFA), the production of saturated end products of the biohydrogenation process and the types and amounts of the accumulating FA intermediates (Fievez et al., 2007). Different *in vivo, in vitro* and *in sacco* techniques are used to investigate rumen biohydrogenation. There are three types of *in vitro* techniques. The most widely used technique is 24-h batch incubations. The time series incubations are used to estimate kinetic parameters. A few experiments used a continuous culture technique, but there seemed to be no improvements to the batch culture system. Fievez et al. (2007) reported that *in vitro* techniques provide an accurate estimate of the *in vivo* biohydrogenation. The *In sacco* technique is limited in simulating *in vivo* biohydrogenation, because of physical loss of UFA from the bag (Fievez et al., 2007).

To determine rumen biohydrogenation *in vivo* it is important to take samples before intestinal hydrolyses occurs when bile salts and pancreatic juices are added to the digesta to digest dietary lipids. A commonly used sampling method is therefore duodenal sampling. With this method a T-cannula is placed in the proximal duodenum. Samples can also be taken via the abomasum, by making use of a T-cannula which is placed in the abomasum. To place this cannula the animal has to undergo an intensive surgical procedure. The T-cannula also requires extensive maintenance and involves longer animal recovery times compared to ruminal sampling. From animal welfare point of view sampling via a ruminal cannula is preferred. Therefore Punia et al. (1988) estimated the flow of N-fractions from the rumen by taking samples from the omasal canal by means of aspiration through a plastic tube via the ruminal cannula. Huhtanen et al. (1997) developed this procedure to an omasal sampling technique (OST) to take samples of rumen outflow through the reticula-omasal orifice. Experiments with OST indicated that OST provides a promising alternative for the duodenal sampling technique to investigate stomach digestion in dairy cows and rumen mineral metabolism (Ahvenjarvi et al., 2000, Tuori et al., 2006).

1.4 Objective of the study

Although OST provides to be a promising alternative for duodenal sampling, the OST is not often applied on experiments which focus on biohydrogenation. Shingfield et al. (2003) investigated the effect of dietary fish oil on biohydrogenation of fatty acids and Lundy et al. (2004) studied ruminal biohydrogenation of fatty acids fed as amides and calcium salts using the OST as sampling method. However, there is no study which focuses on the question if OST provides a promising tool in assessing ruminal fatty acid outflows.

The objective of the current study is therefore to investigate if OST provides a method to quantify different fatty acid flows in the omasal canal as a result of differences in ruminal biohydrogenation. Data of the *in vivo* experiment mentioned above is used. FA flows in the omasal canal will be compared with the FA profile in the rumen and milk. In addition the omasal fatty acid flows will be compared with duodenal flows described in published studies. Consequently in this study the following questions will be answered:

- Is OST a reliable method to determine fatty acid flows in the omasal canal?
- Do the fatty acid flows in the omasal canal measured with OST correspond to the biohydrogenation in the rumen measured in the rumen after fasting?
- Can the fatty acid flows in the omasal canal measured with OST be used to examine differences in ruminal biohydrogenation?

2. Materials and methods

An *in vivo* experiment has been carried out to study the effect of different linseed treatments on biohydrogenation in the rumen of dairy cows. The following chapter will describe how the experiment was carried out, which measurements were taken, which analyses were performed and how the data was analyzed.

2.1 Experimental setup

Within this experiment 4 different diets were tested on 4 different cows during 4 periods. In this experiment a 4x4 Latin square design has been used to assign the different treatments to the cows for each period (table 2.1). The sequence of the treatments was balanced to handle carry over effects. Every period consisted of 21 days. In the first 16 days the cows could adapt to the treatment and the measurements where taken from day 17 until day 21.

Table 2.1 Experimental setup based on 4x4 Latin square design.

	Subject 1	Subject 2	Subject 3	Subject 4
Period 1	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Period 2	Treatment 2	Treatment 4	Treatment 1	Treatment 3
Period 3	Treatment 3	Treatment 1	Treatment 4	Treatment 2
Period 4	Treatment 4	Treatment 3	Treatment 2	Treatment 1

The cows used within this experiment were 4 multiparous Holstein Friesian cows fitted with a ruminal cannula. The cows were selected from the herd of the experiment accommodation 'De Ossekampen' in Wageningen according to an early lactation stage. Pre-experimental data of the cows is shown in table 2.2. The cows were individually housed in the experimental unit of 'De Ossekampen'. The cows were milked twice a day at approximately 7.00 am and at 5.00 pm. The cows were fed twice a day after milking. The feed was offered in individual feedboxes. The cows had continuous access to drinking water.

Table 2.2 Pre-experimental data of cows used in the biohydrogenation experiment.

Cow nr.	Age	Weight (kg)	DIL[1]	Lactation nr.	Milkprod.[2]	Fat %	Protein %	FPCM[3]
2359	6.09	708	25	6	22.5	4.69	3.54	24.6
491	7.10	575	50	6	29.6	5.72	3.82	36.4
247	5.09	552	73	4	37.2	3.88	2.87	35.7
411	4.04	659	60	3	29.6	5.01	3.76	33.9

[1] Days in lactation on 8/11/2008
[2] Milk production before experiment (kg/d)
[3] Fat and Protein Corrected Milk (kg/d)

2.2 Experimental diets

The cows were fed a total mixed ration (TMR) during the experiment. The basic diet consisted of grass silage, maize silage, a commercial concentrate and a mix of soya-, rape- and wheat meal. Chemical composition of these products is shown in table 2.3. The grass silage was produced from rye grass, and consisted of a 3rd and 4th cut of the year 2008 at "De Ossekampen". Maize silage was grown and harvested in 2008 at "De Ossekampen". The commercial concentrate and the blend of soya, rape and wheat meal (ratio 33:33:33) were produced by Rijnvallei mengvoeders BV in Wageningen.

Table 2.3 Chemical composition of basic ingredients of the experimental diets (g/kg DM).

	Dietary ingredients			
	Grass silage	Maize silage	Concentrates	SRT[1]
DM (g/kg)	439	328	872	870
Ash	89	46	70	53
Cfat	33.4	29.4	39.4	27.7
N	30.1	10.2	26.3	45.0
CP	188	64	164	281
NDF	530	399	259	160
ADF	310	231	176	109
ADL	34	24	47	35
Starch	ND[2]	331.6	162.8	198.6
Sugar	34.3	7.5	80.5	74.0
VEM[3]	880	970	1100	1092
DVE[4]	75	45	115	161

DM = Dry Matter; Cfat = Crude Fat; N = Nitrogen; CP = Crude Protein; NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; ADL = Acid Detergent Lignin.
[1] Soya-, Rape- and wheat meal blend
[2] ND = Not Detected
[3] VEM = Voeder eenheid melk, for grass and maize silage analyzed by BLGG Oosterbeek; for concentrates and SRT provided by supplier
[4] DVE = Darm verteerbaar eiwit, for grass and maize silage analyzed by BLGG Oosterbeek; for concentrates and SRT provided by supplier

2.2.1 Experimental feedstuffs

An experimental ingredient was added to the basic diet to form the experimental diet. The four experimental ingredients were all linseed products which were exposed to different technological treatments, namely: crushed linseed, extruded linseed, spraydried linseed oil and linseed oil in combination with marine algae. The linseed used for these different treatments originated from the same batch, which was obtained from Research Diet Services BV (RDS) in Wijk bij Duurstede. The linseed oil for treatment 3 and 4 was obtained from Cehave Landbouwbelang BV, Veghel, the Netherlands. Chemical composition of the experimental feedstuffs is shown in table 2.4.

2.2.2 Crushed linseed

Treatment 1 consisted of crushed linseed (CL). Linseed was milled at the Wageningen Feed Processing Centre (WFPC) in Wageningen with a roller-mill (Ipswich Turner, Christy Turner Ltd, Ipswich UK) with a distance between the rolls of 0.25 mm.

2.2.3 Extruded linseed

The second treatment consisted of extruded whole linseed (EL). Wheat bran was added to the whole linseed in a ratio linseed:wheat of 70:30. Next, this was mixed in a twin-shaft paddle mixer (Forberg F60) for two minutes. The mixture of whole linseed and wheat bran was then extruded with a small-scale single screw extrusion line (Almex Al 150), which was fully equipped with a feeder, pellet press (Robinson/Heesen V2/30) and cooler unit (6% steam and 2% water, 127 °C for 20-30 seconds; Almex, Zutphen, the Netherlands). After extrusion the pellets where dried in a dry-oven at 40 °C.

2.2.4 Spraydried linseed oil

Treatment 3 consisted of spraydried linseed oil (SLO). The SLO was produced by DMV, Veghel, the Netherlands and treated with formaldehyde (0.65 %) at Cehave Landbouwbelang. The method used was described by Scott et al (1971).

Table 2.4 Chemical composition (g/kg DM) of experimental ingredients of the experimental diets.

	Experimental ingredients[1]				
	CL	EL	SLO	MALO	
				MA[2]	MALO
DM (g/kg)	923	950	974	874	916
Ash	34	42	16	90	60
Cfat	459.5	339.1	646.5	77.6	385.0
N	36.8	35.0	49.3	28.3	18.8
CP	230	219	308	177	118
NDF	214	232	ND[3]	170	113
ADF	120	126	ND	95	63
ADL	32	35	ND	23	15
Starch	1.9	44.5	ND	109.0	72.6
Sugar	26.8	36.7	ND	86.4	57.6
VEM[4]	1745	1448	2653	1312	2008
DVE[5]	70	72	204	159	106

DM = Dry Matter; Cfat = Crude Fat; N = Nitrogen; CP = Crude Protein; NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; ADL = Acid Detergent Lignin.
[1] CL = Crushed linseed; EL = Extruded linseed; SLO = Spray dried linseed oil; MALO = Linseed oil in combination with marine algae.
[2] MA = Marine algae
[3] Not Detected
[4] VEM = Voeder eenheid melk, provided by supplier
[5] DVE = Darm verteerbaar eiwit, provided by supplier

2.2.5 Linseed oil in combination with marine algae

The fourth treatment consisted of linseed oil together with marine algae (MALO). Marine algae was fed in pelleted form. Most important component of these pellets was DHA Gold; a dried, whole cell algae product containing a minimum of 18% docosahexaenoic acid (DHA). DHA Gold is produced by Martek, Columbia. The DHA Gold content in the pellets was 11 g/kg DM. The pellets with the DHA were produced in Gent, Belgium.

2.2.6 Diet calculation

Formulation and chemical composition of the experimental diets are shown in tables 2.5 and 2.6. The amount of experimental ingredients added to the basic diets was based on an equal supply of UFA's. The fatty acid compositions of the experimental diets are shown in table 2.7. The amount of commercial concentrates was adjusted to the amount of experimental feedstuff added. The VEM and DVE levels in the diet were based on the average need of a dairy cow in early lactation stage.

