Assessing the Biological Effectiveness of Protected Lipid Supplements for Ruminants

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ABSTRACT

Techniques are described for assessing the effectiveness with which lipids may be protected against ruminal degradation. A simple in vitro test was developed using pancreatic lipase, and this test may have application in quality control of the commercial production of protected lipid supplements, as it is applicable to supplements containing polyunsaturated or saturated lipids. All the in vitro tests overestimate the actual in vivo biological effectiveness, and this is probably due to mastication and greater microbial activity in vivo than in vitro. The poor biological response of some protected lipid supplements is most probably due to the incomplete entrapment of lipid droplets in the protein matrix.

INTRODUCTION

The adipose tissue and milk fat of ruminant animals rarely contains large proportions of polyenoic fatty acids due to the capacity of the rumen microbes to hydrolyse and hydrogenate the dietary lipid (1,2). Quantities of dietary fat in excess of 5% by weight of the ration are not usually fed to ruminants due to the deleterious effect of the fat on the rumen microflora (3,4).

The development of a method for protecting lipids against microbial action, by encapsulating micro-droplets of lipid in a protein matrix subsequently reacted with formaldehyde, has allowed the fatty acid composition of ruminant milk and adipose tissue to be modified (5,6) and has permitted animals to be fed substantial levels of dietary fat without impairing normal rumen function (7).

The essential features of biologically effective ruminant lipid supplements are: (a.) lipid droplets firmly embedded in a continuous matrix of protein. (b.) formaldehyde reactivity with the protein in order to prevent protein solubility in the rumen. (c.) sufficient reversibility of the formaldehyde reaction so that the protein is solubilized after passage through the rumen. (d.) absorption of lipid from the small intestine. The magnitude of the response obtained when protected lipid supplements are fed to ruminants to modify fat or supply energy is related to the amount of lipid fed and to the effectiveness of the lipid protection mechanisms. The effectiveness of lipid protection is largely determined by the technology and processes used to produce these supplements. Until recently the only means of evaluating the quality of lipid supplements for ruminants were to either incubate the supplements with rumen fluid and measure the degree of hydrogenation of unsaturated fatty acids, or to feed the supplements to ruminants and measure the proportion of 18:2 in milk fat. These two methods of evaluation were, of necessity, restricted to those supplements which contained large proportions of a polyunsaturated fatty acid (e.g., 18:2 or 18:3). Furthermore, these methods of evaluation often did not help in understanding the reasons for inferior biological responses when they occurred, and were unsatisfactory for routine quality control of commercial lipid supplement formulations. The present studies report the development and assessment of simple quality control procedures designed to predict the biological effectiveness of lipid supplements prepared from either unsaturated or saturated fats using different sources of protein and different manufacturing technologies.

EXPERIMENTAL PROCEDURES

Preparation of Supplements

Spray-dried oil-casein supplements. Manufacture of spray-dried oil-casein supplements was as previously described (5); formaldehyde was added either before or after spray drying. The dried material was stored in an airtight container prior to use as a precaution against oxidation and to facilitate the reaction between formaldehyde and protein.

Flash-dried oil-casein supplements. In the manufacture of these supplements, a concentrated casein solution was emulsified with oil, formalin (37% formaldehyde w/v) was added to the emulsion to induce gelation, and the gel was dried using a flash (or pneumatic) drier. The initial step was to dissolve hydrochloric or lactic acid casein in five parts by weight of water containing sodium hydroxide (2.5% NaOH by weight of casein) using a colloid mill. Oil was then added to the casein solution, and emulsification was effected by repeated passage through the colloid mill. The temperature of the emulsion was never allowed to exceed 45 C, and the pH was adjusted to 8-8.5 by adding further NaOH. After the pH adjustment, the emulsion was pumped into an enclosed cylindrical batch ribbon mixer where formalin was added (ca. 8% formalin by weight of casein). The addition of formalin caused the emulsion to gel, and the gel was dried in a Raymond flash (or pneumatic) drier. The inlet air temperature was 400 C, and the air temperature at the outlet of the hammer mill was 200 C.

