Acta vet. scand. 1973, *14*, 673—682.

From the Department of Animal Husbandry and Genetics, Veterinary College of Norway, Oslo, and the Department of Physiology and Biochemistry, National Research Institute on Animal Husbandry, Copenhagen, Denmark.

MARINE FAT FED TO YOUNG CALVES

II. EXPERIMENTS WITH RE-ENTRANT DUODENAL FISTULATED CALVES

By

Knut Flatlandsmo

FLATLANDSMO, KNUT: *Marine fat fed to young calves. II. Experiments with re-entrant duodenal fistulated calves.* Acta vet. scand. 1973, *14*, 673—682. — In two calves fed milk replacers containing hydrogenated marine fat, collection of the duodenal chyme was carried out over a 24 hr. period, when the calves were about one and a half weeks old. The fat was partly broken down to free fatty acids (F.F.A.) before entering the duodenum. Hydrolysis was probably due to pregastric esterase. No hydrogenation of the unsaturated fatty acids was observed.

marine fat; re-entrant duodenal fistulae.

The utilization of marine fats has been studied in dairy cows, pigs (*Sundstöl* 1970 a, b) and hens (*Laksesvela* 1966, *Herstad* 1970). Results from these studies are, however, not directly applicable for the evaluation of milk replacers containing marine fats fed to young calves. A study of the digestibility and utilization of the fatty acids in marine fats was therefore undertaken with young calves. Some results from this study have already been published (*Flatlandsmo* 1972).

The present paper describes an experiment carried out with two calves in which re-entrant duodenal fistulae were inserted. The aim of the investigation was to study whether marine fat was broken down in the forestomachs when given as a component of a milk replacer.

MATERIALS AND METHODS

The animals were two Jersey cross calves housed in boxes with peat bedding. Within the first week of life a re-entrant duodenal cannula was inserted into each. Postoperatively, the calves were given an isotonic salt and glucose solution by s.c. infusion in addition to small amounts of a milk replacer given orally.

The two calves were fed milk replacers which were identical except for the marine fat component. The milk replacer fed to calf No. 764 contained marine fat melting at 31—33°C (termed 31—33), while that fed to calf No. 661 contained a more hydrogenated marine fat melting at 38—40°C (termed 38—40). A more detailed description of the milk replacer in which the respective fats were incorporated is published elsewhere (*Flatlandsmo* 1973).

After a recovery period of four days a balance experiment was performed over a period of three days. The calves were fed at 09_{30}, 13_{15} and 18_{20} hours with equal amounts of the respective milk replacers. Chyme from the duodenum was obtained through the proximal cannulae and collected in Erlenmeyer flasks which were kept at 80—85°C (in a hot water bath) in order to inactivate lipolytic enzymes. After homogenization, a sample of 4—4.5 ml was removed for determination of lipid content. The rest of the chyme was returned to the intestine via the adjacent cannulae. A total collection of the chyme that passed the duodenum during a 24 hr. period was undertaken the second day of the balance experiment. About 30 samples were collected from each calf over the 24 hr. collection period.

The intervals between the taking of each sample varied because of variations in the duodenal flow. This decreased when the calves were lying down, and increased greatly when they were standing.

The digestive system of the calves appeared to function normally throughout the collection period, the total digestibility of the fatty acids being high. Calf No. 764 died, however, the day after termination of the test. Post-mortem examination revealed abomasal/intestinal inflammation, and this was considered as being the most probable cause of death. Calf No. 661 died two days later, and the abomasum was found to contain more than 5 l of liquid, mixed with small particles of peat. No sign of intestinal inflammation was observed.

Table 1. Lipid and chyme flow in the re-entrant fistulated calves.

Collection period	Calf No. 764				Calf No. 661			
	lipid in chyme		chyme		lipid in chyme		chyme	
	g/hour	percentage of total	g/hour	percentage of total	g/hour	percentage of total	g/hour	percentage of total
09_{30}—13_{00}	1.57	15	347	22	1.09	7	315	20
13_{01}—18_{20}	1.77	22	444	36	3.39	30	409	33
18_{21}—21_{30}	2.50	27	322	24	2.88	23	361	26
21_{31}—09_{30}	1.09	36	82	18	1.69	40	99	21
Total		100		100		100		100

All samples were analysed with regard to total lipid content, while pH was determined in only 23 samples. Quantitative analysis of fatty acids was performed on seven samples. In two samples from each calf, lipid fractions were separated by thin layer chromatography before gas-chromatographic analyses of fatty acids. The analytical methods are discussed in another publication (*Flatlandsmo* 1973).