Table 2.5 Formulation of experimental diets (g/kg DM)

	Experimental diets[1]			
	CL	EL	SLO	MALO
Grass silage	31.2	31.0	31.2	31.2
Corn silage	29.4	29.2	29.4	29.4
Wheat	6.8	6.4	7.3	7.7
Rapeseed meal	6.5	6.1	6.9	6.9
Soybean meal	4.9	4.7	5.0	4.9
Corn	5.2	4.5	6.0	5.4
Palm kernel expeller	2.2	1.9	2.5	2.2
Soybean hulls	2.1	1.8	2.4	2.2
Rapeseed expeller	1.6	1.4	1.8	1.6
Beet pulp	1.1	0.9	1.2	2.2
Molasses	1.3	1.1	1.5	1.6
Rapeseed meal treated	0.8	0.7	0.9	0.8
Corn gluten feed	0.0	0.0	0.0	0.4
Toasted soybean expeller	0.0	0.0	0.0	0.2
Form treated soybean meal	0.0	0.0	0.0	0.2
DHA Gold[2]	0.0	0.0	0.0	0.4
Premix	0.2	0.1	0.2	0.2
Limestone	0.2	0.1	0.2	0.2
Salt	0.1	0.1	0.1	0.1
Palm oil	0.1	0.1	0.1	0.1
Other components	0.0	0.0	0.0	0.2
Crushed linseed	6.5	0.0	0.0	0.0
Extruded linseed	0.0	9.9	0.0	0.0
Spraydried linseed oil	0.0	0.0	3.3	0.0
Linseed oil	0.0	0.0	0.0	2.1

[1] CL = Crushed linseed; EL = Extruded linseed; SLO = Spray dried linseed oil; MALO = Linseed oil in combination with marine algae.
[2] DHA Gold is a dried, whole cell algae product containing a minimum of 18% docosahexaenoic acid.

The first week of every period the cows had *ad libitum* access to the feed to enhance fast adaptation to the treatment. In the second and third week the feed supply was restricted to 95% of the average feed intake in week 1 of period 1. With the restricted feed supply variations in feed intake were minimized.

Table 2.6 Chemical composition of experimental diets (g/kg DM).

	Experimental diets[1]			
	CL	EL	SLO	MALO
DM (g/kg)	582.66	589.05	582.74	582.13
Ash	67.73	67.03	68.62	69.46
Cfat	62.39	64.52	55.30	56.32
N	27.24	27.31	27.43	26.10
CP	170.22	170.67	171.47	163.15
NDF	360.22	360.65	353.62	354.09
ADF	215.59	214.60	212.82	212.32
ADL	31.76	31.82	31.01	30.82
Starch	148.01	147.39	152.45	152.94
Sugar	38.10	37.84	38.61	40.14
VEM[2]	1034	1027	1044	1048
DVE[3]	84	83	90	87

DM = Dry Matter; Cfat = Crude Fat; N = Nitrogen; CP = Crude Protein; NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; ADL = Acid Detergent Lignin.
[1] CL = Crushed linseed; EL = Extruded linseed; SLO = Spray dried linseed oil; MALO = Linseed oil in combination with marine algae.
[2] Voeder Eenheid Melk, calculated from diet individual components
[3] Darm Verteerbaar Eiwit, calculated from individual diet components

Table 2.7 Fatty acid composition of experimental diets (g/kg DM).

	Experimental diets[1]			
	CL	EL	SLO	MALO
C12:0	1.15	1.01	1.51	1.21
C14:0	0.43	0.38	0.56	0.71
C16:0	4.52	4.63	4.66	5.06
C16:1	0.19	0.19	0.20	0.20
C18:0	1.32	1.36	0.95	1.21
C18:1c9	8.79	8.92	8.99	8.53
C18:1c11	0.68	0.66	0.62	0.70
C18:2n6c	11.78	11.98	11.92	11.60
C18:3n3	17.26	17.88	15.70	15.22
C20:0	0.12	0.12	0.13	0.12
C22:5	0.01	0.01	0.01	0.21
C22:6n3	0.00	0.00	0.00	0.59
Other	0.53	0.51	0.59	0.54
Total UFA[2]	36.15	36.11	36.13	36.40

[1] CL = Crushed linseed; EL = Extruded linseed; SLO = Spray dried linseed oil; MALO = Linseed oil in combination with marine algae
[2] UFA = Unsaturated fatty acids

2.3 Measurements and sampling

2.3.1 Feed

During week 1 in period 1, the feed intake was recorded daily before the afternoon milking by weighing feed residues to calculate the restricted feeding level for weeks 2 and 3 of the four periods. During week 2 and 3 of every period possible feed residues were weighed and a sample was taken for dry matter determination to monitor if selection of certain feedstuffs occurred.
Every Monday a sample was taken from all feed ingredients. The samples were kept at a temperature of –20 °C to keep the samples stable. Before analysis the samples of each period were pooled per feed ingredient.

2.3.2 Weight

To monitor body weight of the animals, the cows were weighed on every first Monday of every adaptation period and on the Monday of the measurement week, using a balance for farm animals. Weighing is performed twice a day, in the morning after milking and in the afternoon prior to milking.

2.3.3 Milk

During the first week of November, two milk samples were taken to collect pre-experimental data. One sample was taken for fatty acid analysis and one sample to analyze fat, protein, lactose and urea contents.

During the experiment milk production was recorded twice a day. In week 3 of each period two milk samples were taken on both Monday evening and Tuesday morning milking. The evening and morning samples were pooled. One sample for fat, protein, lactose and urea content analysis was stored at 4 °C and send to the Milk Control Station in Zutphen, the Netherlands where they were analyzed. The second sample was stored at -20 °C for fatty acid analysis.

2.3.4 Omasal samples

Omasal samples were collected on day 18 and 19. On day 14 the omasal sampling device was placed, to avoid disturbances in digesta flow from reticulum to omasum when placing the device just before sampling. Samples were taken between 12.00 and 22.00 hours with 2-hours intervals. In this way a 12 hour period is covered, which is considered to be representative for the entire feeding cycle. 775 gram of the sample was weighed and stored at -20 °C. Additional 2 samples were taken for VFA and ammonia analysis. To stabilize the samples phosphoric acid was added to the VFA sample and Trichloroacetic acid (TCA) to the ammonia sample as described by Taweel et al. (2005).

The samples of digesta entering the omasal canal were collected using the system described by Huhtanen et al (1997), with the incorporation of the modifications made by Ahvenjärvi et al (2000). During practicing problems occurred with sampling caused by blockage of the sampling device by the leaves of the omasum. Therefore the sampling device in the omasum was slightly changed. The device used within this experiment is shown in figure 2.1. The 'head' of the device which was placed in the omasum was made of Teflon. It was ellipse formed with 4 slots over the long end. To pump up the omasal sample openings were made in the Teflon head which ended in the PVC-tube which was placed through the Teflon head. The openings were placed in the slots to prevent blockage by the omasal leaves. A weight covered with Teflon was attached on the head by meaning of a small rope. The weight was placed in the abomasum to keep the device in place. The PVC-tube (12 mm) was connected to a flexible tube which was placed through a hole in the plug of the ruminal cannula.

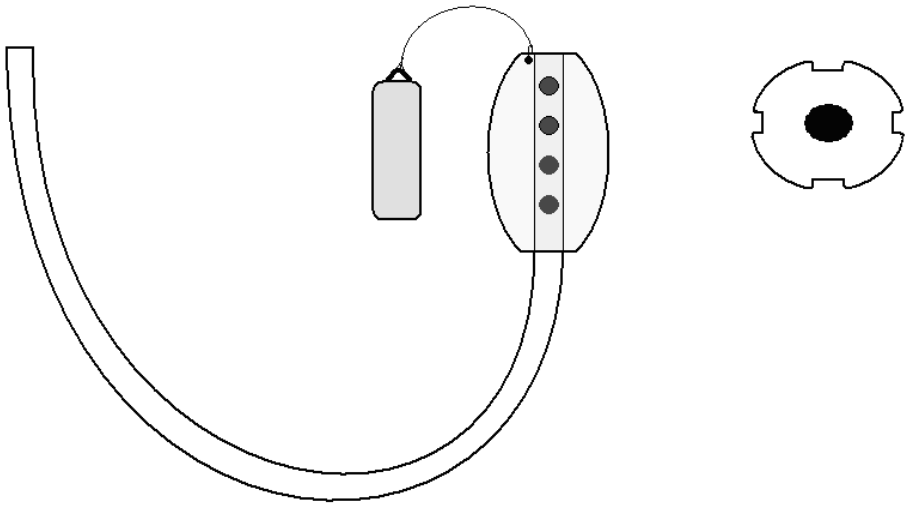

Figure 2.1 Device for the omasal sampling technique which is placed in the omasum.

To determine digesta flow the triple marker method described by France and Siddons (1986) was used. With this method the digesta flow was reconstituted by making use of three markers. Two external indigestible markers were used, where Chrome EDTA (CrEDTA) was associated with the liquid phase and Ytterbium-acetate (Yb-acetate) with the small particle phase. The large particle phase was associated with the internal marker acid-detergent lignin (ADL). From day 14 on the external markers were continuously infused in the rumen by meaning of a peristaltic pump, which was connected to the rumen by tubes which were put through the ruminal cannula plug. Per day 3.0g of Chrome and 2.4g of Ytterbium was added per cow per day both dissolved in 2 liters of water. To enhance the establishment of a steady state situation in the rumen a primer dose of 4.5g Chrome and 3.6g Ytterbium was inserted into the rumen on day 14. The preparation protocols of the CrEDTA and Yb-acetate solutions are given in the appendix.

To assess the digesta flow the omasal samples were separated into the three phases. The separation of the three phases was managed by the following procedure. The omasal canal samples where first squeezed through a double layer cheesecloth. The solids which remained in the cheesecloth were defined as the large particle phase (LP). The LP as well as the filtrate was then pooled for each cow per period. The filtrate was then centrifuged at 10 000*g* for 15 minutes. The supernatant was then poured off and defined as the fluid phase (FP). The remainder was then collected as small particle phase (SP). The three phases were then frozen and freeze-dried to perform chemical analysis.

2.3.5 Faecal collection

To determine feed digestibility and marker recoveries in the faeces, manure was quantitatively collected from day 17 12.00h until day 19 24.00h. A collection period of 60 hours was covered which was considered to give a representative result. Separation of the urine and the faeces was achieved by collecting the faeces as soon as possible before it got mixed with urine.

The 60 hour period was divided into 6 blocks of 8 hours and a last block of 12 hours. At the end of every block, the total faeces production was weighed. A representative sample was taken after intensive mixing per cow. After freeze-drying the faeces, samples were pooled per cow and period.

2.3.6 Ruminal samples

On day 21 ruminal samples were taken to determine biohydrogenation kinetics in the rumen. Sampling started at 12.00h when most of the feed was considered to be consumed. During sampling the animals were fasted so no further consumption was possible during sampling. From 12.00h until 22.00 hours, every two hours a grab sample of solid material and a rumen fluid sample was taken from every cow. The grab sample was taken via the rumen cannula, and consisted of samples from several places divided over the rumen. The fluid sample was taken by inserting a PVC-tube with small openings into the rumen. A sampling tube connected to a plastic bottle was inserted in the PVC-tube and the rumen fluid was collected by pushing the air out of the plastic bottle and let it replace by the rumen fluid through the vacuum created in the bottle. In the fluid sample pH was measured immediately and samples were taken for determination of VFA's and ammonia. The samples were stabilized as described by the omasal samples. Both the grab samples and the fluid samples were frozen at -20 °C until analysis of FA profile.

2.4 Analyses

Analyses were carried out at the Animal Nutrition group of WUR. The grass, maize, omasal LP, faecal and ruminal samples were freeze-dried and milled to pass a 1 mm sieve. The pelleted concentrates were also milled to pass a 1 mm sieve.

