Original article

Influence of pH on lipolysis and biohydrogenation of soybean oil by rumen contents in vitro

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Summary — The effect of different pH values on rumen lipolysis and biohydrogenation was investigated during incubations of the rumen contents with 40 or 80 mg of soybean oil as the sole substrate. Mean pH values studied were 6.8, 6.3, 6.0, 5.6 and 5.2. Lipolysis was calculated from the decrease in fatty acids present in triacylglycerols (TAG), as well as from the accumulation of free fatty acids (FFA) during the incubation. At pH ≤ 6.0 lipolysis was low, and the inhibition became greater with decreasing pH. At the same pH value, the inhibition in incubations with 80 mg of soybean oil was more pronounced than with 40 mg. Even at the lowest pH value, after incubation, no free linolenic acid could be detected because of biohydrogenation, whereas linoleic acid hydrogenation was only partially inhibited at pH 5.2. This means that lipolysis is much more sensitive to low pH values than biohydrogenation. Literature data indicate however that, besides pH, other factors must be involved in the decrease of both lipolysis and biohydrogenation in the rumen of animals fed highly concentrated diets.

rumen metabolism / soybean oil / pH / lipolysis / biohydrogenation

Résumé — Influence du pH sur la lipolyse et la biohydrogénation de l'huile de soja dans le contenu de rumen in vitro. *L'effet de différentes valeurs du pH sur la lipolyse et l'hydrogénation a été étudié dans des incubations avec du contenu de rumen auquel on a ajouté 40 mg ou 80 mg d'huile de soja comme seul substrat. Les valeurs de pH étudiées étaient : 6,8 ; 6,3 ; 6,0 ; 5,6 et 5,2. La lipolyse a été calculée non seulement par la détermination de la diminution de la quantité d'acides gras dans les triglycérides, mais aussi par détermination des acides gras libres, accumulés dans l'incubation. Avec les valeurs du pH ≤ 6,0, l'hydrolyse des triglycérides a été inhibée, et l'inhibition est devenue plus importante au fur et à mesure que le pH diminuait. Pour un même pH, l'inhibition a été plus prononcée dans les incubations avec 80 mg d'huile de soja qu'avec 40 mg. Même au pH le plus bas, après l'incubation, l'acide linolénique libre était complètement absent par suite du processus de biohydrogénation, tandis que l'hydrogénation de l'acide linoléique était partiellement inhibée à pH 5,2. Apparemment, la*

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lipolyse est plus sensible à un pH faible que l'hydrogénation des acides gras polyinsaturés libérés. Cependant, il ressort de la littérature qu'en dehors du pH d'autres facteurs jouent un rôle déterminant dans la diminution de la lipolyse et l'hydrogénation dans le rumen des bovins ou des ovins recevant des rations riches en concentrés.

métabolisme ruminal / huile de soja / pH / lipolyse / hydrogénation

INTRODUCTION

Although there are few experimental results, it is accepted that feeding rations with a low roughage content decreases the lipolysis of triacylglycerols (TAG) and biohydrogenation of unsaturated long chain fatty acids in the rumen (Harfoot and Hazlewood, 1988). Changing the diet of cows from a high to a low roughage type decreased the number of lipolytic bacteria in the rumen, while the in vitro lipolytic and biohydrogenating activity was also lowered (Latham et al, 1972). Demeyer et al (1972) found that the administration of sucrose (80 g twice daily) into the rumen of a fistulated sheep resulted in lower amounts of free fatty acids liberated from linseed oil in in vitro incubations with the rumen fluid. A similar observation was made by Gerson et al (1985), who found that the in vitro rates of lipolysis and biohydrogenation were reduced when a portion of fibre in the diet of sheep was replaced by starch. More recently, the inhibitory effect of starch supplementation on lipolysis was confirmed in experiments using the rumen simulation technique (RUSITEC) (Abel et al, 1990).

To ascertain whether these effects are due to the lower pH values in the rumen that result from the feeding of low roughage/high starch diets, we investigated the effect of pH on the lipolysis and hydrogenation of soybean oil during incubations with strained rumen contents. A brief report of some of these results has been published previously (Van Nevel and Demeyer, 1996).