RESULTS

During the collection period calf No. 764 received a total of 71.3 g of 31—33, and calf No. 661 received 70.9 g of 38—40. During the same period, only 36.6 g were found to pass the duodenum in the case of calf No. 764, the corresponding figure for calf No. 661 being 50.9 g.

The total amount of chyme collected from the two calves was 5.485 and 5.578 g respectively, and was over 60 % more than the amount of feed ingested. A detailed description of the lipid and chyme flow during the test is given in Table 1. The chyme flow between 9_{30}—13_{00} represents the first feeding, and that between 13_{01}—18_{20} the second feeding. In both calves, the lipids passed at a slower rate than the total chyme during the first 9—12 hrs., while the reverse was observed later on.

Table 2 shows that the percentage of fatty acids in total chyme lipids varied considerably. There was a general increase in the fatty acid content of chyme at the end of the collection period. In calf No. 764 the abomasum was not empty at the start

Table 2. Fatty acid content and distribution in chyme from the re-entrant fistulated calves at different times of collection.

Calf No.		764							661						
Time of collection		09_{15}	10_{08}	11_{00}	14_{30}	19_{10}	00_{05}	07_{55}	09_{20}	09_{55}	13_{05}	14_{15}	16_{30}	19_{25}	04_{55}
Total flow (g)	Chyme	4.1	134.8	88.4	162.0	232.5	87.3	188.2	3.9	136.4	181.1	147.0	218.0	176.3	233.1
	Lipid	0.017	1.186	0.192	0.466	1.325	1.432	2.599	0.0105	0.391	0.650	0.775	1.559	2.555	6.207
Fatty acids (%)	Chyme	0.26	0.58	0.12	0.17	0.40	1.16	1.19	0.16	0.17	0.24	0.30	0.42	0.96	1.12
	Lipid	63	67	55	60	70	71	86	59	60	67	56	59	66	42
C14:0		5.8	5.6	6.1	6.3	5.9	5.6	6.4	6.0	7.9	6.0	7.1	3.9	7.0	5.9
C14:1		0.4	0.2	0.2	0.5	0.2	0.3	0.3	—	0.3	0.4	0.2	0.2	0.4	0.4
C16:0		20.5	16.7	17.8	14.5	15.0	13.9	14.8	21.0	19.7	21.0	19.4	20.2	17.3	16.6
C16:1		7.7	6.9	7.7	7.7	9.0	9.2	9.2	6.2	6.9	6.9	7.2	7.4	7.2	6.8
C18:0		6.8	6.3	6.5	5.5	4.2	4.2	5.8	9.0	8.7	8.4	9.2	8.4	7.6	7.9
C18:1		14.1	11.4	14.0	13.1	12.4	11.9	14.4	13.7	13.7	12.7	14.5	13.8	12.3	12.1
C18:2		2.2	1.1	2.9	2.1	1.4	1.5	0.8	4.0	4.2	8.8	4.8	3.1	2.9	2.9
C20:0		3.2	3.3	4.7	3.6	2.6	3.0	1.5	6.8	5.6	6.8	6.5	6.8	5.8	5.9
C18:3/20:1		15.3	18.2	17.3	17.5	19.0	18.7	17.6	13.3	14.0	10.4	13.9	14.7	13.8	13.7
C22:0		2.8	3.4	1.8	3.4	3.1	3.3	2.7	3.7	3.3	4.1	3.9	4.9	5.3	5.8
C22:1		18.5	20.8	15.6	19.8	21.5	21.6	21.4	10.8	11.2	10.3	9.3	11.3	13.5	13.9
Cx		2.7	6.1	5.4	6.0	5.7	6.8	5.1	5.5	4.5	4.2	4.0	5.3	6.9	8.1

Table 3. Lipid composition in chyme at two stages of collection from the calf fed the lesser hydrogenated fat (31—33).