2.4.1 DM, Ash, CP, Cfat, NDF, ADF, ADL, Sugars and Starch analysis.

Samples of feed and omasum were analyzed for DM, Ash, CP, Cfat, NDF, ADF and ADL. Feed samples were analyzed on Sugars and Starch as well. In the feacal samples, Cfat and ADL were determined. Dry matter (DM) values were determined by oven drying at 103 °C for 4 hours (ISO 6496; ISO, 1999a). Ash content (Ash) was determined by incineration at 550 °C for 3 hours (ISO 5984; ISO, 2002). Nitrogen was determined according to the Kjehdahl method with $CuSO_4$ as the catalyst (ISO 5983; ISO, 1997). Crude protein (CP) was calculated by multiplying the N content by 6.25. Crude fat (Cfat) was determined gravimetrically after 6 h extraction with petroleum-ether (ISO 6492). Neutral detergent fiber (NDF) was determined according to the method described by van Soest et al. (1991) with the modifications which are described by Goelema et al. (1998). Acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according the method described by Van Soest (1973). Sugar was determined by the method described by Van Vuuren et al. (1993) with the use of a 40% ethanol solution. Modifications to this method were as described by Abrahamse et al. (2008). Starch was determined as glucose using the amyloglucosidase method (ISO 15914; ISO, 2004) after the starch was liberated by heating in a boiling water bath in the presence of 2 n HCL (Cone, 1991).

2.4.2 Marker analysis

Marker concentrations of Chrome and Ytterbium were determined in the samples of the omasum and in the feaces and were performed at the laboratory of the Animal Science Group in Lelystad following a standard protocol.

2.4.3 Volatile fatty acids and Ammonia-N

Volatile fatty acids (VFA's) and ammonia-N (NH3) were determined in the liquid fraction of the omasal and rumen samples. VFA concentration was determined using gas chromatography (GC type Fisons HRGC MEGA2, Fisons Instruments, Milan, Italy) as described by Taweel et al. (2005). NH3 was determined by spectrophotometry using the Berthelot reaction as described by Taweel et al. (2005). For the statistical analysis the average VFA and NH3 concentrations were calculated per treatment and per period.

2.4.4 Fame analysis

Fatty acid composition was determined in the feed, omasal and ruminal samples. Feed, rumen and OST samples were extracted using the Folch method (Folch et al., 1957). A sample of 375 mg of the (freeze-dried) material was extracted for 1h with 15 mL of chloroform-methanol (2:1 vol/vol) and 375µL of distilled water. The homogenized extracts were filtered and 2.2 mL of distilled water was added. After phase separation samples were centrifuged at 1000 rpm for 5 minutes. Wash solution (480 mL methanol and 470 mL NaCl solution [7.3 g/L of water]) was added before removing the upper layer. This washing procedure was repeated twice. Approximately 3 ml of the bottom layer was collected and

solvent was evaporated by vacuum centrifugation. Residual lipids were collected and FA methylated following Raes et al. (2001). Glycerides were converted to methyl esters by transestification with 3 mL of Sodium hydroxide/methanol (0.5 *M*). After heating for 30 minutes at 50 °C in an oven 2 mL of hydrochloric/methanol was added (1:1) to esterify free FA. After heating for 10 minutes at 50 °C 2 mL of distilled water and 2 mL of hexane were added. The suspension was centrifuged for 5 minutes at 2000 rpm. Hexane fraction was collected and 2 mL of hexane was added to the non-hexane fraction. The suspension was again centrifuged for 5 minutes at 2000rpm and the hexane fraction was added to the earlier collected hexane fraction. This was evaporated in a vacuum centrifuge and re-suspended in 1 ml of hexane and transferred in a GC-vial.

Milk fat extraction was performed following the method of Röse-Gottlieb. A sample 10 gram of fresh milk was weighed in a majonnier flask. Subsequently 1.25 mL of ammonia, 10 mL of ethanol and 25 mL of diethyl ether were added. Then 25 mL petroleum ether was added. After thoroughly shaking, the ether-fat was separated and the remaining solution was two times extracted with 15 mL diethyl ether and 15 mL petroleum ether. Ether solution was again separated and then evaporated at 40-50 °C, vented with nitrogen and milk fat was determined. The remaining fat was dissolved in 15-20 mL petroleum ether and petroleum ether was evaporated. The remaining fat was than dissolved in 5 mL of hexane and 100 µL of sodium methylate (30%) was added. Then 1 gram of $NaHSO_4$ was added and this was dried with Na_2SO_4. This was then transferred in a GC-vial.

Fatty acid methyl esters were quantified using gas chromatography (Trace GC Ultra™, Thermo Fisher Scientific, Waltham MA, USA) with a fused silica capillary column (100 m x 0.250 mm and 0.2 µm film thickness; Supelco; SP2560, Bellefonte PA, USA) using helium as a carrier gas at a constant flow of 1.5 ml/min. The flame ionization detector was set at 280 ºC. The time temperature program used, started with an initial temperature of 140 ºC for 4 min, raised 4 ºC per min to a final temperature of 240 ºC for 20 min. Fatty acid methyl esters were identified using external standards (S37, Supelco, Bellefonte PA, USA; odd and branched chain fatty acids, *trans*11-C18:1, *cis*9, *trans11*-C18:2, *trans*10, *cis12*-C18:2, Larodan Fine Chemicals AB, Malmö, Sweden). Fatty acids *trans*6+7+8-C18:1, *trans*12-C18:1, *trans*13+14-C18:1, *cis*12-C18:1, *cis*13-C18:1, *cis*14+trans16-C18:1, *cis*15-C18:1, *trans*11*cis*15-C18:2 were identified according to the elution sequence reported by Loor et al. (2004) and Shingfield et al. (2006). Separation of the isomers *trans*10-C18:1 and *trans*11-C18:1 was done by using a second time temperature program, starting with an initial temperature of 70 ºC for 4 min, raised 1 ºC/min to 165 ºC for 20 min, raised with 2 ºC/min to 170 ºC for 10 min, and raised with 4 ºC/min to a final temperature of 215 ºC for 20 min.

2.5 Calculations

Digesta flows into the omasum were calculated by using the triple marker method described by France and Siddons (1986). Feacal marker recoveries were used as daily marker administrations. Ruminal nutrient digestibilities were calculated as ([omasal nutrient flow – nutrient intake] / nutrient intake) ·

100%. Whole tract digestiblities were calculated as ([feacal nutrient flow – nutrient intake] / nutrient intake) · 100%. Biohydrogenation values were calculated as ([omasal FA flow – FA intake] / FA intake) · 100%. Daily milk FA production (g/day) was calculated as milk fat production (g/day) · 0.933 as described by Glasser et al. (2007) and not as Stamey et al. (2010), because we are more interested in FA production rather than fatty acyl production. Transfer efficiencies of FA from intake to milk were calculated as (milk FA / FA intake) · 100%. Transfer efficiencies of FA from omasum to milk were calculated as (milk FA / omasal FA flow) · 100%.

2.6 Statistical analysis

Statistical analyzes were carried out in SAS version 9.1 (SAS Institute, Cary, NC). The effect of the different treatments was assessed by the general model for a Latin square design:

$Y_{ijk} = \mu + \alpha_k + \beta_i + \gamma_j + \varepsilon ijk$ where the terms could be defined as follows:

Y_{ijk}: The individual values for the dependent variables

μ: Overall mean

α_k: The effect of the treatment

β_i: The effect of the period

γ_j: The random effect of the cow

εijk: A random error

This model was tested in SAS with the proc MIXED procedure. Comparisons of means of the different treatments were done using Tukey's adjustment for the probability. Significance was declared when $P < 0.05$.

3. Results

3.1 Feed intake

Nutrient intake is shown in table 3.1. No significant effects were found on nutrient intake. Average daily DM intake was 20.6 kg. The crude fat intake tended to differ between the treatments with a higher crude fat intake for the EL treatment compared to the MALO treatment ($P < 0.10$).

Table 3.1. Effects of feeding different sources of linseed in the diet on nutrient intake (kg/day) of lactating dairy cows.

	Dietary Treatment[1]					
	CL	EL	SLO	MALO	SEM	P-value
DM	20.4	20.7	21.6	19.8	1.30	0.502
OM	19.0	19.4	20.1	18.4	1.21	0.494
Cfat	1.28	1.34	1.19	1.12	0.081	0.058
N	0.55	0.57	0.59	0.52	0.035	0.200
NDF	7.32	7.48	7.63	7.02	0.467	0.513
ADF	4.39	4.45	4.59	4.21	0.281	0.506
ADL	0.65	0.66	0.67	0.61	0.041	0.396
Starch	3.04	3.07	3.28	3.03	0.189	0.392
Sugar	0.74	0.77	0.84	0.80	0.055	0.431

DM = Dry Matter; OM = Organic Matter; Cfat = Crude Fat; N = Nitrogen; NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; ADL = Acid Detergent Lignin.
[1] CL = Crushed linseed; EL = Extruded linseed; SLO = Spray dried linseed oil; MALO = Linseed oil in combination with marine algae

Fatty acid intake was different between the treatments for some fatty acids (table 3.2). Intake of C12:0 was highest for the SLO treatment ($P < 0.001$). C14:0 intake was higher for the SLO and MALO treatments compared to the CL and EL treatments ($P < 0.01$). C18:0 intake was higher ($P < 0.05$) for the CL and EL treatment compared to the SLO treatment. Intake of C18:3n3 was higher for the EL treatment ($P < 0.05$) compared to the MALO treatment and the other treatments showed an intermediate intake. For the MALO treatment the C22:5 as well as the C22:6n3intake were higher compared to all other three treatments ($P < 0,001$). No significant differences were found for total saturated, monounsaturated, polyunsaturated and total FA intake.

3.2 Fermentation

The different dietary treatments did not differ in rumen pH, ammonia-N and VFA content (table 3.3). For the MALO treatment rumen fermentation pattern was modified, caused by a higher proportion of propionate at the expense of acetate. The ration acetate:propionate of the MALO treatment was significantly lower compared to the SLO treatment ($P < 0.05$), while the other treatments showed intermediate results.

Table 3.2. Effects of feeding different sources of linseed in the diet on fatty acid intake (g/day) of lactating dairy cows.

	Dietary Treatment[1]					
	CL	EL	SLO	MALO	SEM	P-value
C12:0	23.50 [b]	21.05 [b]	32.71 [a]	24.05 [b]	1.843	0.002
C14:0	8.70 [b]	7.82 [b]	12.05 [a]	14.15 [a]	0.671	<0.001
C16:0	92.41	96.12	100.71	100.11	5.988	0.378
C16:1	3.82	3.85	4.34	3.91	0.283	0.249
C18:0	27.11 [a]	28.37 [a]	20.77 [b]	23.91 [ab]	2.188	0.024
C18:1c9	180.35	185.76	193.75	168.84	11.452	0.148
C18:1c11	13.92	13.82	13.24	13.92	0.777	0.874
C18:2n6c	241.44	248.98	257.45	229.37	15.371	0.253
C18:3n3	351.70	371.59	338.48	301.35	22.832	0.062
C20:0	2.47 [ab]	2.50 [a]	2.74 [ab]	2.36 [b]	0.155	0.101
C22:5	0.16 [b]	0.16 [b]	0.17 [b]	4.09 [a]	0.124	<0,001
C22:6n3	0.00 [b]	0.00 [b]	0.00 [b]	11.62 [a]	0.373	<0,001
Total saturated[2]	239.82	242.73	261.77	248.68	16.131	0.425
Total monounsaturated[3]	199.19	204.40	212.61	187.60	12.479	0.198
Total polyunsaturated[4]	596.24	623.63	598.72	549.44	41.655	0.663
Total fatty acids	1035.26	1070.75	1073.10	985.73	66.778	0.428

[1] CL = Crushed linseed; EL = Extruded linseed; SLO = Spray dried linseed oil; MALO = Linseed oil in combination with marine algae
[2] C4:0, C6:0, C8:0, C10:0, C11:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0 and C24:0
[3] C14:1, C15:1, C16:1, C17:1, C18:1tr9, C18:1tr10+11, C18:1c9, C18:1c11, C20:1, C22:1n9 and C24:1
[4] C18:2n6t, C18:2tr11c15, C18:2n6c, C18:3n6, C18:3n3, CLAc9tr11, CLAtr10c12, C20:2, C20:3n6, C20:3n3, C20:4n6, C22:2, C20:5n3, C22:5 and C22:6n3
[a-c] Means within a row with different superscript differ (P < 0.05)

Table 3.3. Effect of feeding different sources of linseed in the diet on rumen fermentation characteristics in lactating dairy cows.