MATERIALS AND METHODS

Animals and rations

Rumen contents (ca 0.5 L) were taken from a rumen cannulated wether (ca 80 kg), just before the morning feeding. The animal received 300 g of commercial concentrate (14.9% of crude protein; 4.0% of crude fibre) and 300 g of meadow hay (12.4% of crude protein; 26.1% of crude fibre). The ration was fed at 9 and 16 h.

Incubation method

The rumen contents were filtered under anaerobic conditions (CO_2-flushing) through a metal kitchen sieve (mesh width ca 1.5–2 mm). The rumen fluid (10 mL) was incubated for 6 h with Burroughs solution (10%; 40 mL; saturated with CO_2; initial pH 6.8) (Burroughs et al, 1950) in glass flasks (incubation gas: CO_2) at 39 °C in a shaking waterbath. As a nitrogen source, 10 mg of N as NH_4HCO_3 were added per flask. The different pH values in the different incubation flasks were obtained by adding HCl (5 N) just prior to incubation, and were 6.8, 6.3, 5.9, 5.6 and 5.2. In order to avoid large pH changes during the incubation, 40 or 80 mg of soybean oil (SO) was added as the sole substrate. A preliminary experiment showed that in incubations at pH 6.9 and with 50 mg of SO, the addition of 0.5 g of hay as a fermentation substrate had no effect on the lipolytic activity in the incubation, but an effect on $C_{18:1}$ accumulation was observed (table I).

The SO was added to the flasks dissolved in 1 mL diethylether, and the solvent was evaporated at 39 °C under a stream of N_2 gas; the rumen fluid and buffer were then added. The fatty acid (FA) composition of the SO was: $C_{16:0}$, 10.81%; $C_{18:0}$, 4.02%; $C_{18:1}$, 21.81%; $C_{18:2}$, 55.36%; $C_{18:3}$, 6.50%; and 1.50% other (not identified).

Table I. Lipolysis and overall biohydrogenation in incubations of rumen fluid with soybean oil (SO) in the presence or absence of substrate (0.5 g of ground hay) [a].

| | Fatty acid | | | | | | Sum [c] | Recovery [d] (%) |
	14:0	16:0	18:0	18:1	18:2	18:3		
Fatty acids (mg) liberated from TAG								
SO (*n* = 3)	0	4.18 (0.88)[b]	1.70 (0.62)	8.92 (1.70)	18.45 (1.16)	3.00 (0.27)	36.59 (3.08)	
SO + hay (*n* = 2)	0	4.01 (0.64)	0.85 (1.20)	5.19 (1.87)	20.06 (3.60)	3.02 (0.47)	33.49 (0.30)	
Fatty acids (mg) in FFA fraction								
SO (*n* = 3)	0.33 (0.21)	4.50 (0.68)	5.87 (2.26)	25.91 (2.70)	0.91 (0.12)	0	38.62 (5.95)	106.0 (16.0)
SO + hay (*n* = 3)	0.19 (0.17)	3.12 (0.60)	10.09 (1.45)	14.81 (1.81)	0.59 (0.21)	0	29.71 (3.42)	83.2 (5.6)

[a] Rumen fluid (10 mL) and Burroughs solution (40 mL) were incubated (6 h) under CO_2 with 50 mg of SO and with or without 0.5 g of ground hay; [b] in parentheses standard deviation; *n* = number of repetitions; [c] Sum of all fatty acids (identified and unidentified; [d] 100 x (fatty acids in FFA) / (fatty acids liberated from TAG).

After incubation, the pH was measured (PHM 62 Standard pH Meter, Radiometer, Copenhagen, Denmark) and the incubation contents were acidified with 0.7 mL H_2SO_4 (10 N). The next morning, the total amount of lipids were extracted and the triacyglycerols (TAG) and free fatty acids (FFA) were separated by thin layer chromatography (TLC). The fatty acid composition of both lipid classes was determined by gas chromatography (GLC). There were five repetitions for each incubation series except for the pH 5.2 series ($n =$ 3).

