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Production of milk with a high content of polyunsaturated fatty acids. 1. Experiments in relation to the efficiency of production

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Summary

A high-yielding dairy cow fitted with a re-entrant cannula at the beginning of the small intestine was fed with rations containing sunflower seeds (1), protected sunflower seeds (2) or defatted sunflower seeds and an infusion of sunflower oil in the small intestine (3). Three other lactating cows were also fed with rations containing protected sunflower seeds. Protection was made against biohydrogenation of polyunsaturated fatty acids in the rumen.

Protection had no influence on the overall apparent digestion of the ration, but caused a shift of the digestive process from the stomachs towards the intestines. Infusing sunflower oil in the small intestine reduced the apparent digestion of the lipid fraction and of the other dietary ingredients.

Feeding the protected supplement had no influence on milk yield or on milk fat production. The fatty acid composition of the milk fat changed markedly after feeding the protected supplement and after infusing sunflower oil. Protection of the sunflower seeds against biohydrogenation in the rumen increased the linoleic acid ($C_{18:2}$) content of the milk fat from 3.8 to 11.0 %, but decreased the palmitic acid ($C_{16:0}$) and oleic acid ($C_{18:1}$) content. Infusing sunflower oil increased the linoleic acid content of the milk fat further to 21.4 %, but decreased the stearic ($C_{18:0}$) and oleic acid content.

Feeding the protected supplement had only a minor influence on the linoleic acid content of the body fat taken from the right flank.

It was estimated that after feeding the unprotected supplement only 11 % of the dietary linoleic acid was incorporated in the milk fat. Feeding the protected supplement increased this value to 23 % in low-yielding, and to 48 % in high-yielding cows. After infusing sunflower oil 35 % of the linoleic acid infused was incorporated in the milk fat. It was also estimated that less than 7 % of the dietary linoleic acid was incorporated in the body fat of a low-yielding cow after protection of the linoleic acid against biohydrogenation in the rumen.

1 Introduction

From a technological and nutritional point of view it would be desirable to control the fatty acid composition of animal fats as found in meat and milk. This is particularly true of polyunsaturated fatty acids such as linoleic acid. High contents of polyunsaturated fatty acids are found mainly in vegetable oils and fats, but not usually in animal fats. Two possible ways exist to increase the polyunsaturated fatty acid content of animal products, viz replacing animal fats by vegetable oils or increasing the polyunsaturated fatty acid content of animal fats. As far as monogastric animals are concerned, increase of the polyunsaturated fatty acid content of the fat seems possible by incorporating vegetable oils in the ration, such as maize oil, soya oil, sunflower oil, safflower oil. This measure is not without problems, as it softens animal fat and makes it more susceptible to oxidation. However, a large part of the animal fats consumed in human nutrition originate from ruminants, e.g. milk, beef, mutton, lamb, etc. The incorporation of vegetable oils in the ration of these animals scarcely results in any increase of the polyunsaturated fatty acid content of milk or meat, because unsaturated fatty acids are biohydrogenated in the rumen due to the action of rumen microbes (1).

Not only are unsaturated fatty acids attacked by micro-organisms in the rumen, but also other dietary ingredients such as proteins and carbohydrates. The microbial fermentation in the rumen usually causes losses, and to control these losses research has been going on for several years to find ways to protect dietary ingredients against microbial attack in the rumen. So far, the best results have been achieved with protective protein. It has been possible, however, to cover other dietary ingredients such as fats with a layer of protein and, by protecting the protein against microbial attack, the enveloped fat will also be protected (2). The method of protecting protein is based on a treatment with formaldehyde (3). Formaldehyde forms chemical bonds with protein which are resistant at a neutral pH such as is usually found in the rumen. The drop in pH which takes place in the abomasum weakens the chemical bond between formaldehyde and protein and therefore the protein is no longer resistant to the action of proteolytic enzymes in the digestive tract, such as pepsin and trypsin. Due to the destruction of the protective protein layer the enveloped fat also becomes available for digestion and absorption.