Flash-dried oil seed supplements. Whole sunflower seeds or sunflower seed kernels were dry mixed with protein (casein or soybean), and the mixture was augered into a 22.5 Kw performated plate mill (Fryma ML-250/R) (4 mm diameter hole) at a constant rate (ca. 10 kg/min). At the same time, water and 3.5 M NaOH were continuously introduced at rates of 20-22 l/min and ca. 2.2 l/min respectively. The slurry from the plate mill was held in a balance tank prior to pumping through a 45 Kw Carborundum stone mill (Fryma, MK 360). The pH of the emulsion was 10-11 when leaving the plate mill and 8-10 when leaving the stone mill, and the temperature of the emulsion was never allowed to exceed 45 C. The emulsion was then treated with formalin to induce gelation and dried in a pneumatic flash drier as described above.

In vitro Biological Evaluation of Supplements

Ruminal protein solubility. The release of ammonia during in vitro incubation with rumen fluid was used as a measure of the solubility of the proteins. To 10 ml of strained rumen fluid sufficient lipid supplement was added to supply 75 mg of protein, and the mixture was incubated anaerobically at 37 C for 20 hr. The reaction flasks including rumen fluid blanks were treated with 5 ml of 0.2 M H_2SO_4. The mixtures were centrifuged to remove suspended matter, and ammonia was estimated in the supernatant

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after steam distillations (8,9). Net ammonia production was calculated from the difference between the incubated and blank values corrected for ammonia initially present.

Ruminal hydrogenation of unsaturated lipids. Samples of unsaturated lipid supplements (containing ca. 40-50 mg of oil) were incubated in test tubes with 10 ml of strained rumen fluid obtained as described previously (5). The tubes were flushed with nitrogen, capped with rubber serum caps and incubated in a shaking water bath at 38 C for periods up to 20 hr. the incubated and corresponding unincubated reaction mixtures were saponified and the fatty acids extracted and methylated (5). The methyl esters were analysed by gas liquid chromatography (GLC) (10), and the extent of protection against ruminal hydrogenation was calculated using the formula:

$$\text{Protection (\%)} = \frac{\text{\% 18:2 after incubation}}{\text{\% 18:2 before incubation}} \times 100$$

The endogenous level of polyunsaturated fatty acids in the rumen fluid was always less than 2% by weight of the total fatty acid, and thus had little effect on the above calculations. The hydrogenating capacity of each batch of rumen fluid was verified by incubating the rumen fluid with samples of polyunsaturated oil-casein supplements prepared without formalin.

Ruminal lipolysis of triacylglycerol. Samples of the lipid supplements (containing ca. 40-50 mg of lipid) were incubated with 10 ml of strained rumen fluid as described in the previous section. When the extent of triacylglycerol (TG) hydrolysis was measured by GLC, heptadecanoic acid (17:0)(20 mg) was added to each reaction tube as an internal standard.

The incubated and corresponding unincubated reaction mixtures were extracted with 10 ml of chloroform-methanol (C/M 2:1 v/v) containing 0.5 ml of 5 M HCl. The mixtures of rumen fluid and acidic C/M were vigorously shaken and allowed to stand for 2-4 hr until two phases were clearly distinguished.

The upper aqueous phase was removed and discarded and the lower organic phase was filtered to remove suspended matter. The filtrate was evaporated to dryness using rotary film evaporator, and the extent of TG hydrolysis was estimated using either thin layer chromatography (TLC), or if 17:0 was added, GLC methods described below.

(i) TLC analysis of the extraced lipids was carried out using silica gel G and a solvent system of petroleum ether: diethyl ether:acetic acid (84:15:1; v/v/v). The separated lipids were visualised by spraying with an ethanolic solution of 2,7 dichlorofluorescein (0.2% w/v) and viewing under UV light. The extent of TG hydrolysis could only be estimated qualitatively by comparing the relative intensities and sizes of the TG and free fatty acid (FFA) spots in both the incubated and the unincubated reaction mixtures.

(ii) GLC analysis was used in conjunction with the 17:0 internal standard to assess the degree to TG lipolysis. This method is similar to that used by Storry (personal communication) and relies on the determination of the proportion of 17:0 in the FFA fraction of the incubated and the unincubated lipid extracts. The dilution of 17:0 in the FFA fraction which occurs during incubation was used as an index of ruminal lipolysis. The FFA in the lipid extracts were methylated with diazomethane and the methyl esters separated by GLC (5). In addition, samples of the total lipid extracts were saponified, acidified, and extracted with petroleum ether, and the total fatty acids obtained by this means were also methylated with diazomethane and analyzed by GLC. The GLC 17:0 measurements were used to estimate the following values:

$TFA\ t_0$ = Total fatty acids at 0 hr
$TFA\ t_{20}$ = Total fatty acids at 20 hr
$FFA\ t_0$ = Free fatty acids at 0 hr
$FFA\ t_{20}$ = Free fatty acids at 20 hr
$EFFA\ t_0$ = Endogenous ruminal free fatty acids at 0 hr (from unincubated rumen fluid controls)
$EFFA\ t_{20}$ = Endogenous ruminal free fatty acids at 20 hr (from incubated rumen fluid controls).