	Dietary Treatment[1]					
	CL	EL	SLO	MALO	SEM	P-value
pH	6.14	6.11	6.21	6.10	0.115	0.610
Ammonia-N, mg/l	129	169	123	130	40.2	0.452
Volatile fatty acids, mmol/l	111	111	112	118	7.06	0.710
Molar proportions, mmol/mol						
Acetate	632 [ab]	628 [ab]	641 [a]	605 [b]	15.8	0.044
Propionate	211 [ab]	211 [ab]	189 [b]	228 [a]	19.5	0.028
Butyrate	117	122	133	129	6.33	0.109
Isobutyrate	8.66	8.36	8.27	8.22	0.555	0.202
Valerate	15.5	15.1	15.2	15.8	1.54	0.879
Isovalerate	15.0	15.5	14.4	14.3	0.832	0.702
Acetate:propionate	3.08 [ab]	3.09 [ab]	3.49 [a]	2.77 [b]	0.368	0.025

[1] CL = Crushed linseed; EL = Extruded linseed; SLO = Spray dried linseed oil; MALO = Linseed oil in combination with marine algae
[a-b] Means within a row with different superscript differ (P < 0.05)

3.3 Omasal flows

Table 3.4 shows the daily nutrient flows of the omasum. Feeding the different sources of linseed showed no effects on the nutrient flows to the omasal canal. Average daily DM omasal flow was 13.06 kg and did not significantly differ between the four treatments. The daily NDF flow averaged 2.88 kg and did not differ between treatments. Figure 3.1 shows the correlation of the nutrient intake and the nutrient omasal flows. DM, OM and N intake showed to have a strong correlation with the omasal flows (R^2 > 0.8). Correlation for Cfat and NDF was R^2 > 0.6.

Table 3.4 Effect of feeding different sources of linseed in the diet on omasal flows of nutrients (g/day) in lactating dairy cows.

	Dietary Treatment[1]					
	CL	EL	SLO	MALO	SEM	P-value
DM	13398	13232	13148	12474	1282.2	0.667
OM	8650	8564	8612	8180	806.4	0.806
Cfat	1311	1268	1146	1191	93.5	0.491
N	490	496	510	463	42.8	0.678
NDF	2998	2950	2800	2782	428.4	0.659

DM = Dry Matter; OM = Organic Matter; Cfat= Crude Fat; N = Nitrogen; NDF = Neutral Detergent Fiber.
[1] CL = Crushed linseed; EL = Extruded linseed; SLO = Spray dried linseed oil; MALO = Linseed oil in combination with marine algae

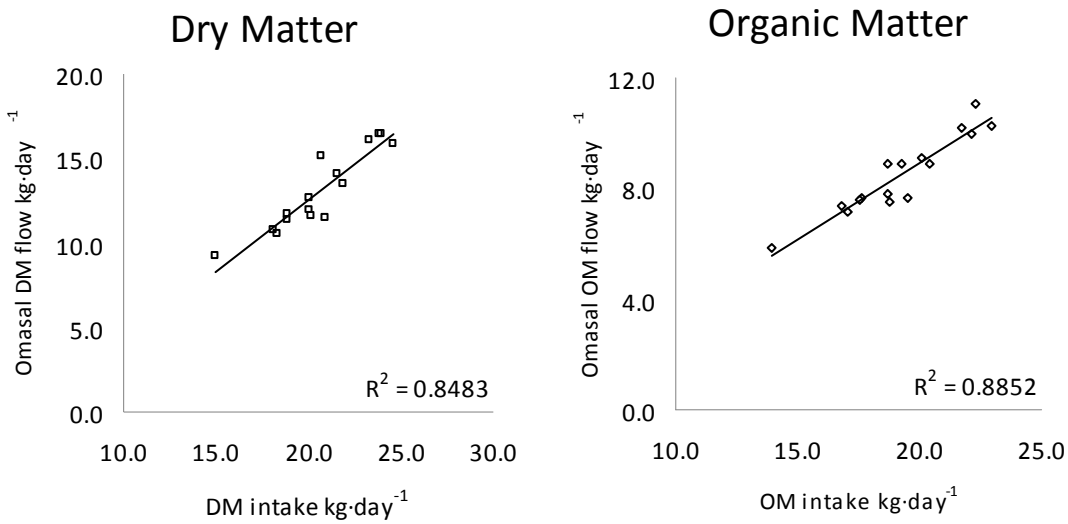

Figure 3.1a Correlation of intake and omasal flow for dry matter (DM), and organic matter (OM) in lactating dairy cows fed different sources of linseed.

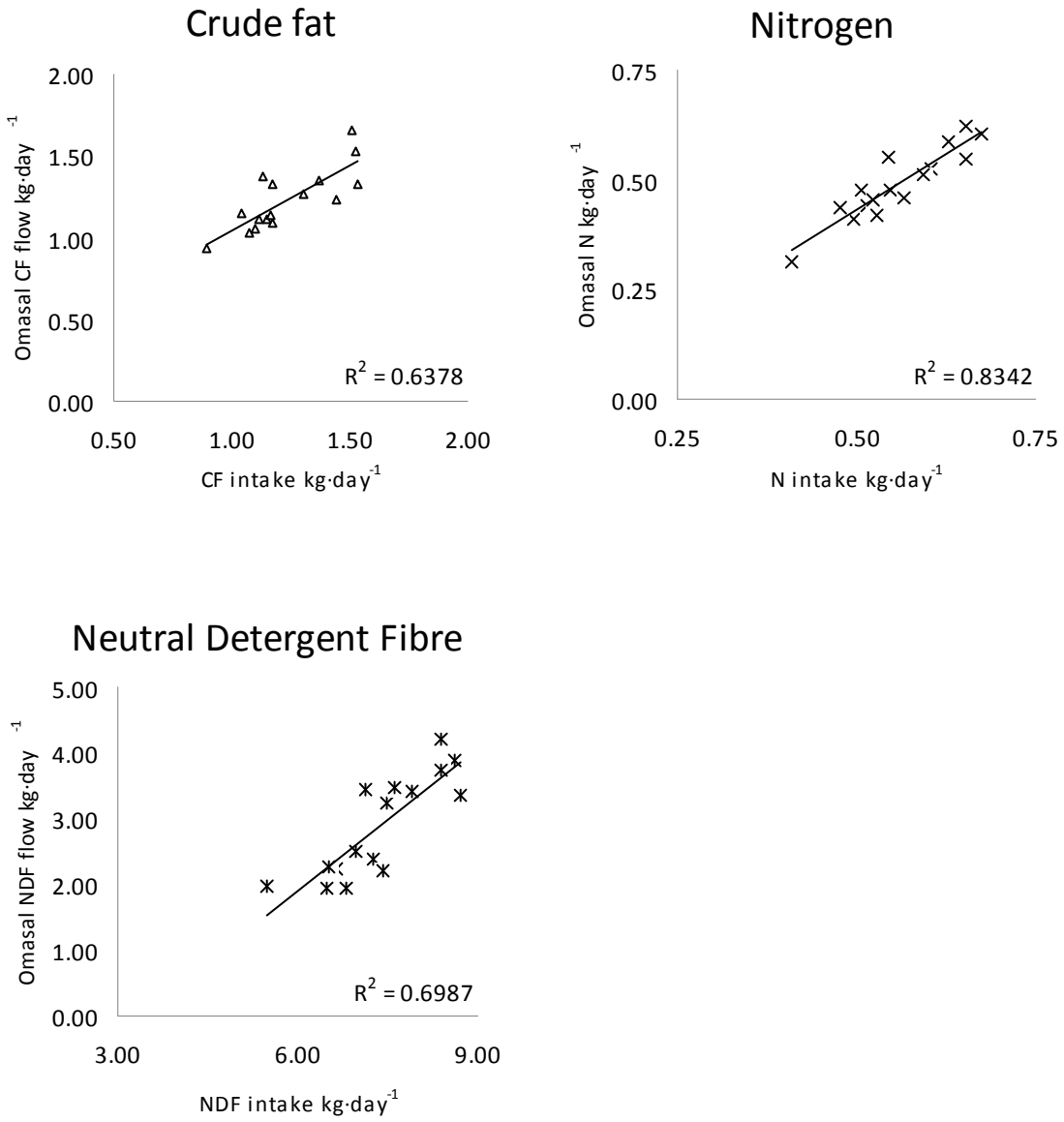

Figure 3.1b Correlation of intake and omasal flow for crude fat (Cfat), nitrogen (N), and neutral detergent fiber (NDF) in lactating dairy cows fed different sources of linseed.

Individual fatty acid flows in the omasum were affected by dietary treatment (table 3.5). C12:0 flow was higher for the SLO treatment compared to the El treatment ($P < 0.05$). The CL and MALO treatments showed intermediate C12:0 flows. The flow of C14:0 FA for the MALO treatment was higher compared to the CL and EL treatments ($P < 0.01$) and the SLO treatment ($P < 0.05$). C16:1 flow was higher for the MALO treatment compared to the CL and EL treatments ($P < 0.05$) and tended to be higher compared to the EL treatment ($P < 0.1$) C18:0 flow was significantly lower in the MALO treatment compared to the SLO and EL treatments ($P < 0.05$) and the CL treatment ($P < 0.01$). Omasal flow of total C18:1 *trans* fatty acids was higher for the MALO treatment compared to the other treatments ($P < 0.01$). Large part of the difference is caused by the C18:1 *trans* 10 flow which is higher for the MALO treatment compared to all other treatments ($P < 0.01$). Flow of C18:1 *trans* 4, 5, 6+7+8 is significantly higher for the MALO treatment compared to the other three treatments ($P < 0.05$). For the C18:1 *trans* 9 the MALO treatment was also higher compared to the SLO and EL treatments ($P < 0.01$) and CL ($P < 0.05$). C18:1 *trans* 11 and 12 flows were significantly higher ($P < 0.05$) for the MALO treatment compared the SLO and EL treatments. Total C18:1 *cis* flow was not significantly different for the four treatments, but C18:1 *cis* 11 flow for the MALO treatment was higher compared to the EL treatment ($P < 0.05$) and tended to be higher compared to the CL and SLO treatments ($P < 0.1$). C18:2n6 *cis* flow was higher for EL treatment compared to the MALO treatment ($P < 0.05$) and tended to be higher compared to the CL treatment ($P < 0.1$). Flow of C18:3n3 was significantly lowest for the MALO treatment compared to the CL and SLO treatment ($P < 0.01$) and compared to the EL treatment ($P < 0.001$). The El treatment had a significantly higher C18:3n3 flow in the omasum compared to CL ($P < 0.05$) and SLO ($P < 0.01$). A higher flow of CLA *trans* 10 *cis* 12 was found for the MALO treatment compared to the CL and EL treatments ($P < 0.05$) and a tendency ($P < 0.1$) towards a higher flow for the MALO treatment was found compared to the SLO treatment. Only in the MALO treatment a C22:6n3 flow was detected. Total saturated fatty acid flow was higher for the CL treatment compared to the MALO treatment ($P < 0.05$), while the other treatment showed intermediate results ($P < 0.1$). Total monounsaturated fatty acid flow was highest for the MALO treatment ($P < 0.01$). No differences were found in total polyunsaturated and total fatty acid flows.