Component	Cholesterol esters		Triglycerides		F.F.A.		Diglycerides/ cholesterol		Mono- glycerides		Phospho- lipides	
Time of collection	10_{08}	07_{55}	10_{08}	07_{55}	10_{08}	07_{55}	10_{08}	07_{55}	10_{08}	07_{55}	10_{08}	07_{55}
Fatty acids (% of total)	0	6	31	38	68	41	1	13	0	1	0	1
C14:0		11.8	6.8	6.6	5.1	10.3	3.7	6.8		1.3		
C16:0		10.5	16.0	15.4	21.1	21.9	65.4	18.1		29.4		
C16:1		5.8	10.1	9.8	4.9	8.8		8.4		0.1		
C18:0		19.2	4.8	3.8	10.2	7.6		5.9		39.4		27.7
C18:1		7.0	13.4	12.8	10.8	12.9		12.7		22.7		38.8
C18:2		—	—	—	—	—		—		—		—
C20:0		2.4	4.0	3.2	3.7	2.4		4.4				21.7
C20:1/18:3		10.0	20.8	20.6	16.0	14.1		19.1				
C22:0		2.7	2.8	2.9	2.8	2.3		3.8				
C22:1		4.6	20.3	22.5	16.9	12.9		18.9				
Cx		26.0	1.0	2.4	8.5	6.8		1.9				

of the experiment. This may explain the high percentage of fatty acids in the first two chyme samples. No marked difference was observed as regards the distribution of fatty acids in chyme as compared to that in the ingested fat.

Tables 3 and 4 show the results of more detailed analyses of lipids collected at two stages. The samples at 10_{08} and 10_{50} were the first and fourth after the first feeding. The two other samples were analysed to show the lipid composition and fatty acid ratio at a later stage. In both calves the triglycerides were partially hydrolysed before entering the duodenum.

The fatty acid ratio of the triglycerides found in the chyme from calf No. 764 (Table 3) differed only slightly from that in the corresponding marine fat. A similar ratio was seen in the diglycerides-cholesterol. In the F.F.A. fraction an increased proportion of the longer chained fatty acids was observed.

Neither did the fatty acid ratio of the triglycerides in the chyme from calf No. 661 (Table 4) show any marked difference from the corresponding marine fat. In the diglycerides-cholesterol, there was an increased proportion of unsaturated acids. The two fractions showing the fatty acid ratio of F.F.A. differed markedly.

Table 4. Lipid composition in chyme at two stages of collection from the calf fed the more hydrogenated fat (38—40).

Component	Cholesterol esters		Triglycerides		F.F.A.		Diglycerides/ cholesterol		Mono- glycerides		Phospho- lipides	
Time of collection	10_{50}	04_{55}	10_{50}	04_{55}	10_{50}	04_{55}	10_{50}	04_{55}	10_{50}	04_{55}	10_{50}	04_{55}
Fatty acids (% of total)	2	3	86	43	11	31	0	20	0	2	1	1
C14:0	11.2	10.2	6.9	7.4	3.4	16.5		6.4		3.9	8.6	
C16:0	12.0	11.4	23.2	21.0	34.6	27.5		19.3		24.6	43.4	59.6
C16:1	3.1	4.9	6.5	7.5	1.1	10.9		6.0		—	1.3	
C18:0	8.8	6.0	9.2	7.5	19.4	7.5		12.1		24.3	20.3	37.5
C18:1	6.3	5.9	10.9	12.4	4.6	14.5		12.0		3.9	13.5	
C18:2	—	—	—	—	—	1.2		—		—		
C20:0	8.3	6.2	8.3	7.3	8.6	3.4		10.3		18.1		
C20:1/18:3	9.4	11.2	15.5	16.4	7.9	9.5		12.5		4.4		
C22:0	9.0	3.2	5.9	5.8	7.4	2.3		8.4		13.2		
C22:1	10.2	4.9	13.6	14.6	6.3	1.5		10.9		3.3		
Cx	21.7	36.1		0.1	6.7	5.2		2.1		4.3		

Compared to the ingested fat, the sample collected at 10_{50} showed an increased proportion of saturated acids. In the sample from 04_{55}, there were greatly increased proportions of C14:0, C16:1 and C18:1 while the largest decrease was in C18:0.