From these values it was possible to calculate the following two other values:

$$RFA\ t_0 \text{ (released fatty acids at 0 hr)} = FFA\ t_0 - EFFA\ t_0$$

and

$$RFA\ t_{20} \text{ (released fatty acids at 20 hr)} = FFA\ t_{20} - EFFA\ t_{20}.$$

The resistance to ruminal lipolysis was then calculated using the formula:

$$\text{Resistance (\%)} = \frac{TFA\ t_{20} - RFA\ t_{20}}{TFA\ t_0 - RFA\ t_0} \times 100$$

Pancreatic lipolysis of triglycerides. Samples of supplement containing ca. 40 mg of lipid were weighed into test tubes. To each tube was also added 40 mg pancreatic lipase (Sigma type II), 1 ml 0.05 M tris buffer solution pH 7.4 (37 C), 50 μl $CaCl_2$ solution (45% w/v) and 250 μl bile salt solution [0.1% solution of bile salt mixture (Baltimore Biological Laboratories, Baltimore MD) in 0.05 M tris buffer tris buffer at pH 7.4].

The tubes were stoppered, well mixed and shaken in a water bath at 38 C for periods up to 3 hr using a wrist action laboratory shaker (120 strokes/min).

Immediately after incubation 5 ml of chloroform/methanol (2:1, v/v) (C/M) were added and well mixed to destroy the lipase activity and to extract the lipid and liberated FFA. Similarly, with the zero time unincubated tubes, 5 ml of C/M solution were added prior to the addition of pancreatic lipase. The incubated and unincubated tubes containing the C/M were centrifuged at 1000 r.p.m. for 3 min, and the organic phase was removed. The residual aqueous layer was again extracted with a further 3 ml of C/M. The combined chloroform extracts were evaporated to dryness under a stream of nitrogen in a water bath at 60 C and the residue dissolved in 3 ml of light petroleum. The petroleum ether (PE) extract was shaken with 1 ml of distilled water and the two phases allowed to separate. The aqueous layer was removed, and the PE layer was evaporated to dryness in a stream of nitrogen. The residue was dissolved in 2 ml of ethanol, three drops of nile blue indicator (0.2% in ethanol) were added, and the mixture was titrated to a pale pink end point against a standard .01M ethanolic NaOH solution. The titrations were corrected by subtracting the equivalent blank (zero hour) titrations, and from these values the following were calculated:

$$\text{\% observed hydrolysis (OH)} = \frac{\text{milli-equivalents NaOH}}{\text{total milli-equivs of FA in supplement sample}} \times \frac{100}{1}$$

$$\text{\% absolute resistance to hydrolysis} = 100 - OH$$

The theoretical total milli-equivalents of FA in supplement were calculated as follows:

$$\frac{\text{Wt of oil in supplement} \times 0.9}{263}$$

0.9 is a correction factor used to compensate for the glycerol moiety of the TG. 263 is the mol. wt. of linoleic acid (18:2) moiety of TG. The efficiency of the technique was always checked by the incubation of a standard unprotected TG formulation.

In vivo Biological Evaluation of Supplements

Ruminal hydrogenation of unsaturated lipids. This technique was adapted from the work of Hogan et al. (11) and depended on evidence that the total long chain fatty acids passing from the abomasum was approximately equal to the intake in the diet. Hence the change in concentration of 18:2 and 18:3 gave an approximation of the degree of hydrogenation. The animals were fed basal diets of chopped alfalfa hay and oats (1:1,w/w) 800 g/day. The abomasal digesta was sampled via an abomasal fistula at various time periods and ca. 20 ml of digesta was saponified and fatty acids extracted as described for the rumen fluid incubations. The extracted fatty acids were methylated and analysed by GLC. The proportion of polyunsaturated fatty acid (e.g., 18:2) in the abomasal lipids was compared with a theoretical level estimated by assuming (a.) that all of the 18:2 in the lipid supplement was protected against ruminal hydrogenation; (b.) that all of the 18:2 in the basal diet was hydrogenated; and (c.) that there was no significant synthesis or degradation of the carbon skeleton of fatty acids by micro-organisms. Assumptions (b.) and (c.) appear to be justifiable (5,12). The in vivo protection of these supplements was calculated using the formula:

$$\% \text{ protection} = \frac{\text{Actual \% 18:2 in abomasum}}{\text{Theoretical \% 18:2 in abomasum}} \times 100$$