Analysis

After the addition of 10 mg of heptadecanoic acid (Riedel-de-Haën, Seelze, Germany) as an internal standard, lipid extraction was performed, mainly as described by Folch et al (1957). The total contents of the flasks were extracted three times with chloroform/methanol (2:1; vol/vol; C/M) without homogenization. The extract was washed three times with H_2O to avoid methylester formation during further analysis of the samples, and the final volume was brought to 100 mL with C/M. The TAG and FFA fractions were quantified as FA by a combination of TLC and GLC as previously described (Demeyer et al, 1978). From the extract, 2 mL was dried by evaporation at approximately 40 °C under a stream of N_2. The residue was dissolved in 200 µL chloroform and spotted on glass plates (20 x 20 cm), coated with Silicagel G (Merck, Darmstadt, Germany). The solvent system was hexane/diethylether/acetic acid (80:20:2; vol/vol/vol). Lipid fractions were detected under UV light after being sprayed with a 0.1% (wt/vol) solution of 2,7-dichlorofluorescein in ethanol. The TAG and FFA bands were scraped from the plate into glass centrifuge tubes with screwcaps. Transesterification was performed in the presence of 0.5 mL benzene, and the methylesters were extracted as previously described (Demeyer et al, 1978). Individual FA were determined by GLC using a Shimadzu GC-14A apparatus (Shimadzu Corporation, Tokyo, Japan) with a flame ionization detector, equipped with a J & W capillary column DB 225 (30 m length, 0.25 mm id, film 0.25 µ) (Alltech Associates, Inc, Deerfield, IL) using H_2 as the carrier gas. The peaks were identified based on the standard's retention times and quantified using the peak area of the internal standard (IS), which appeared in the FFA fraction. These values were also used for the quantification of FA in the TAG fraction. Reported $C_{18:1}$ values in the FFA fraction after incubation are the sum of three incompletely separated peaks, which are oleic acid and no identified positional or stereoisomers. The isomers of octadecapolyenoic acids were also not identified.

Calculation and statistical treatment

From the difference in the amount of FA in the TAG fraction before and after incubation, the amount of FA liberated from the TAG can be calculated. The difference between the FFA before and after incubation gives a measure of the accumulation of FA, and both values representing lipolysis should more or less agree. This can be expressed as a recovery value, calculated as 100 x (sum of FFA accumulated) (sum of FA released from TAG). Theoretically, this value should be approximately 100%.

Because of the accumulation of free $C_{18:1}$ in the incubations, a common phenomenon in in vitro incubations (Harfoot and Hazlewood, 1988), only the biohydrogenation of linoleic and linolenic acids ($C_{18:2}$ and $C_{18:3}$ respectively) was calculated as 100 x ($C_{18:2}$ or $C_{18:3}$ accumulated in FFA) / ($C_{18:2}$ or $C_{18:3}$ liberated from TAG). In such calculations, we assumed that the linoleic and linolenic acids not recovered as FFA after incubation were (potentially) available for complete hydrogenation as is the case in vivo, despite the fact that we also observed a slight accumulation of unidentified acids in the 80 mg incubations (max 3.13 mg, except at pH 6.29 where 6.88 mg were measured), that were most probably $C_{18:2}$ isomers. Isomerization is known to be the first step in the biohydrogenation process (Harfoot and Hazlewood, 1988).

Data were submitted to an analysis of variance with one factor (pH) and comparison of means was done by Tukey's test (SPSS Inc, 1990).

RESULTS

The preliminary experiment showed that the addition of hay as substrate for the microbes has no effect on lipolytic activity (table I). However, with the hay, $C_{18:1}$ (oleic acid: cis-

9 and positional or stereoisomers) was hydrogenated to stearic acid ($C_{18:0}$) to a larger extent, although in both cases no free linolenic acid ($C_{18:3}$, cis-9, 12, 15) and very little linoleic acid ($C_{18:2}$, cis-9, 12) could be detected.

In table II, the pH values before and after incubation are shown. Depending on the pH before incubation, small changes were sometimes observed. At higher pH values (6.9–6.4), a decrease of 0.2 units was noted, but at a pH of 6.0 and lower, the changes were negligible. In the following tables, the incubation pH was calculated as the mean value of the initial and final pH.