Table 3.5 Effect of feeding different sources of linseed in the diet on omasal flows of fatty acids (g/day) in lactating dairy cows.

	Dietary Treatment[1]					
	CL	EL	SLO	MALO	SEM	P-value
C12:0	7.66 [ab]	6.29 [b]	10.76 [a]	8.55 [ab]	1.247	0.014
C14:0	9.63 [bc]	8.27 [c]	11.16 [b]	14.55 [a]	1.285	0.001
C16:0	95.55	91.03	100.37	123.74	13.713	0.097
C16:1	1.11 [b]	1.17 [ab]	0.86 [b]	2.57 [a]	0.364	0.023
C18:0	368.45 [a]	342.62 [a]	331.58 [a]	147.98 [b]	32.199	0.007
C18:1tr4	0.59 [b]	0.56 [b]	0.52 [b]	1.45 [a]	0.199	0.016
C18:1tr5	0.39 [b]	0.34 [b]	0.37 [b]	1.45 [a]	0.204	0.007
C18:1tr6+7+8	5.38 [b]	4.23 [b]	4.44 [b]	13.28 [a]	1.729	0.019
C18:1tr9	3.38 [b]	2.92 [b]	2.97 [b]	9.13 [a]	0.999	0.005
C18:1tr10	7.62 [b]	6.24 [b]	5.42 [b]	149.59 [a]	20.758	0.005
C18:1tr11	35.62 [ab]	25.97 [b]	32.56 [b]	92.22 [a]	14.717	0.034
C18:1tr12	6.74 [ab]	5.08 [b]	5.49 [b]	13.71 [a]	1.624	0.021
C18:1tr13+14	28.53	22.39	22.23	52.43	7.383	0.060
C18:1c9	42.50	40.98	51.96	57.43	8.927	0.217
C18:1c11	5.99 [ab]	5.28 [b]	5.89 [ab]	12.21 [a]	1.956	0.030
C18:1c12	6.79	5.09	6.23	2.68	1.397	0.179
C18:1c13	9.61	8.55	8.06	11.13	1.946	0.703
C18:1c14+tr16	4.45 [ab]	3.63 [ab]	2.86 [b]	11.75 [a]	2.021	0.043
C18:1c15	1.55	1.09	1.31	4.76	1.004	0.091
C18:2n6c	17.69 [ab]	20.15 [a]	16.29 [ab]	10.69 [b]	2.353	0.025
C18:3n3	21.81 [b]	33.83 [a]	15.47 [b]	4.59 [c]	2.594	0.000
CLAc9tr11	4.74	3.47	8.69	3.99	1.524	0.153
CLAtr10c12	0.02 [b]	0.11 [b]	0.24 [ab]	0.59 [a]	0.094	0.014
C22:5	3.99	3.70	4.41	3.07	0.654	0.564
C22:6n3	0.00 [b]	0.00 [b]	0.00 [b]	1.00 [a]	0.086	0.000
Total C18:1 *cis*[2]	66.44	60.99	73.44	88.21	13.892	0.291
Total C18:1 *trans*[3]	88.25 [b]	67.73 [b]	74.01 [b]	333.27 [a]	37.464	0.002
Total > C20[4]	17.28 [ab]	14.55 [b]	29.90 [a]	17.37 [ab]	3.558	0.044
Total saturated[5]	515.98 [a]	478.99 [ab]	486.69 [ab]	331.62 [b]	45.238	0.027
Total monounsaturated[6]	160.82 [b]	134.01 [b]	153.40 [b]	438.29 [a]	52.159	0.004
Total polyunsaturated[7]	66.33	77.48	67.51	76.43	14.145	0.837
Total fatty acids	747.71	693.06	722.23	847.94	100.610	0.414

[1] CL = Crushed linseed; EL = Extruded linseed; SLO = Spray dried linseed oil; MALO = Linseed oil in combination with marine algae

[2] Sum of C18:1c9, C18:1c11, C18:1c12, C18:1c13 and C18:1c15

[3] Sum of C18:1tr4, C18:1tr5, C18:1tr6+7+8, C18:1tr9, C18:1tr10, C18:1tr11, C18:1tr12, C18:1tr13+14, C18:1tr15

[4] Sum of C20:0, C20:1, C21:0, C 20:2, C22:0, C20:3n6, C22:1n9, C20:3n3, C20:4n6, C23:0, C22:2, C24:0, C20:5n3,C24:1, C22:5, C22:6n3

[5] Sum of C12:0, C13:0 iso, C13:0 anteiso, C14:0 iso, C14:0 anteiso, C14:0, C15:0 iso, C15:0 anteiso, C15:0, C16:0 iso, C16:0 anteiso, C16:0, C17:0 iso, C17:0 anteiso, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0 and C24:0

[6] Sum of C15:1, C16:1, C17:1, C18:1tr4, C18:1tr5, C18:1tr6+7+8, C18:1tr9, C18:1tr10, C18:1tr11, C18:1tr12, C18:1tr13+14, C18:1c9, C18:1tr15, C18:1c11, C18:1c12, C18:1c13, C18:1c14+tr16, C18:1c15, C20:1 and C 22:1n9

[7] Sum of C18:2n6t, C18:2c9tr13, C18:2tr8c13, C18:2c9tr12, C18:2tr9c12, C18:2tr11c15, C18:2n6c, C18:c9c15, C18:3n6, C 18:3n3, CLAc9tr11, CLAtr10c12, CLAtr9c11, CLAc9c11t11c13, CLAtr11tr13, CLAt9t11t10t12, C20:2, C20:3n6, C20:3n3, C20:4n6, C22:2

[a-c] Means within a row with different superscript differ ($P < 0.05$)

3.4 Digestibility

Table 3.6 shows the nutrient digestibility in the rumen and feacal digestibility of DM and crude fat. DM, OM, N and NDF rumen digestibilities did not differ between the different dietary treatments. The whole tract digestibility of DM in the MALO treatment was higher ($P < 0.05$) compared to the EL treatment, and CL and SLO treatments showed intermediate results. Crude fat digestibility was lower for the EL treatment compared to the SLO and MALO treatments ($P < 0.001$) and CL treatment ($P < 0.05$).

Table 3.6 Effect of feeding different sources of linseed in the diet on rumen and whole tract digestibility of nutrients as % of intake in lactating dairy cows.

	Dietary Treatment[1]					
	CL	EL	SLO	MALO	SEM	P-value
DM	34.90	36.58	39.16	37.23	2.750	0.370
OM	53.34	54.28	56.05	54.38	1.595	0.339
N	12.53	12.74	13.99	10.54	3.559	0.885
NDF	60.09	60.95	63.57	60.64	3.778	0.439
Whole tract digestibility						
DM	73.28 [ab]	72.65 [b]	74.49 [ab]	74.72 [a]	0.925	0.025
Cfat	71.25 [b]	64.78 [c]	78.49 [a]	80.41 [a]	1.057	<0.001

DM = Dry Matter; OM = Organic Matter; Cfat = Crude Fat; N = Nitrogen; NDF = Neutral Detergent Fiber
[1] CL = Crushed linseed; EL = Extruded linseed; SLO = Spray dried linseed oil; MALO = Linseed oil in combination with marine algae
[a-c] Means within a row with different superscript differ ($P < 0.05$)

Ruminal biohydrogenation of the different UFA as % of FA intake is shown in table 3.7. Biohydrogenation of C18:1 *cis* 9 did not significantly differ between the different treatments. Biohydrogenation of C18:1 *cis* 9 tended to be lower for the MALO treatment compared to the EL treatment ($P < 0.1$), where CL and SLO show intermediate results. Biohydrogenation of C18:2n6c was higher for the MALO treatment compared to the EL treatment ($P < 0.05$) and CL and SLO showed intermediate results. C18:3n3 biohydrogenation was higher in the MALO treatment compared to the EL treatment ($P < 0.001$) and CL and SLO ($P < 0.05$). Biohydrogenation of C22:6n3 was determined only for the MALO treatment, since intake of C22:6n3 in the CL, EL and SLO treatments were approximately zero.

Table 3.7 Effect of feeding different sources of linseed in the diet on biohydrogenation of unsaturated fatty acids in the rumen as % of intake in lactating dairy cows.

	Dietary Treatment[1]					
	CL	EL	SLO	MALO	SEM	P-value
C18:1c9	77.40	78.00	73.52	65.89	3.825	0.080
C18:2n6c	92.90 [ab]	91.88 [b]	93.70 [ab]	95.34 [a]	0.060	0.015
C18:3n3	93.99 [bc]	90.86 [c]	95.43 [b]	98.49 [a]	0.049	<0.001
C22:6n3	ND[2]	ND	ND	91.03		

[1] CL = Crushed linseed; EL = Extruded linseed; SLO = Spray dried linseed oil; MALO = Linseed oil in combination with marine algae
[2] ND: Biohydrogenation is not determined because C22:6n3 intake of CL, EL and SLO were approximately zero
[a-c] Means within a row with different superscript differ ($P < 0.05$)

3.5 Milk production

Table 3.8 shows the milk production characteristics. Milk yield did not differ between the treatments. Milk fat content was lower for the MALO treatment compared to the other three treatments ($P < 0.05$). C12:0 production per day was higher for the EL treatment compared to the MALO treatment ($P < 0.05$). C16:0 production was higher for the SLO treatment compared to the CL treatment ($P < 0.05$) and the MALO treatment ($P < 0.001$). The EL treatment showed also a higher C16:0 production compared to the CL treatment ($P < 0.05$) and the MALO treatment ($P < 0.01$). C18:0 outflow in the milk was lower for the MALO treatment compared the other treatments ($P < 0.01$). C18:1 *trans* 10 was higher ($P < 0.001$) for the MALO treatment compared to the other three treatments. C18:1 *cis* 9 production is lower for the MALO treatment compared to the CL and EL treatments ($P < 0.001$) and the SLO treatment ($P < 0.01$). Milk C18:1 *cis* 12 production was lower for the MALO treatment compared to the other treatments ($P < 0.05$). C18:2n6 *cis* production was higher for the SLO treatment compared to the CL and EL treatments ($P < 0.01$) and the MALO treatment ($P < 0.001$). C18:2n6 *cis* production was lower for the MALO treatment compared to the EL treatment ($P < 0.05$). The MALO had a significantly lower production for C20:0 ($P < 0.001$) and C20:1 ($P < 0.01$) compared to the other treatments. C18:3n3 production was higher for the SLO treatment compared to the CL and EL treatments ($P < 0.01$) and the MALO treatment ($P < 0.001$). CLA *cis* 9 *trans* 11 production was higher for the MALO treatment compared to the EL treatment ($P < 0.05$). CL and SLO showed intermediate results. C22:5 production was higher for the SLO treatment compared to the MALO treatment ($P < 0.05$). CL and EL showed intermediate results. C22:6n3 could not be determined in the milk

Total C18:1 *cis* production was significantly lower in the MALO treatment compared to the other treatments ($P < 0.001$). Total milk C18:1 *trans* production was higher for the MALO treatment compared to the other treatments ($P < 0.01$). Total >C20 production was lower for the MALO treatment compared to the SLO treatment ($P < 0.01$) and EL treatment ($P < 0.05$), where CL showed intermediate results. Total saturated milk fatty acid production was lower for the MALO treatment compared to the EL treatment ($P < 0.01$) and the CL and SLO treatments ($P < 0.05$). Total monounsaturated fatty acid production was lower in the MALO treatment compared to the EL treatment ($P < 0.01$) and the CL and SLO treatments ($P < 0.05$). Total polyunsaturated fatty acid production is higher in the SLO treatment compared to the other treatments ($P < 0.01$). Total milk fatty acid production was lower for the MALO treatment compared to the other treatments ($P < 0.01$).