The practical possibilities of such a technique are highly dependent on the biological efficiency of the transfer of polyunsaturated fatty acids from vegetable oils to animal products such as milk fat. A second criterion is the technological quality of the milk and dairy products produced from it. The first aspect was studied at the Institute for Animal Feeding and Nutrition

Table 1. Composition of the daily rations.

Experiment	Animal No	Meadow hay (kg)	Concentrates (kg)	Sunflower oil (kg)
I	93	8	10 (A)	
II	93	8	10 (B)	
II	53	8	10 (B)	
II	30	7	5 (B)	
II	37	7	5 (B)	
III	93	8	9 (C)	1

Research 'Hoorn', and this report deals with the results. The second aspect was studied at the Netherlands Institute for Dairy Research and the results of this study are reported in a subsequent paper (4).

2 Materials and methods

2.1 Experimental animals and rations

The experiments were performed with four lactating cows. Two animals produced between 5 and 10 kg, and the other animals between 24 and 28 kg of milk per day. One of the latter (animal No 93) was fitted with a rumen fistula and a re-entrant cannula at the beginning of the small intestine.

All animals were fed rations consisting of long meadow hay and concentrates. The composition of the rations is shown in Table 1. Supplement A contained 18 % sunflower seeds + 2 % casein; supplement B contained 18 % sunflower seeds + 2 % casein, protected with formaldehyde (Alta Lipids Pty, Ltd, Sydney, Australia), supplement C contained 10 % defatted sunflower seeds. In addition to supplement C sunflower oil was infused continuously into the small intestine. Concentrates containing sunflower seeds (exp. I and Exp. II) and the sunflower oil (Exp. III) were stabilized against oxidation with 30 and 150 mg ethoxyquin per kg, respectively.

2.2 Experimental design

All experiments consisted of an adaptation period of three weeks and an experimental period of one week. In Experiment I the cannulated animal was fed with the unprotected supplement (A). In the fourth week of the experiment milk production was measured and sampled for two consecutive days. Duodenal flow and faecal production were also measured and sampled continuously for 96 h. In Experiment II all animals were fed with the pro-

tected supplement (B). The cannulated animal was treated as in Experiment I. Just before the start of the experiment and in the fourth week, milk production of the other animals was measured and sampled for two consecutive days. Also, just before and in the fifth week after the beginning of Experiment II, body fat samples were taken from the right flank of two animals by the biopsy technique. In Experiment III the cannulated animal was fed with a supplement containing defatted sunflower seeds. Simultaneously sunflower oil was continuously infused into the small intestine at a rate of approx. 40 g/h. In the fourth week of Experiment III milk production was measured and sampled for two consecutive days. Faecal production was also sampled for four consecutive days.

2.3 Sampling and analysis

Measuring and sampling of the intestinal flow was performed according to a method described earlier (5). Samples of concentrates, duodenal content and faeces were freeze-dried and analysed for dry matter, organic matter, energy, ether extract, total lipids and fatty acid composition.

Milk was stabilised with 10 mg BHA (butylated hydroxy anisole) per litre and cooled to below 4 °C immediately after milking.

Total lipids were determined in the duodenal digesta, faeces and samples of body fat according to the method of Folch et al. (6). A further clean-up of the lipid fraction from duodenal digesta and faeces was carried out by column chromatography (cellulose powder) according to Rhodes & Lea (7). Total lipids in milk were isolated according to the corresponding IDF standards (1969). Fatty acid composition of lipid fractions was determined by gas chromatography of the methyl esters using glass capillary columns (8).

3 Results

3.1 Influence of the supplement of protected fat on the digestion of other dietary ingredients

Protection of certain dietary constituents against microbial attack in the rumen could possibly influence the digestive process. This was studied with the cannulated animal (cow No 93) and the results are shown in Table 2.

The results of Experiments I and II indicate that the protection of vegetable oils enveloped by protein has no negative influence on the apparent digestion of the other components of the ration. There seems to be a shift of the digestion from the stomachs towards the intestines, which is thought to be the result of the protection.

Infusing the oil in the small intestine (Experiment III), however, seems to have a negative influence on the apparent digestion.