The effect of the pH on the liberation of FA from SO, calculated from TAG analysis is shown in table III (40 mg of SO in the incubation) and table IV (80 mg of SO). With 40 or 80 mg of SO as substrate, an incubation pH of 6.3 had no significant effect on the lipolysis (sum of FA) as compared with pH 6.8. At pH 6.0–5.9, the amount of lipolysis decreased, and the inhibition became greater with decreasing incubation pH. It was also interesting to note that at a similar pH value, inhibition of lipolysis in incubations with 80 mg of SO was more pronounced than with 40 mg. Calculation of the degree of inhibition of lipolysis, caused by the lower pH values, for each FA separately, demonstrated that only at the lowest pH (5.2), was the liberation of polyunsaturated fatty acids (PUFA; $C_{18:2}$ and $C_{18:3}$) from TAG more inhibited than the liberation of the other FA. At the other pH values, there was relatively good agreement between the inhibition of the individual FA liberation and the overall inhibition calculated from the sum of the FA.

In tables V and VI, the effect of decreasing incubation pH on lipolysis calculated from the determination of FFA is presented. In this case again, only pH values lower than 6.3 seemed to inhibit lipolysis, but the percentage inhibition was somewhat higher than that calculated from the TAG analysis.

As $C_{18:1}$ fatty acids partially accumulated without further hydrogenation to $C_{18:0}$, it did not seem very meaningful to calculate the overall biohydrogenation and the effect of pH upon it. Therefore, the disappearances of $C_{18:2}$ and $C_{18:3}$ were calculated separately and it was assumed that although an important fraction of $C_{18:1}$ accumulated, once both acids were no longer found in the incubation, the amount which had disappeared could have potentially been completely hydrogenated as occurs in vivo. These results are presented in table VII. It is clear that even at the lowest pH values, linolenic acid always completely disappeared. The disappearance of linoleic acid was almost complete (98–95%) above pH 6.3, but was only 60% at pH 5.2.

DISCUSSION

Because of the sometimes large pH changes in the rumen in vivo during the daily feeding cycle, the influences of pH on rumen metabolism can be better studied in in vitro systems where the pH values can be controlled. As we found that the lipolytic activity was independent of the presence of a fermentable substrate (hay), the incubations could be undertaken with SO as the sole substrate. This rendered the pH changes very small and made rigorous control of the pH unnecessary. The fact that the lipolysis was not influenced by the presence or the absence of ground hay as the fermentation substrate is not so surprising, as lipolytic bacteria are able to grow on the glycerol formed by the hydrolysis of TAG or other glycerolesters (Hobson and Mann, 1961; Henderson, 1975; Prins et al, 1975). It should be mentioned here that it is not certain whether microbes respond in the same way to pH variations with or without addition of an energy substrate, which is a possible limitation of our incubation technique.

Table II. pH changes in incubations of rumen fluid with soybean oil (SO) with different initial pH values [a].

	40 mg of SO in incubation				80 mg of SO in incubation		
Mean pH	pH 0 [b]	pH 6 [c]	Δ pH	Mean pH	pH 0 [b]	pH 6 [c]	Δ pH
6.78 ± 0.04	6.91 ± 0.04	6.65 ± 0.04	–0.25 ± 0.03	6.78 ± 0.03	6.89 ± 0.04	6.67 ± 0.04	–0.21 ± 0.04
6.34 ± 0.07	6.45 ± 0.07	6.24 ± 0.08	–0.21 ± 0.05	6.29 ± 0.06	6.39 ± 0.06	6.19 ± 0.10	–0.20 ± 0.12
5.98 ± 0.06	6.04 ± 0.03	5.92 ± 0.12	–0.12 ± 0.13	5.92 ± 0.08	5.98 ± 0.09	5.86 ± 0.10	–0.12 ± 0.10
5.56 ± 0.06	5.58 ± 0.07	5.54 ± 0.09	–0.04 ± 0.09	5.53 ± 0.10	5.56 ± 0.12	5.50 ± 0.09	–0.06 ± 0.09
5.22 ± 0.06	5.20 ± 0.09	5.24 ± 0.03	0.05 ± 0.07	5.25 ± 0.01	5.23 ± 0.04	5.27 ± 0.02	0.04 ± 0.04

[a] Mean value ± standard deviation ($n = 5$, except pH 5.2–5.3 where $n = 3$); [b] pH 0 = pH at start of incubation; [c] pH 6 = pH at end of incubation (6 h).