Table 3.8 Effect of feeding different sources of linseed in the diet on milk yield, composition and fatty acid content in lactating dairy cows.

	Dietary Treatment[1]					
	CL	EL	SLO	MALO	SEM	P-value
Milk production, kg/day	33.08	31.44	33.67	29.68	2.408	0.402
Milk fat, g/kg	42.98 [a]	47.50 [a]	46.70 [a]	32.73 [b]	5.966	0.002
Milk protein, g/kg	31.78	32.70	32.55	30.88	1.166	0.552
Milk fatty acid , g/day						
C12:0	44.58	42.40	57.16 [a]	31.09 [b]	7.161	0.056
C14:0	135.38	140.80	157.38	107.91	13.711	0.072
C16:0	303.81 [b]	354.05 [a]	372.58 [a]	255.33 [b]	49.156	0.001
C16:1	19.62	23.68	18.61	17.95	3.799	0.237
C18:0	182.16 [a]	214.96 [a]	194.53 [a]	63.59 [b]	26.770	0.001
C18:1tr6+7+8	4.02	3.76	3.61	7.55	0.936	0.068
C18:1tr9	3.14	2.77	2.86	5.20	0.704	0.142
C18:1tr10	4.97 [b]	6.54 [b]	4.50 [b]	58.46 [a]	3.809	<0.001
C18:1tr11	15.89	8.87	14.94	28.58	4.230	0.076
C18:1tr12	5.23	4.44	6.43	9.14	1.121	0.097
C18:1tr13+14	17.44	14.76	16.57	23.86	2.969	0.250
C18:1c9	283.20 [a]	322.06 [a]	270.21 [a]	93.83 [b]	42.749	0.000
C18:1tr15	6.65	6.15	5.64	7.29	1.046	0.725
C18:1c11	5.90	6.47	6.22	6.26	0.597	0.812
C18:1c12	3.98 [a]	3.52 [a]	4.15 [a]	1.31 [b]	0.449	0.014
C18:1c13	0.34	0.50	0.45	0.35	0.252	0.960
C18:1c14+tr16	0.81 [ab]	0.83 [ab]	0.04 [b]	1.61 [a]	0.302	0.049
C18:1c15	7.70	7.12	6.55	7.34	1.109	0.899
C18:2tr11c15	3.66	2.82	3.69	8.96	1.572	0.104
C18:2n6c	16.15 [bc]	17.31 [b]	29.92 [a]	9.68 [c]	1.437	<0.001
C18:3n3	10.80 [b]	11.02 [b]	44.70 [a]	3.93 [b]	3.106	<0.001
CLAc9tr11	6.66 [ab]	4.53 [b]	6.03 [ab]	11.66 [a]	1.269	0.032
CLAtr10c12	0.11	0.00	0.16	0.00	0.104	0.624
C22:5	0.91 [ab]	0.99 [ab]	1.37 [a]	0.58 [b]	0.125	0.014
Total C18:1c[2]	301.93 [a]	340.51 [a]	287.62 [a]	110.69 [b]	42.987	<0.001
Total C18:1tr[3]	57.34 [b]	47.30 [b]	54.54 [b]	140.08 [a]	11.034	0.003
Total >C20[4]	7.39 [ab]	8.02 [a]	10.99 [a]	4.01 [b]	0.808	0.004
Total saturated[5]	817.65 [a]	920.56 [a]	865.08 [a]	409.96 [b]	77.428	0.007
Total monounsaturated[6]	391.58 [a]	427.35 [a]	374.37 [a]	281.50 [b]	44.655	0.003
Total polyunsaturated[7]	53.50 [b]	51.03 [b]	100.81 [a]	50.42 [b]	6.788	0.005
Total fatty acids	1262.72 [a]	1398.94 [a]	1340.26 [a]	741.88 [b]	97.821	0.002

[1] CL = Crushed linseed; EL = Extruded linseed; SLO = Spray dried linseed oil; MALO = Linseed oil in combination with marine algae
[2] Sum of C18:1c9, C18:1c11, C18:1c12, C18:1c13 and C18:1c15
[3] Sum of C18:1tr6+7+8, C18:1tr9, C18:1tr10, C18:1tr11, C18:1tr12, C18:1tr13+14 and C18:1tr15
[4] Sum of C20:0, C20:1, C21:0, C22:0, C22:1n9, C24:1, C20:2, C20:3n6, C20:3n3, C20:4n6, C20:5n3, C22:2, C22:5, C22:6n3 C23:0 and C24:0
[5] Sum of C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0 iso, C13:0 anteiso, C13:0, C14:0 iso, C14:0 anteiso, C14:0, C15:0 iso, C15:0 anteiso, C15:0, C16:0 iso, C16:0 anteiso, C17:0 iso, C16:0, C17:0 anteiso, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0 and C24:0
[6] Sum of C14:1, C15:1, C16:1, C17:1, C18:1tr6+7+8, C18:1tr9, C18:1tr10, C18:1tr11, C18:1tr12, C18:1tr13+14, C18:1c9, C18:1tr15, C18:1c11, C18:1c12, C18:1c13, C18:1c14+tr16, C18:1c15, C20:1, C22:1n9 and C24:1
[7] Sum of C18:2n6tr, C18:2c9tr13, C18:2tr8c13, C18:2c9tr12, C18:2tr9c12, C18:2tr11c15, C18:2n6c, C18:2c9tr15, C18:3n6, C18:3n3, CLAc9tr11, CLAtr10c12, C20:2, C20:3n6, C20:3n3, C20:4n6, C20:5n3, C22:2, C22:5 and C22:6n3
[a-c] Means within a row with different superscript differ (P < 0.05)

Recoveries of intake and omasal flow of C18:2n6c and C18:3n3 are show in table 3.9. Recovery for C18:2n6c from intake to milk is higher for SLO compared to the CL and EL treatments ($P < 0.01$) and the MALO treatment ($P < 0.001$). The MALO treatment had a lower C18:2n6c recovery compared to CL and EL ($P < 0.05$). C18:3n3 had a significantly higher recovery from intake for the SLO treatment compared to the other treatments ($P < 0.001$). Recovery from omasal flow to milk was for C18:2n6c higher for the SLO treatment compared to the CL treatment ($P < 0.05$) and the EL and MALO treatments ($P < 0.01$). C18:3n3 recovery from the omasal flow was higher for the SLO treatment compared to all other treatments ($P < 0.001$).

Table 3.9 Effect of feeding different sources of linseed in the diet on recoveries of C18:2n6c and C18:3n3 in lactating dairy cows.

	Dietary Treatment[1]					
	CL	EL	SLO	MALO	SEM	P-value
Recovery from intake (%)[2]						
C18:2n6c	7.2 ^{b}	7.2 ^{b}	11.6 ^{a}	4.2 ^{c}	0.65	<0.001
C18:3n3	3.2 ^{b}	3.0 ^{b}	13.0 ^{a}	1.3 ^{b}	0.63	<0.001
Recovery from omasal flows (%)[3]						
C18:2n6c	110.9 ^{b}	88.7 ^{b}	184.1 ^{a}	94.6 ^{b}	17.60	0.006
C18:3n3	59.2 ^{b}	33.5 ^{b}	287.6 ^{a}	89.0 ^{b}	16.30	<0,001

[1] CL = Crushed linseed; EL = Extruded linseed; SLO = Spray dried linseed oil; MALO = Linseed oil in combination with marine algae
[2] Calculated as (milk fatty acid production : fatty acid intake) x 100
[3] Calculated as (milk fatty acid production : omasal fatty acid flow) x 100

3.6 Ruminal biohydrogenation

Figure 3.2 shows the C18:3n3 concentration in the rumen over a period 10 hours. EL showed to have a clearly higher C18:3n3 concentration in the rumen compared to CL, SLO and MALO with a disappearance of 42% during the time period. Start concentrations were almost equal for CL and SLO, but disappearance of SLO (80%) was slightly higher then for the CL treatment (76%). MALO showed to have lower concentrations compared to the other treatments and disappearance was 66%.

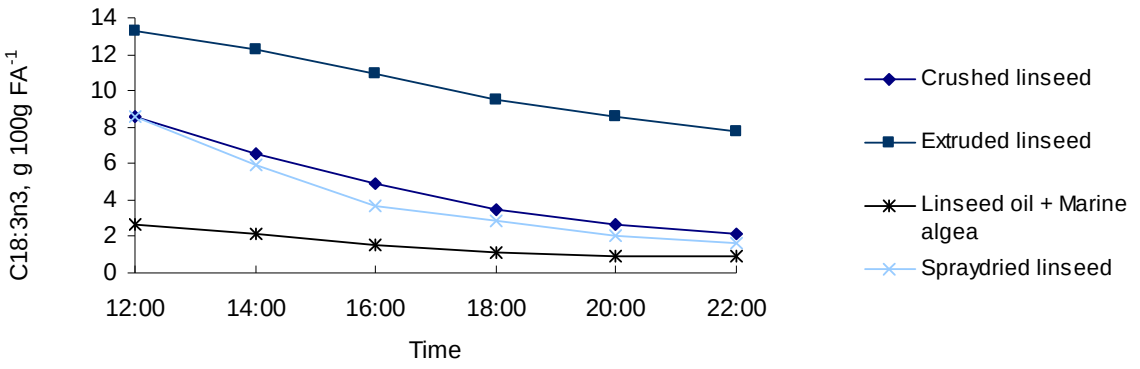

Figure 3.2. Effect of feeding four different sources of linseed in the diet on C18:3n3 content (g/100g FA) in the rumen over a 10h time period.

4. Discussion

4.1 Sampling procedure

Punia et al. (1988) developed a technique to take samples from the omasum by inserting a tube via the rumen cannula through the reticula-omasal orifice and collect material with the use of a vacuum pump. Disadvantage of this technique was insertion of the tube which was needed each sampling time. Therefore Huhtanen et al. (1997) further developed this procedure by placing a device in the omasum which could stay in the omasum during the measuring period. A compressor/vacuum pump was used to collect samples. During sampling problems of blockage of the tube were encountered and therefore, Ahvenjärvi et al. (2000) further developed the OST procedure by adjusting the pump and using a larger tube for the sampling device however, problems during sampling remained. Therefore, another sampling device was developed for this study. The device was tested several times and no problems occurred when sampling. During the measurements in period 1 however, the device was sometimes blocked by the leaves of the omasum. Therefore, during a few sampling times intervention was needed by means of unblocking the device by placing a hand around the device. After period 1 the curve in the tube connected to the device was slightly changed so the device could be placed a bit further behind the reticula-omasal orifice. With this adjustment sampling improved and the next periods intervention was needed only occasionally. On visual appearance the digesta samples, which needed intervention during collection did not show to differ from other samples. Unfortunately, pH of the omasal samples was not measured.