Table 2. Digestion of the rations.[1]

	DM[2] (kg/day)			OM[2] (kg/day)			Energy (MJ/day)		
	I	II	III	I	II	III	I	II	III
Intake	15.4	15.4	15.6	14.2	14.2	14.6	292.6	290.3	308.6
Duodenal flow (% of intake)	72.2	76.9	—	61.6	67.8	—	74.3	81.9	—
Apparently digested (% of intake)	69.1	73.5	67.2	71.6	75.3	68.9	67.3	71.8	63.0
Disappeared from the stomachs (% of app. dig.)	40.3	31.4	—	53.6	42.7	—	38.1	25.2	—

[1] Duodenal flow and faecal production were corrected for 100 % recovery of chromic oxide as an indigestible marker.
[2] DM = dry matter; OM = organic matter.

3.2 The digestion of the lipid fraction of the ration

In Table 3 a survey is given of the digestion of the ether extract, the total fatty acids and linoleic acid, the main representative of the polyunsaturated fatty acids of the rations as found in the cannulated animal.

Protection seemed to have a small negative influence on the apparent digestion of the lipid fraction of the ration. Infusing the oil also had a negative influence on the apparent digestion of the ether extract, but this may also have been the result of an increased flow of lipids in the small intestine.

The protection had a marked effect on the fatty acid composition of the lipids in duodenal content. After feeding the unprotected supplement the distribution of the C_{18} acids between stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic

Table 3. Digestion of the lipid fraction of the ration.

	Ether extract (g/day)			Total fatty acids (g/day)			Linoleic acid (g/day)		
	I	II	III	I	II	III	I	II	III
Intake	1006	958	1479	583	542	899	297	248	500
Duodenal flow (% of intake)	183.6	174.8	—	107.7	139.5	—	11.4	57.3	—
Apparently digested (% of intake)	85.0	82.1	78.8	88.7	85.8	84.3	99.7	99.2	98.6
Disappeared from the stomachs (% of app. dig.)	—98.4	—91.3	—	—8.7	—46.0	—	88.5	42.3	—

Table 4. Milk production and fat content of the milk.

Animal	Experiment I		Experiment II		Experiment III	
	milk yield (kg/day)	fat (%)	milk yield (kg/day)	fat (%)	milk yield (kg/day)	fat (%)
93	23.80	3.60	25.30	3.58	18.56	4.43
53	27.80	5.09	27.30	4.55		
30	5.80	4.45	6.12	4.40		
37	8.75	4.30	8.00	4.12		

acid ($C_{18:2}$) was 69, 24 and 7 % respectively. Feeding the protected supplement changed these percentages to 56, 19 and 25 respectively.

After infusing sunflower oil the fatty acid composition of the lipids in faeces changed markedly. In the previous two experiments (I and II) stearic acid accounted for about 64 % of the total acids, and oleic acid for about 10 %. After the infusion these percentages were 20 and 52, respectively.

3.3 The transfer of linoleic acid from the diet to the milk

In Table 4 the results are given of milk production and milk fat content of the cannulated cow (No 93), in Experiments I, II and III, and of the other animals just before and during Experiment II. Feeding the supplement had no significant effect on milk production or fat content of the milk.

After infusing sunflower oil into the small intestine the fat content of the milk was increased by nearly 25 %, but because of a drop in milk production of more than 35 % the total milk fat production was slightly reduced.

In Table 5 the estimated overall transfer of linoleic acid from the diet to the milk is shown in high-yielding animals as well as in low-yielding cows. In this table the possible contribution of linoleic acid present in meadow hay was not taken into account. It is known that grassland products contain be-

Table 5. The transfer of linoleic acid from the diet to milk.

Animal(s)	Intake (g/day)	In milk (g/day)	Transfer (%)
93	297	32.8	11
93	248	95.6	38
53	248	145.3	59
30 + 37	124	28.6	23
93	500	175.9	35

tween 15 and 20 % linoleic acid in their lipid fraction (9, 10, 11), but the estimated contribution of fat from the roughage part of the rations fed was only of minor importance, as the fat content (ether extract) was only approx. 2 %. Moreover the linoleic acid in the meadow hay was not protected against biohydrogenation in the rumen, and the transfer of linoleic acid from the hay to the milk is therefore suggested to be no more than 5 %.