Table III. Influence of pH on lipolysis in vitro, calculated from fatty acids (mg) liberated from TAG (substrate: 40 mg of SO) [1].

pH	16:0	18:0	18:1	18:2	18:3	Sum [2]	Inhibition [3] (%)
6.78 ± 0.04	3.10[a]	1.16[a]	7.34[a]	16.53[a]	2.16[a]	30.89[a]	–
6.34 ± 0.07	2.97[a] (4.2)	1.12[a] (3.5)	7.11[a] (3.1)	15.97[a] (3.4)	2.04[a] (5.6)	29.56[a]	4.13[a]
5.98 ± 0.06	2.78[a] (10.3)	0.96[a] (17.2)	6.43[a] (12.4)	15.16[a] (8.3)	2.01[a] (6.9)	27.34[a]	11.44[a]
5.56 ± 0.06	1.67[b] (46.1)	0.74[a] (36.2)	4.84[a] (34.1)	9.61[b] (41.9)	1.21[b] (44.0)	18.90[b]	39.05[b]
5.22 ± 0.06	0.97[b] (68.7)	0.82[a] (29.3)	7.11[a] (3.1)	4.36[c] (73.6)	0.48[c] (77.8)	14.54[b]	52.38[b]
RSD [4]	0.34	0.29	1.58	1.72	0.23	2.55	7.89

[1] Values per column bearing different superscripts are significantly different ($P \leq 0.05$); [2] sum of identified and unidentified fatty acids; [3] inhibition was calculated relative to pH 6.78 values; [4] residual standard deviation. In parentheses, % inhibition (relative to pH 6.78) calculated per fatty acid.

Table IV. Influence of pH on lipolysis in vitro, calculated from fatty acids (mg) liberated from TAG (substrate: 80 mg of SO) [1].

pH	16:0	18:0	18:1	18:2	18:3	Sum [2]	Inhibition [3] (%)
6.78 ± 0.03	5.93[a]	2.29[a]	13.11[a]	30.77[a]	4.12[a]	58.72[a]	–
6.29 ± 0.06	6.05[a] (−2.0)	2.30[a] (−0.4)	13.08[a] (0.2)	30.84[a] (−0.2)	4.09[a] (0.7)	58.62[a]	−0.82[a]
5.92 ± 0.08	3.91[b] (34.1)	1.48[b] (35.4)	8.30[b] (36.7)	20.16[b] (34.5)	2.99[b] (27.4)	38.58[b]	33.29[b]
5.53 ± 0.10	3.17[b] (46.5)	1.39[b] (39.3)	7.05[bc] (46.2)	15.30[bc] (50.3)	2.00[b] (51.5)	30.86[b]	46.62[b]
5.25 ± 0.01	1.54[c] (74.0)	1.07[b] (53.3)	4.69[c] (64.2)	6.10[c] (80.2)	0.75[c] (81.8)	14.96[c]	74.52[c]
RSD [4]	0.56	0.28	1.33	3.51	0.54	5.74	10.45

[1] Values per column bearing different superscripts are significantly different ($P \leq 0.05$); [2] sum of identified and unidentified fatty acids; [3] inhibition was calculated relative to pH 6.78 values; [4] residual standard deviation. In parentheses, % inhibition (relative to pH 6.78 values) calculated per fatty acid (negative values represent stimulation).

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Table V. Influence of pH on lipolysis in vitro, calculated from FFA accumulation after incubation (substrate: 40 mg of SO) [1].

pH	16:0	18:0	18:1	18:2	18:3	Sum [2]	Inhibition (%) [3]	Recovery (%) [4]
6.78 ± 0.04	4.14[a]	5.88[a]	19.44[a]	0.86[a]	0.0[a]	32.45[a]	–	105.5[a]
6.34 ± 0.07	3.78[ab] (8.7)	4.48[ab] (23.8)	17.02[a] (12.5)	0.36[a] (58.1)	0.0[a]	27.82[ab]	13.9[ab]	94.2[ab]
5.98 ± 0.06	3.38[b] (18.4)	3.71[ab] (36.9)	13.90[b] (28.5)	0.69[a] (19.8)	0.0[a]	26.02[b]	19.6[b]	95.1[ab]
5.56 ± 0.06	2.22[c] (46.4)	2.28[bc] (61.2)	7.39[c] (62.0)	1.66[a] (−193.0)	0.09[a]	15.07[c]	52.3[c]	80.7[b]
5.22 ± 0.06	1.71[c] (58.7)	0.87[c] (85.2)	4.35[d] (77.6)	1.95[a] (−226.7)	0.11[a]	10.54[c]	67.53[c]	72.6[b]
RSD [5]	0.36	1.20	1.29	0.97	0.11	2.77	7.60	11.4