A major shortcoming of the OST arises from the collection of samples that are not representative for true digesta entering the omasal canal (Ahvenjärvi et al., 2000; Huhtanen et al., 1997). FA mainly attach to large particles in the digesta, and consequently unrepresentative sampling can cause an important over- or underestimation of fatty acid flows. Ahvenjärvi et al. (2003) reported the triple marker method, described by France and Siddons (1986), to provide accurate estimates of omasal flows. Therefore this method is also used in present study.

4.2 Feed intake

Huhtanen et al. (1997) reported a reduced feed intake during the omasal sample collection period. To prevent variations in feed intake, and subsequently fatty acid intake, feeding level was restricted from week 2 onwards within this experiment. Unfortunately, this could not prevent the occurrence of feed residues, but residues were only found occasionally and on both adaptation and sampling days. Therefore, no differences were found in feed intake between the adaptation and sampling periods.

The slightly lower DM intake and a lower Cfat content in the diet for the MALO treatment compared to the EL treatment caused a tendency of a lower Cfat intake for the MALO treatment compared to the EL

treatment. But differences were too small to have an effect on further results. The higher C18:3n3 content of the EL diet compared the MALO diet together with the higher DM intake for the EL treatment explains the higher C18:3n3 intake for the EL treatment compared to the MALO treatment. A higher C18:3n3 intake could result in a higher omasal C18:3n3 flow. Although C18:3n3 was higher for the EL treatment compared to the MALO treatment this difference was probably caused by a difference in biohydrogenation rather then by a difference in intake.

4.3 Fermentation

Rumen fermentation characteristics, like pH, ammonia-N and VFA content were similar for the different treatments. The lower acetate:propionate ratio for the MALO treatment could indicate a lower fiber digestion for the MALO treatment (Chilliard et al., 2000), but this was not reflected in NDF digestibility.

4.4 Omasal flows

To evaluate the occurrence of variation between different measurements, correlation coefficients between omasal nutrient flows and nutrient intake are determined. High correlations with no outliers indicate that large sampling errors are minimal.

Titgemeyer (1997) described digestibilities of OM and NDF to be more useful in comparing ruminal digestion compared to DM digestibility because duodenal contents contain high levels of ash. The same results are found in experiments with omasal sampling by Avenjärvi et al. (2000) and Punia et al. (1988) who found higher ash flows in the omasum compared to the duodenum indicating a high absorption of minerals in the omasum, which was also concluded in a mineral absorption study by Tuori et al. (2006). These results also explain our low DM rumen digestibility compared to the OM digestibility, which is also reported in other OST experiments (Shingfield et al., 2003; Shingfield et al., 2008; Lundy et al., 2004). OM digestibility of 53- 56% are slightly higher compared to digestibilities found (47.2 ± 4.73) in a meta-analysis study by Huhtanen et al. (2010) on 32 studies using OST, but comparable to other studies (Ahvenjärvi et al., 2000, Owens et al., 2008) and within the range described by Titgemeyer (1997). Huhtanen et al. (2010) found a NDF digestibility of 55.1% ± 12.50. NDF digestibility within this study is again slightly higher with values ranging from 60-63%. Shingfield et al. (2003) reported digestibilities of 55-67% and Owens et al. (2008) between 43-71%. A large part of the fiber digestion occurs in the rumen (Van Soest, 1994; Huhtanen et al., 2006). This makes total NDF digestibility a good indirect measure to determine the accuracy of the ruminal NDF digestibility. Unfortunately, within this study the NDF is not analyzed in the feaces samples, so no total tract NDF digestibilities could be calculated. Ahvenjärvi et al. (2000) reported omasal NDF flow could be influenced by the sampling method. Because the sampling device is located close to the reticulo-omasal orifice, it is possible that during a reticular contraction particles which are not likely to escape the rumen are included in the sample. These particles are likely to have a higher fiber content compared to the actual omasal flow, which causes an overestimation of the NDF flow. This error cannot be corrected by any marker method. Within

this study NDF digestibility was slightly higher compared to other studies which indicates that this error is minimal in the present study.

The whole tract Cfat digestibility was lowest for the EL treatment. Sterk et al. (2010) reported that extrusion ruptured the whole linseed only to a certain extent. This is also observed in the present study where whole linseeds were found in the feaces. The seed coat of the linseed prevents the oil to be released post-ruminal, and the FA to be absorbed (Jenkins, 2006) resulting in a lower Cfat digestibility. This might have accounted for the CL as well. Crushing might not have damaged all the seeds so leaving some seed coats still intact.

Duodenal fatty acid flows are derived from FA adherent to feed particles and microbial cells, lipids from rumen bacteria, protozoa, endothelial cells and bile (Noble, 1981; Doreau and Ferlay, 1994). The advantage of omasal sampling comparing to duodenal sampling when measuring ruminal biohydrogenation is that the fatty acids entering the omasum are less contaminated with endogenous secretions. Total fatty acid flow in the omasum accounted for approximately 70 % of dietary intake. In a literature study, Doreau and Chilliard (1997) reported that in many experiments a net loss of FA from intake to duodenum was observed. More recent studies however, showed an increase in fatty acid flow in the omasum compared to fatty acid intake (Lundy et al., 2004; Shingfield et al., 2008). This is consistent with the expectation that there is a minimal absorption of fatty acids across the rumen epithelium (Noble, 1981). The 70% recovery found in this study is low compared to these findings. Omasal samples from this study were also analyzed on FA profile at the Ghent University (Belgium) by B. Vleaminck, who uses another procedure to quantify the FA compared to the procedure used in Wageningen. He found total omasal FA flows of 1118.67, 1027.69, 905.77 and 945.21 gram/day (SEM 80.2792 and P = 0.234) for the CL, EL, SLO and MALO treatment respectively. This difference was mainly caused by differences in SFA which were approximately two times higher. The unsaturated *cis* FA however, were approximately two times lower which could not be explained. Therefore these results are not included in this study. The total FA flow determined with the FA analysis method from the Ghent University indicates that the total FA acid flows reported in this study are an under-estimation, likely being the result of an incomplete extraction or methylation in the FA analysis then to be the result of unrepresentative sampling.

Biohydrogenation of C18:2n6 and C18:3n3 was reported to be on average 80 and 92% respectively (Doreau and Chilliard, 1997). With biohydrogenation values of 92 - 95% for C18:2n6 and 91 – 98% found in the present study there seems to be an overestimation of biohydrogenation, especially when dietary treatments were expected to inhibit biohydrogenation. This overestimation of hydrogenation is probably caused by the underestimation of FA flows in the omasum. Figure 3.1 supports this thought by comparing the transfer efficiency of C18:3n3 from omasum to milk. Transfer efficiencies found in the present study are compared with data gathered from literature, which measured post-ruminal C18:3n3 flow in the duodenum. The figure clearly shows the higher transfer efficiencies found for the MALO and CL treatment compared to the transfer efficiencies found in earlier studies. Transfer efficiency for

C18:3n3 in the SLO treatment was with 288% an outlier. The values therefore support the idea of underestimating C18:3n3 flow in the omasum. EL C18:3n3 transfer efficiency does not show the same trend, probably because a large part of the C18:3n3 found in the omasum is incorporated into whole seeds and C18:3n3 is not available for absorption and milk production (Jenkins, 2006). This also explains the higher C18:3n3 flow in the omasum. The intact seed coat provides an effective protection against biohydrogenation (Jenkins, 2006).

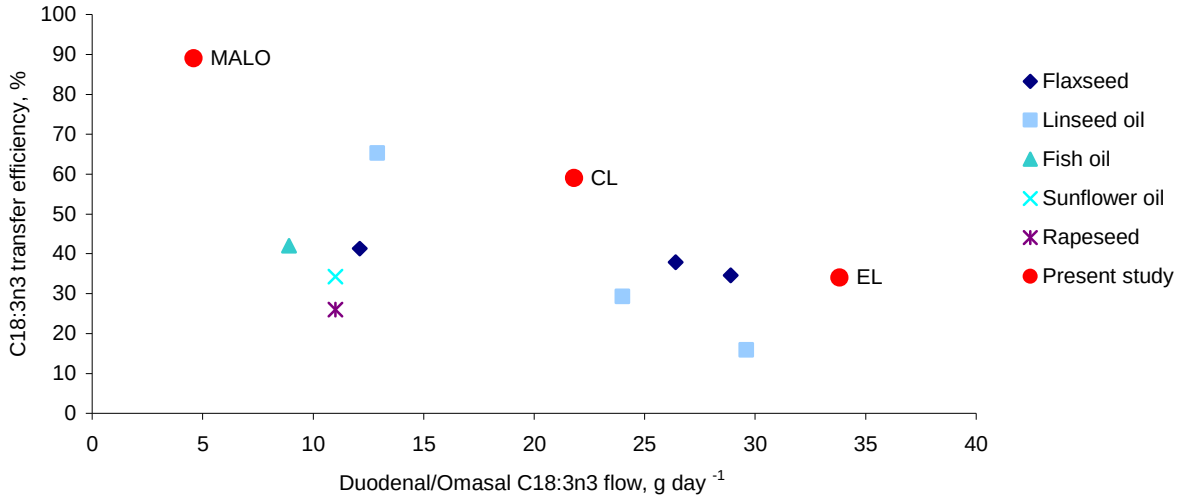

Figure 4.1 C18:3n3 transfer efficiency (milk C18:3n3 / duodenal or omasal C18:3n3) from duodenal/omasal flow into milk (with data from Chilliard et al., 1991; Gonthier et al., 2004, 2005; Loor et al., 2004; Loor et al., 2005a, 2005b, 2005c). Results of SLO from present study not shown.

In vitro experiments showed that formaldehyde treated linseed offered a proper protection against ruminal biohydrogenation. *In vivo* experiments showed the same promising results (Tymchuk et al., 1998; Sinclair et al., 2005), but Petit (2003) did not find higher UFA levels in milk feeding dairy cows with formaldehyde treated whole flaxseed or sunflower seed. Pretreatment of the oilseeds seems to be essential to ensure the proper protection against biohydrogenation (Sinclair et al., 2005; Fievez et al., 2007). In this study linseed oil encapsulated in a layer of formaldehyde treated protein was used. Disappearance of C18:3n3 in the rumen of 95% indicated low protection against biohydrogenation. In the milk however, C18:3n3 production per day was 2.88 times higher than the daily omasal canal flow. This indicates that an error is made in determining the omasal C18:3n3 flow, because polyunsaturated fatty acids are not synthesized by ruminant tissues (Chilliard et al., 2000) The origin of this error is unclear, but it is possible that SLO behaves different in the digesta compared to CL and EL, because the material of. SLO was very fine. FA are known to accumulate on feed particles in the digesta (Harfoot and Hazlewood, 1997). In the omasal samples C18:3n3 was found more in the large particle phase for the CL and El treatments compared to the SLO and MALO treatments where more C18:3n3 was found in the small particle phase. Because of these characteristics it is possible that the SLO product had a very low retention time in the rumen. It could therefore be possible that during sampling most of the SLO already

passed on the post-ruminal part of gastrointestinal tract and could not be included in the omasal samples. Unfortunately this matter could not be further investigated because the samples of the individual time points were pooled.