The total digestibility of the fatty acids was as high as 93 and 94 %. The shortest chained unsaturated acids showed an apparent digestibility of 97—98 %, while the corresponding figure for the long chained fatty acids was only about 80 %.

DISCUSSION

In a review article, *Radostits & Bell* (1970) describe various fat sources used as components in milk replacers fed to calves. The present experiment suggests the possibility that hydrogenated marine fat may be digested as efficiently as other fats by young calves. The digestibility coefficient seems to be normal. Rumen function may not be a necessary prerequisite to achieve normal digestibility (*Flatlandsmo* 1972).

It is not possible to evaluate the exact effect of the heat treatment of the chyme. However, heat treatment may have altered

the intestinal flora in the lower intestines, and may thus have been a contributory factor in the death of the calf with intestinal inflammation.

Only 51 and 72 % of the ingested lipids seemed to pass the duodenum during the collection period. The retarded passage of lipids is probably due to several factors. Firstly, the breaking down of the protein coagulum in the abomasum may considerably influence lipid digestion since the fat is enclosed in it. Secondly, fat stimulates enterogastrone secretion which decreases the motor activity of the stomach. Thirdly, the slow passage of higher fatty acids may be partly due to their low solubility in water. In addition, the re-entrant fistulae may have interfered with the normal passage of the chyme.

These factors may also have contributed to the difference in chyme flow observed between the two calves. As shown in Table 1, the chyme collected during the period 09_{30}—13_{00} from the calf fed 31—33 had a higher lipid content than the chyme collected during the same period from the calf fed 38—40. This difference may have been due to the fact that the abomasum of calf No. 764 contained chyme at the start of the experiment.

The amount of chyme was in both calves about 60 % greater than the ingested volume of the milk replacer. *Porter* (1969) found that the volume of chyme could be double the volume of feed ingested.

While there was a lower concentration of lipids in chyme during the last 12 hrs. of collection than in the two preceding periods (Table 1), the percentage of fatty acids increased (Table 2). This may be partly due to the low chyme flow during the last 12 hrs. The high content of fatty acids in chyme from the last samples (07_{55} and 04_{50}) as compared to that in the samples taken before feeding (09_{15} and 09_{20}), suggests that not all the ingested lipid passed on further from the abomasum.

That there was little variation in fatty acid distribution throughout the collection period (Table 2), suggests that the different fatty acids were liberated at the same rate from the protein coagulum.

As regards the calf fed 31—33, the sample at 10_{08} had a higher proportion of fatty acids in the F.F.A. fraction as compared to the corresponding sample from the other calf. In addition, it showed an increased proportion of the longest chained unsaturated fatty acids. This indicates hydrolysis of the triglyce-

rides. Both these changes had probably occurred in lipids which were in the abomasum at the start of the experiment.

In the calf fed 38—40, little of the triglycerides seemed to have been broken down in the sample collected at 10_{50} hours. The total percentage of fatty acids present in the F.F.A. fraction showed little difference as compared to the ingested fat, but the increase in saturated acids was marked. This increase was probably caused by hydrolysis of the diglycerides-cholesterol, which constituted 11 % of the total of fatty acids in the ingested fat.

In the two samples which showed the composition at a later stage of collection, about 40 % of the fatty acids was present in the triglyceride fraction. In both calves, there was a lower proportion of the longer chained fatty acids in the F.F.A. fraction as compared with the triglyceride fraction. This may indicate a slower passage from the abomasum, which could have been partly due to lower solubility in water. The proportion of F.F.A. in chyme lipids was, however, much higher than that in the ingested fat.

In adult ruminants, lipids enter the duodenum mostly as free fatty acids (*Lennox et al.* 1968). The observations of *Otterby et al.* (1964) suggest that a substantial lipolysis of milk fat takes place in the abomasum of nipple fed calves. The present experiment suggests a similar hydrolysis of hydrogenated marine fat. In contrast to adult ruminants, no hydrogenation of unsaturated fatty acids occurred.

Young calves have a special enzyme, pregastric esterase, which acts upon lipids in the feed. As regards milk fat, this enzyme has high specificity for the glyceride linkage involving butyric acid, while higher fatty acids are less affected (*Otterby et al.*). According to *Ramsey & Young* (1961) pregastric esterase probably consists of at least two different esterases. If these possess different substrate specificity this could in part explain the hydrolysis of higher fatty acids observed in this experiment. Gastric lipase activity is probably low in young calves (*Otterby et al.*).