[1] Values per column bearing different superscripts are significantly different ($P \leq 0.05$); [2] sum of identified and unidentified fatty acids; [3] inhibition was calculated relative to pH 6.78 values; [4] recovery: 100 x (fatty acids in FFA) / (fatty acids released from TAG); [5] residual standard deviation. In parentheses, % inhibition (relative to pH 6.78 values) calculated per fatty acid (negative values represent stimulation).

Table VI. Influence of pH on lipolysis in vitro, calculated from FFA accumulation after incubation (substrate: 80 mg of SO) [1].

pH	16:0	18:0	18:1	18:2	18:3	Sum [2]	Inhibition (%) [3]	Recovery [4] (%)
6.78 ± 0.03	6.09[a]	5.94[a]	29.82[a]	1.00[a]	0,0[a]	50.59[a]	--	86.6[a]
6.29 ± 0.06	5.70[a] (6.4)	4.49[a] (24.4)	25.37[a] (14.9)	1.53[ab] (−53.0)	0.0[a]	46.06[a]	7.9[a]	78.8[a]
5.92 ± 0.08	3.72[b] (38.9)	4.27[a] (28.1)	15.21[b] (49.0)	2.47[bc] (−147.0)	0.0[a]	30.99[b]	38.1[b]	80.9[a]
5.53 ± 0.10	2.42[c] (60.3)	2.02[b] (66.0)	9.19[c] (69.2)	2.96[c] (−196.0)	0.10[a]	19.78[c]	59.7[c]	68.6[a]
5.25 ± 0.01	1.84[c] (69.8)	0.64[b] (89.2)	5.61[c] (81.2)	1.94[ac] (−94.0)	0.0[a]	12.13[d]	76.0[c]	81.7[a]
RSD [5]	0.51	1.09	2.35	0.57	0.06	3.44	8.49	14.4

[1] Values per column bearing different superscripts are significantly different ($P \leq 0.05$); [2] sum of identified and unidentified fatty acids; [3] inhibition was calculated relative to pH 6.78 values; [4] recovery: 100 x (fatty acids in FFA) / (fatty acids released from TAG); [5] residual standard deviation. In parentheses, % inhibition (relative to pH 6.78 values) calculated per fatty acid (negative values represent stimulation).

Table VII. Effect of pH on disappearance (%) of linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids as a consequence of biohydrogenation [1].

40 mg of SO in incubation			80 mg of SO in incubation		
pH	$C_{18:2}$	$C_{18:3}$	pH	$C_{18:2}$	$C_{18:3}$
6.78	94.6[a]	100.0[a]	6.78	96.6[a]	100.0[a]
6.34	97.8[a]	100.0[a]	6.29	94.9[ab]	100.0[a]
5.98	95.2[a]	100.0[a]	5.92	87.6[ac]	100.0[a]
5.56	80.5[ab]	89.8[a]	5.53	76.7[bc]	91.8[a]
5.22	59.5[b]	68.8[a]	5.25	66.7[c]	100.0[a]
RSD [2]	14.4	21.0		10.0	6.7

[1] Values per column bearing different superscripts are significantly different ($P \leq 0.05$); [2] residual standard deviation.

From our experiments, it seemed that at pH ≤ 6.0 the lipolytic activity was significantly inhibited, and that the intensity of the inhibition was greater with the larger amount (80 mg) of SO as the TAG substrate. In order to obtain an inhibition of approximately 50%, the pH had to decrease to 5.5, a value only observed naturally in the rumen of animals consuming considerable amounts of high-concentrate diets.

The inhibition of lipolysis calculated from the FFA analysis was somewhat higher than that calculated from TAG analysis. This difference was obviously related to the sometimes low recovery of FFA compared to the amount of FA released from TAG and calculated as explained above. In general, the recovery was lower in incubations with 80 mg of SO compared to the 40 mg incubations. The lowest values were observed at pH values lower than 6.0 and, in this case, the $C_{18:1}$ accumulation was less than expected from lipolysis and biohydrogenation of the PUFA. The incorporation in microbial matter is not excluded, but rather doubtful as this would suggest that the microbial lipids were not completely extracted with the method used (Demeyer et al, 1978). The reason for the low recovery phenomenon in our work is as yet unknown, but it was also observed by Hino et al (1993).