It was estimated that in the experiments reported here the overall transfer of linoleic acid from duodenal digesta to milk fat was 95, 67 and 40 % in Experiments I, II and III, respectively.

3.4 The transfer of linoleic acid from the diet to body fat

One of the reasons for the low efficiency of the transfer of linoleic acid from the diet to the milk fat could be an appreciable incorporation of linoleic acid into the body fat. This might also explain the difference between the high-yielding and the low-yielding cow, if it is taken into account that the high-yielding animal had a negative energy balance, whereas the low-yielding animals were in a positive energy balance. For that reason body fat samples were taken by biopsy and the isolated body fat was analysed for the fatty acid composition. The results (given in Table 6) are not very conclusive. Only minor differences were found in the fatty acid composition between the

Table 6. Fatty acid composition of the depot fat.

Fatty acids	Low-producing cows		High-producing cows	
	control	after feeding protected fat	control	after feeding protected fat
14:0	3.3	3.1	4.7	4.5
15:br	0.2	0.2	0.4	0.3
14:1	2.3	2.4	2.2	1.7
15:0	0.4	0.4	0.6	0.5
16:br	0.3	0.3	0.5	0.4
16:0	28.5	28.1	29.6	29.4
16:1	8.8	9.0	5.3	5.3
17:0	1.5	1.3	1.6	2.0
18:br	0.9	1.0	0.7	1.0
17:1	1.3	1.3	1.0	1.0
18:0	8.4	7.9	10.4	11.6
18:1	40.8	41.3	37.7	38.1
19:0	1.2	1.0	1.2	1.0
?	0.2	0.4	0.5	0.5
18:2	0.8	1.1	1.0	1.0
20:0	0.3	0.4	1.1	0.5
18:3	0.8	0.8	1.2	1.0
?	—	—	0.2	0.2

different samples. In all cases the proportion of oleic acid is very high. There seems to be a small increase in the linoleic acid content of the body fat of the low-yielding animal after feeding the protected supplement, but not in the body fat of the high-yielding cow.

3.5 The influence of the diet on the fatty acid composition of the milk fat

In Table 7 the fatty acid composition of the milk fat produced after feeding the different rations to low- and high-yielding cows is shown. In low-yielding cows feeding the *protected* supplement resulted in increased percentages of the C_{18} acids, particularly linoleic acid ($C_{18:2}$). These increases were mainly at the expense of palmitic acid ($C_{16:0}$). Compared with the control ration, feeding the *unprotected* sunflower seeds containing supplement to high-yielding cows also increased the total amount of C_{18} acids slightly. However, only stearic ($C_{18:0}$) and linoleic acid were increased, mainly at the expense of palmitic acid and oleic acid ($C_{18:1}$). Feeding the protected supplement to high-yielding cows increased stearic and linoleic acid further (particularly linoleic acid), but palmitic and oleic acid were further decreased. Infusing sunflower oil strongly increased the linoleic acid content of the milk fat, mainly at the expense of palmitic and oleic acid.

4 Discussion

The results indicate that protection of dietary ingredients does influence the digestive system of a ruminant. This procedure thus seems to make it possible to manipulate the digestive system of the ruminant and to restrict some of the undesirable losses due to microbial fermentation, without reducing the overall apparent digestibility. The results reported here are in good agreement with the results of fat-coating experiments in sheep (12). The negative influence on the apparent digestion of infusing sunflower oil into the small intestine is probably the result of a decreased apparent absorption from the small intestine. This is possibly due to the large amount of apolar substances in the intestines after the infusion, or due to an increased flow rate. The capacity of the intestines to absorb fatty acids seems to be limited. After termination of the experiment the infusion rate was gradually increased and at a rate of 60 g/h the animal showed severe diarrhoea.

The disappearance of linoleic acid, which was almost complete between intake and faeces as well as between small intestine and faeces does not necessarily mean that this acid was absorbed. A transfer is possible due to microbial biohydrogenation of linoleic acid into oleic acid and stearic acid. This is particularly true in the rumen if no protecting measures are taken. It is

Table 7. Fatty acid composition[1] of the milk fat.