The pH of the abomasal contents considerably influences the activity of pregastric esterase. While optimum pH seems to be about 6, activity has been observed down to about 3 (*Siewert & Otterby* 1970). In both calves involved in this experiment the pH of chyme decreased to such a level (i.e. 3) between each feed. Values above 3 were observed during 15 of the 24 hrs. the col-

lection lasted. Thus pregastric esterase activity on the triglycerides present in the feed was possible during this period.

ACKNOWLEDGEMENT

The experiments were carried out during the author's stay at the Department of Physiology and Biochemistry, National Research Institute on Animal Husbandry, Copenhagen, and the author wishes to thank P. E. Jakobsen, professor, B.Sc. and head of the department, and P. M. Riis, associate professor, B.Sc., B.V.Sc., Ph.D., D.V.Sc., for their interest in the work.

REFERENCES

Flatlandsmo, K.: Marine Fat. Digestibility of its fatty acids in young calves. Acta vet. scand. 1972, *13*, 260—262.

Flatlandsmo, K.: Marine fat fed to young calves. I. Content and distribution of fatty acids in ingested and excreted fat. Acta vet. scand. 1973, *14*, 666—672.

Herstad, O.: Effekt av feitt-tilskott til broilerfôr. (The effect of additional fat to broiler food). Melding fra Norges landbrukshögskole 1970, *49*, 5.

Laksesvela, B.: Sildefett som kyllingfôr. (Herring fat to chicks). Melding fra Sildeolje og Sildemelsindustriens Forskningsinstitutt 1966, nr. 4, 92—102.

Lennox, A. M., A. K. Lough & G. A. Garton: Observations on the nature and origin of lipids in the small intestine of the sheep. Brit. J. Nutr. 1968, *22*, 237—246.

Otterby, D. E., H. A. Ramsey & G. H. Wise: Lipolysis of milk fat by pregastric esterase in the abomasum of the calf. J. Dairy Sci. 1964, *47*, 993—996.

Porter, J. W. G.: Digestion in the pre-ruminant animal. Proc. Nutr. Soc. 1969, *28*, 115—121.

Radostits, O. M. & J. M. Bell: Nutrition of the pre-ruminant dairy calf with special reference to the digestion and absorption of nutrients: A review. Canad. J. Animal Sci. 1970, *50*, 405—452.

Ramsey, H. A. & I. W. Young: Substrate specificity of pregastric esterase from the calf. J. Dairy Sci. 1961, *44*, 2304—2306.

Siewert, K. L. & D. E. Otterby: Effects of in vivo and in vitro acid environments on activity of pregastric esterase. J. Dairy Sci. 1970, *53*, 571—574.

Sundstöl, F.: Forsök over herdet marint fett (HMF) i kraftfôrblandinger til melkekyr. (Experiments on hydrogenated marine fat (HMF) in formula feeds for dairy cows). Husdyrforsöksmötet, Norges landbrukshögskole 1970 a, 99—104.

Sundstöl, F.: Forsök over herdet marint fett (HMF) i kraftfôrblandinger til slaktesvin. (Experiments on hydrogenated marine fat (HMF) in formula feeds for pigs). Husdyrforsöksmötet, Norges landbrukshögskole 1970 b, 146—149.

SAMMENDRAG

Marint fett til unge kalver.

II. Forsök med kalver med re-entrant duodenalfistel.

Artikkelen omhandler oppsamling av duodenalchyme fra to kalver med re-entrant duodenalfistel. Forsöket varte ett dögn og kalvene var ca. 1½ uke gamle. De ble fòret med en melkeerstatning, hvor hydrogenert marint fett, smeltepunkt 31—33°C og 38—40°C, var fettkilde. Triglycerider ble delvis hydrolysert til frie fettsyrer i löpen. Dette var trolig forårsaket av pregastric esterase. Det skjedde ingen hydrogenering av de umettede fettsyrene.

(Received August 3, 1973).

Reprints may be requested from: Knut Flatlandsmo, the Department of Animal Husbandry and Genetics, Veterinary College of Norway, Postboks 8146, Oslo Dep., Oslo 1, Norway.