Comparison of the liberation of individual FA from TAG showed that only at extreme low pH values (5.2) in the rumen, was there preferential inhibition of the PUFA release.

Although our results have clearly shown that lipolysis can be inhibited at low pH values, biohydrogenation of the liberated PUFA was influenced to a much lower extent. At pH values lower than 5.5, no unequivocal effect on hydrogenation of linolenic acid was observed, but in the case of linoleic acid, some inhibition was noted. It seemed that the lipolytic activity in the rumen was more sensitive to pH changes than biohydrogenation. This meant that the inhibition of biohydrogenation in the rumen due to low pH values after the feeding of high concentrate diets was the consequence of an inhibited lipolysis, as only FFA can be hydrogenated by the rumen microorganisms (Hawke and Silcock, 1970; Kepler et al, 1970).

Whether the inhibitory effect of pH on lipolysis was due to the effect of the pH on the growth and metabolism of lipolytic bac-

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teria and/or on direct interference with their lipase activity could not be deduced from this experiment. It has been noted that the growth of *Anaerovibrio lipolytica*, a lipolytic rumen bacterium, was lowered at pH 5.7 and completely inhibited at pH 5.3 (Hobson, 1965). The growth yield of *Butyrivibrio fibrisolvens*, a lipolytic and hydrogenating species, was 75% of its maximum yield when pH was 5.75, while at pH 5.5 the organism was washed out in continuous culture (Russell and Dombrowski, 1980). Studies in batch culture with lipase produced by *A lipolytica* revealed that the activity was severely inhibited at pH 5.8, an effect which could be avoided by maintaining the pH above 6.3 (Henderson et al, 1969). In another study, with olive oil as the substrate, Henderson (1971) found that the lipase activity was most active at pH 7.4, while at pH 6.6 only 50% of the maximum activity level was observed.

An experiment by Gerson et al (1985), however, indicated that other factors in addition to pH must be involved in the control of lipolysis and biohydrogenation. Indeed, these authors could lower the rates (about 50%) of both activity levels, measured in vitro by changing the feed ration of the sheep donating the rumen fluid for incubation, from one with a high fibre content to a high starch one. Surprisingly, the pH in the rumen was not significantly altered by the different diets and remained between 6.1 and 6.7, the highest value being measured on the high starch diet. The inhibition of biohydrogenation was not due to a diminished lipolytic activity, as the authors incubated ^{14}C-labelled linoleic acid for the determination of hydrogenation activity. Apparently, independent of the pH level in the rumen, highly concentrated diets cause changes in the composition of the microflora, such as a drastic decrease in the numbers of *B fibrisolvens* or reduced numbers of lipolytic vibrios, which could explain the inhibition of lypolysis and hydro-

genation (Latham et al, 1972). Mackie et al (1978), however, found an increased proportion of *A lipolytica* in the rumen of sheep that were stepwise adapted to high-concentrate diets. The lowest pH values recorded in the rumen of these sheep were 5.5–5.3. It is possible that higher viable counts of lipolytic bacteria are not necessarily linked to higher lipase activities. Furthermore, besides a change in the composition of the microflora, direct effects of the feed ration composition on lipase activity are possible, as it is well known that a high carbohydrate substrate (eg, glucose) in the growth medium reduced the production of lipase by pure strains of bacteria grown under aerobic conditions (Papon and Talon, 1988; Jaeger et al, 1994). It seems that very little is known about the mechanisms controlling biohydrogenation in the rumen (Harfoot and Hazlewood, 1988).

Finally, based on our results, it can be speculated that the establishment of low pH values in the rumen through the feeding of high-concentrate diets could be a strategy for protecting unsaturated lipid supplements (oils) against biohydrogenation. However, a negative aspect of such a strategy is the fact that at pH values lower than 6.2, fibre digestion in the rumen is depressed (Mould et al, 1983). This is however of relative importance because lipid supplements are usually fed in combination with low roughage diets. This strategy would permit unsaturated fatty acids to reach the small intestine, where they can be absorbed and made available for incorporation in body and milk fat.

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