Fatty acids	Low-producing cows			High-producing cows		
	control	after feeding protected supplement	control	after feeding unprotected supplement	after feeding protected supplement	after infusing sunflower oil
	0*	II	0	I	IIA, IIB, IIC	III
	(30 + 37)**	(30 + 37)	(53)	(93)	(53 + 93)	(93)
4:0	3.2	3.2	4.4	4.1	4.5	3.3
6:0	2.4	2.1	2.6	2.6	3.0	2.5
8:0	1.4	1.3	1.4	1.6	1.8	1.7
10:0	3.2	2.5	2.3	2.9	3.1	3.1
12:0	4.4	3.8	2.9	4.0	4.2	3.9
14:0	12.4	10.4	8.7	10.9	10.0	10.2
16:0	30.6	21.4	25.6	20.8	18.0	23.3
18:0	6.8	12.1	10.3	12.3	13.9	6.7
18:1	20.2	25.5	29.6	26.9	23.4	16.9
18:2	1.3	9.0	1.7	3.8	11.0	21.4
others	14.1	8.7	10.5	10.1	7.1	7.0

[1] Only major fatty acids are listed.
* The figures on this line refer to the experimental periods.
** The figures on this line refer to the cows' numbers.

believed, however, that the almost complete disappearance of linoleic acid from the intestines is at least partly the result of biohydrogenation in caecum and large intestine.

The efficiency of protection of linoleic acid against biohydrogenation in the rumen after feeding the protected supplement (57 %) was considerably less than was expected. In sheep a protection of 66 % was found (12). The low protection may be the result of the pelleting procedures, since pelleting seems to destroy part of the protection (B. S. James, pers. commun.). Despite this, the efficiency of the transfer of linoleic acid from the diet to the milk was still about 38 %. From the results it is concluded that without protection against microbial biohydrogenation the transfer of linoleic acid from the diet to milk is very low. However, even after protection the transfer remained on average below 40 %, partly due to insufficient protection, but also due to losses between small intestine and milk. These results agree fairly well with earlier findings (13, 14, 15).

The small increase in the linoleic acid content of the body fat of low-yielding cows and the absence of such an increase in high-yielding cows is in agreement with the difference in efficiency of transfer from the diet to milk fat between the high- and low-yielding cows. If it is assumed that the low-yielding animal contained about 100 kg of body fat, the increase in linoleic acid content of 0.3 % during five weeks means that less than 7 % of the dietary linoleic acid was incorporated in the body fat, provided that the depot from which the fat was taken is representative of the entire animal. It is known that body fat in the cow from different depots differs in fatty acid composition (16), but the differences in linoleic acid content were rather small.

Feeding the protected supplement had an effect only on the fatty acid composition and it did not increase milk yield or milk fat content, as is usually found, even with rather high milk fat contents after feeding the control diets (2, 15, 17). The changes in fatty acid composition of milk produced after feeding the protected supplement is in good agreement with earlier findings in that the increased linoleic acid content mainly affected oleic and palmitic acid (13, 15). The difference between infusing sunflower oil and feeding the protected supplement is that the increased linoleic acid content after feeding the infused sunflower oil was only at the expense of oleic acid and not palmitic acid, and this is difficult to explain.

5 Conclusions

It is possible to produce milk and dairy products with a rather high content of polyunsaturated fatty acids by protecting vegetable oils against microbial

biohydrogenation in the rumen.

The efficiency of the transfer of linoleic acid ($C_{18:2}$) from the diet to the milk is fairly low, not only as a result of biohydrogenation in the rumen, but also due to the conversion and/or breakdown of linoleic acid absorption from the small intestine.

If polyunsaturated milk has to be produced it seems advantageous to use high-yielding cows.

The economic potential of the production of polyunsaturated milk does not seem to be very promising because of the high cost of the supplement and the low efficiency of the transfer from the diet to the milk.