The C18:3n3 provided by the linseed oil in the MALO treatment was biohydrogenated to a large extent, which is in accordance with results found by Doreau et al. (2009) and Loor et al. (2005). The addition of DHA was expected to result in an accumulation of *trans* intermediate C18:1 FA and not to influence C18:3n3 biohodrogenation (Sterk et al., 2010; Vleaminck et al., 2007; Boeckaert et al., 2007, 2008). The DHA has an inhibiting effect on rumen microbes which are involved in the last step of the biohydrogenation from a *trans* C18:1 tot C18:0 (Boeckaert et al., 2007). Figure 4.2 shows the main biohydrogenation pathways of C18:3n3, but many different C18:1 intermediates are produced (Chilliard et al., 2000; Loor et al., 2005c). Vleaminck et al. (2008) and Sterk et al. (2010) both reported an accumulation of *trans-11* C18:1 when adding DHA *in vitro. In vivo* studies (Boeckaert et al., 2007; Boeckaert et al., 2008) also showed an increase of *trans-10* and *trans-11* C18:1 when adding DHA to the diet The inhibition of the last step of biohydrogenation is clearly shown in the results of the present study with a lower C18:0 flow through the omasum for the MALO treatment. *Trans* C18:1 accumulation is clearly shown by a high increase of *trans-10* and *trans-11* C18:1.

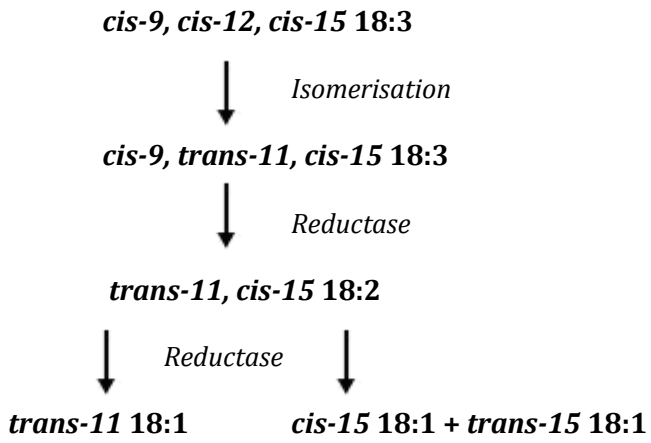

Figure 4.2 Ruminal biohydrogenation pathways of C18:3n3 (following*: Jenkins et al., 2008).*

4.5 Milk production

Milk yield and milk protein content were not different for the different treatments. The low milk fat content for the MALO diet indicates a milk fat depression caused by the DHA (Shingfield and Griinari, 2007). This is in accordance with results of Boeckaert et al. (2008) who also found a milk fat depression when feeding DHA. The increase of the *trans-10* C18:1 has been reported to play an important role in milk fat depression (Griinari et al. 1998; Chilliard et al., 2000), however a direct relationship is not proven (Shingfield and Griinari, 2007).

Milk FA content is calculated as 93.3% of total milk fat production as described by Glasser et al (2007). The lower FA content for the MALO treatment was mainly caused by a lower saturated FA content in the milk. The lower C18:0 production for the MALO treatment is in accordance with the omasal flow. A higher *trans-10* and *trans-11*C18:1 flow in the omasum led to a higher production of *trans-10* C18:1 and a tendency for a higher *trans-11* C18:1 production in the milk. The higher *trans*-11C18:1 flow for the MALO treatment resulted in a higher *cis-9, trans-11* CLA production in the milk under influence of the Δ^9-desaturase activity in the mammary gland (Chilliard et al., 2000; Destaillats et al., 2005). The Δ^9-desaturase activity is also shown in the *cis-9* C18:1 production which was higher for the CL, EL and SLO treatment. This was caused by a higher omasal C18:0 flow which resulted in a higher C18:0 uptake in the mammary gland and increased *cis-9* C18:1 production (Chilliard et al, 2000; Glasser et al., 2008).

4.6 Ruminal biohydrogenation

The best way to test omasal flows is to compare with data obtained from the same experiment (Titgemeyer, 1997). Ahvenjärvi et al. (2000) tested the OST by comparing omasal flows with duodenal flows. Since duodenal sampling is a commonly used method to assess rumen lipid metabolism (Fievez *et al.,* 2007) it would be preferred to compare OST with duodenal flows. In the present study a comparison is tried to be made with rumen samples. Doreau and Gachon (2004) determined FA kinetics in the rumen by infusing a pulse dose of a linseed product in the rumen and FA were analyzed in rumen fluid and total contents. Differences between treatments found with this method were comparable to results found by duodenal sampling. Lourenço et al. (2005) determined biohydrogenation rate by using C18:3n3 intake and pool sizes of C18:3n3 in the rumen. This approach was reported to generate reliable biohydrogenation values. Fievez et al. (2007) reported the limitations of both techniques as fasting might disturb substrate pool size and a pulse dose might result in a UFA overload on rumen biohydrogenation microbes and cause a change in biohydrogenation routes.

The intention of the rumen sampling in present study was to calculate biohydrogenation rate. Therefore cows were fasted from 12.00h to 22.00h during the rumen sampling, because a continuous lipid intake hampers biohydrogenation calculation (Fievez et al., 2007). However, the pool of C18:3n3 present in the rumen at the first sampling time was unknown. Only variations of FA concentrations from 5h till 15h after feeding were known. With this data we tried to calculate biohodrogenation rate. However, the calculated biohydrogenation rates were unlikely to be accurate. Therefore these results were not included in this study.

The C18:3n3 concentrations in the rumen of the different treatments which are shown in figure 3.1 show comparable differences between the treatments as are found in the omasal flows. EL treatment showed the highest C18:3n3 concentration. Probably this is again caused by the whole linseeds which were also found in the rumen material for the EL treatment. The unexpected low C18:3n3 flow in the omasum for the SLO treatment shows also in the rumen where a low concentration of C18:3n3 for the SLO treatment is measured. This could support the idea that C18:3n3 has a very short retention time in

the rumen and a large part of the C18:3n3 is already flowed out the rumen, because it behaves differently than the other treatments. This can also be seen by the steeper slope (figure 3.2) for the SLO treatment compared to the other treatments, which indicates a more rapid disappearance of C18:3n3 out of the rumen. Although the slope of CL is also quite steep this is expected to be a result of high biohydrogenation rates in stead of a higher outflow compared to the other treatments. Unfortunately rumen sampling started 5h after feeding and no data is available directly after feeding. The very low concentration of 18:3n3 in the rumen in the MALO diet is in accordance with the low omasal flow of C18:3n3. This supports the idea that addition of DHA does not inhibit the biohydrogenation of the C18:3n3 itself (Boeckaert et al., 2008).

4.7 Recommendations

The results of the study show that OST provides a promising sampling method to determine differences in FA outflow from the rumen. However more research is necessary to further support these findings and to be able to quantify FA flows in the omasum. For further research the following recommendations can be made.

To evaluate the OST procedure as an accurate sampling method to determine biohydrogenation comparison with data gathered in same experiment is preferred. Duodenal sampling and rumen sampling can both be used in determining biohydrogenation. To evaluate OST comparison with duodenal sampling is preferred rather then comparing with rumen samples, as it is hard to determine accurate biohydrogenation from ruminal sampling.

In this study four different treatments of linseed with the same amount of C18:3n3 were tested. In a subsequent study it could be interesting to include two different levels of C18:3n3 into the experiment to test the effect of different amounts of C18:3n3 intake on omasal C18:3n3 flows.

To compare nutrient omasal flow with data from the same experiment it could be useful to analyze feaces on OM and NDF, so whole tract digestibilities of OM and NDF can be compared with ruminal digstibilities.

When quantifying omasal FA flows, a more accurate method is needed for extraction and methylation when preparing samples for FAME analysis. The method used in this study probably caused an underestimation of FA flows in the omasum.

When investigating biohydrogenation for products with different technological or chemical treatments it can be useful to analyze FA at different time points after feeding, so differences in behavior in digesta can be determined.

5. Conclusion

Clear differences were observed in FA flows in the omasum when using OST as sampling procedure. The MALO treatment showed an accumulation of *trans* C18:1 intermediates in the omasum caused by the addition of DHA in this diet. Protection against biohydrogenation of C18:3n3 was not visible in the omasum for the SLO diet. This is expected to be caused by the different behavior of the SLO in digesta.

Quantification of the different FA flows in the omasum was not completely accurate as only 70% of FA intake recovered in the omasum. This was more likely the result of an inadequate method of extraction or methylation when preparing samples for FAME analyses than an error caused by OST. However, this can not be concluded from this study. The underestimation of the FA flows in the omasum resulted in an over prediction of biohydrogenation of C18:3n3 and transfer efficiencies to the milk.

The omasal FA flows determined in this study could not be evaluated by comparison with rumen biohydrogenation, as rumen sampling in this study was inadequate to determine rumen biohydrogenation.

OST provides a promising sampling method to determine differences in FA outflows from the rumen when feeding different linseed sources. Therefore, OST can provide a good alternative for duodenal sampling as it requires less surgical intervention and it avoids contamination from abomasal secretions. However, quantification of FA flows in the omasum with OST needs further evaluation.

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Appendix

Primer dose

De primer dosis wordt aan het begin van de meetperiode verstrekt in ongeveer een liter oplossing. De dosis is anderhalf keer de dagelijkse infusie dosis.

Voor Ytterbium geldt: Primer dose Ytterbium 3.6 gram
Molmassa Ytterbium 173.04
Molmassa Ytterbiumacetaat 350.17

3.6 x (350.17 / 173.04) = **7.285 gram ytterbiumacetaat** moet dus worden opgelost in 1 liter water voor de primer dose.

Voor Chroom geldt: Primer dose Chroom 4,5 gram
Molmassa Chroom 52.00
Molmassa Chroom(lll)chloride hexahydraat 266.45
Molmassa Titriplex lll dihydraat 372.24
Verhouding Chroomchloride:Titriplex = 42.6 : 66.4

4.5 x (266.45 / 52.00) = **23.058 gram Chroomchloride**
23.058 x (66.4 / 42.6) = **35.940 gr Titriplex III**
Chroom oplossen in 800 ml water daarna Titriplex + 200 ml water toevoegen.
Oplossing 2 uur in de koch bij 100 °C afkoelen. Voeg toe 6.09 ml Calciumchloride 1 M en met NaOH op pH 6.5 brengen.

Ytterbium-acetaat infuus

Dosering 2.4 gram Ytterbium per dag.
Twee liter infusievloeistof per dag per koe.
4 koeien 4 perioden en 7 dagen per periode geeft 4x4x7x2 = 224 liter oplossing.

In 2 liter oplossing gaat 2.4 gram x (350.17 / 173.04) = 4.86 gram Ytterbiumacetaat.
Dat geeft 4.86/2 = **2.43 gram Ytterbiumacetaat per liter**

Chroom-EDTA infuus

Dosering 3.0 g chroom per dag.
Twee liter infusievloeistof per dag per koe.
4 koeien, 4 perioden, 7 dagen per periode 4x4x7x2 = 224 liter oplossing.

In 2 liter 3 gram x (266.45 / 52.00) = 15.37 gram Chroomchloride hexahydraat.
Dat geeft **7.69 gram Chroomchloride hexahydraat per liter**
en (66.4 / 42.6) x 7.69 = **11.98 gram titriplex III**
Chroom oplossen in 800 ml water daarna Titriplex + 200 ml water toevoegen.
Oplossing 2 uur in de koch bij 100 °C afkoelen. Voeg toe 2.16 ml Calciumchloride 1 M en met NaOH op pH 6.5 brengen.