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Samenvatting

S. Tamminga, A. Steg-Beers, W. van Hoven & H. T. Badings, *Produktie van melk met een hoog gehalte aan meervoudig onverzadigde vetzuren. 1. Proefnemingen met betrekking tot de efficiency van de produktie*

Twee koeien in een vroeg laktatiestadium (produktie 25 tot 30 kg melk/dag) en twee koeien in een laat laktatiestadium (produktie 5 tot 10 kg melk/dag) werden gevoerd met een rantsoen waarvan het krachtvoer zonnebloemzaad bevatte dat beschermd was tegen microbiële verzadiging van de onverzadigde vetzuren in de pens. Het doel hiervan was melk te produceren met een hoog gehalte aan meervoudig onverzadigde vetzuren. De bescherming was verkregen door het omhullen van de in de zaden aanwezige olie met een laagje eiwit dat vervolgens was behandeld met formaldehyde. Een van de nieuwmelkse dieren, dat voorzien was van een zg. re-entrant fistel in het begin van de dunne darm, werd bovendien gevoerd met een rantsoen waarvan het krachtvoer ongecoat zonnebloemzaad bevatte en met een rantsoen waarvan het krachtvoer geëxtraheerd zonnebloemzaad bevatte. In het laatste geval werd zonnebloemolie door middel van een continu infuus in het begin van de dunne darm aan het dier toegediend.

Het coaten van het zonnebloemzaad had geen nadelige invloed op de schijnbare verteerbaarheid van de verschillende rantsoenbestanddelen, maar de vertering verplaatste zich enigszins van de magen naar de darm. Het infuseren van zonnebloemolie verlaagde de schijnbare verteerbaarheid van de verschillende rantsoenbestanddelen.

Het opnemen van een hoeveelheid ongecoat zonnebloemzaad in het rantsoen verhoogde het gehalte aan linolzuur ($C_{18:2}$) in het melkvet van gemiddeld 1,5 naar 3,8%. Het voeren van gecoat zonnebloemzaad had een nog groter effect op de vetzuursamenstelling van het melkvet. Het linolzuurgehalte steeg van 3,8 naar 11,0 %, wat gepaard ging met een daling van het gehalte aan palmitinezuur ($C_{16:0}$) van gemiddeld 20,8 naar 18,0 en van een daling van het gehalte aan oliezuur ($C_{18:1}$) van 26,9 naar 23,4 %. Door het infuseren van zonnebloemolie steeg het gehalte aan linolzuur in het melkvet verder tot 21,4 %. Ook het palmitine-zuurgehalte steeg en wel van 20,8 naar 23,3 %. De ge-

halten aan stearinezuur ($C_{18:0}$) en oliezuur vertoonden een daling na het infuseren van zonnebloemolie van respectievelijk van 12,3 naar 6,7 % en van 26,9 naar 16,9 %.

Het beschermen van onverzadigde vetzuren tegen microbiële verzadiging in de pens verhoogde de hoeveelheid linolzuur die het begin van de dunne darm bereikte van 11 % van de opgenomen hoeveelheid na het voeren van het ongecoate produkt tot 57 % na het voeren van het gecoate produkt. De efficiëntie waarmee linolzuur uit het ongecoate zonnebloemzaad werd ingebouwd in het melkvet was laag en bedroeg slechts 11 % van de opgenomen hoeveelheid. Het coaten van zonnebloemzaad verhoogde dit tot 23 % bij oudmelkse koeien en tot gemiddeld 48 % bij nieuwmelkse koeien. De efficiëntie waarmee linolzuur uit geïnfuseerde zonnebloemolie werd ingebouwd in het melkvet, bedroeg 35 %. De lage overdrachtspercentages waren het gevolg van zowel een onvoldoende bescherming tegen verzadiging in de pens, als van de omzetting en/of afbraak van linolzuur nadat het de dunne darm had bereikt.

Het voeren van gecoat zonnebloemzaad had weinig invloed op de vetzuursamenstelling van het lichaamsvet in melkkoeien, dat werd verkregen met behulp van vetbiopsie uit het onderhuids vetdepot in de rechterflank. Uit de lichte stijging van het linolzuurgehalte in het depotvet van een laag producerende koe werd geschat dat minder dan 7 % van het linolzuur uit het gecoate rantsoen werd ingebouwd in lichaamsvet.

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