As an example, a sheep receiving 400 g of alfalfa hay, 400 g of crushed oats and 300 g of a formaldehyde treated safflower oil/casein (2:1 w/w) supplement would receive 3% of the basal diet of alfalfa and oats as fatty acids, i.e., 24 g, and 178 g of fatty acids from the lipid supplement (corrected for glycerol moiety).

The 18:2 content of the supplementary fatty acids is 75% or 134 g. Using the above assumptions, the content of 18:2 in the abomasal fatty acids should be 134/(178 + 24) = 66%. If the actual 18:2 content of abomasal fatty acids is 53%, then the percentage protection $= \frac{53}{66} \times 100 = 80\%$.

Milk fatty acid response. Dietary long chain fatty acids are transferred unaltered from the small intestine into the milk of lactating ruminants (5,11). Consequently the concentration of the polyunsaturated fatty acid (e.g., 18:2) in the milk fat is an index of the degree of ruminal protection. The supplements were fed for periods of at least 4 days, the milk fat was extracted (5), and the content of the respective polyunsaturated fatty acid in the milk lipids was determined by GLC procedures.

Other Chemical Analyses

Moisture content of feed ingredients was determined by heating at 100 C for at least 12 hr. Protein content was determined by the Kjeldahl method. Formaldehyde content of supplements was determined by the method of Van Dooren (13).

Statistical Analysis

In Figures 1 and 5 non-linear three parameter functions of the Mitscherlich form

$$y = A - Be^{-cx}$$

were fitted using a modified Levenberg-Morrison-Marquardt (14) least squares minimization procedure. The relationship was constrained to pass through the origin (A=B) in Figure 5 and asymptote at 100% (A=100) for the variable, % protection, in Figure 1. The curves in Figure 1 have been fitted for descriptive purposes only and need to be viewed cautiously due to the small sample size.

Where there was a linear relationship, linear structural relationships were fitted as both the variables were measured with error. This relationship was derived by the method of maximum likelihood (15) assuming that the ratio of error variances of the two variables was unity. The asymptotic variance-covariance matrix for the parameter estimates was estimated using a procedure developed by Patefield (16).

RESULTS AND DISCUSSION

The early protected lipid supplements were prepared by spray drying homogenates of polyunsaturated oil and casein and treating the homogenate or the dried particles with formaldehyde (5,6). Figure 1 shows the effect of formaldehyde treatment of the homogenate on the protection against ruminal hydrogenation in vitro and on the concentration of polyunsaturated fatty acid in the milk fat of goats fed these spray-dried particles. The data of Figure 1 show (a) that the biological effectiveness of these spray-dried materials is proportional to the concentration of added formaldehyde (up to 5% HCHO by weight of protein), and (b) that the in vitro estimate of ruminal protection is closely related with the in vitro milk fatty acid response.

The latter relationship is shown in Figure 2 where in vitro resistance to ruminal hydrogenation was well correlated ($r = .79$, $n = 38$) with the 18:2 content in the milk of lactating goats fed supplements prepared from sunflower seeds. These supplements have been prepared by a different technology to those in Figure 1; the replacement of spray drying with flash (pneumatic) drying and the replacement of expensive casein and purified oils with mixtures of oilseeds has led to reductions in the cost of protected lipid supplements, but in some instances there have also been reductions in the bioloigcal effectiveness of these supplements, as shown by the large number of values less than 85% in vitro in Figure 2.

Until recently the in vitro and in vivo techniques in Figures 1 and 2 were the only means of evaluating lipid supplements which contained large proportions of a polyunsaturated fatty acid (e.g., 18:2 or 18:3). Furthermore, these methods of evaluation often did not help in understanding the reasons for inferior biological responses and were unsatisfactory for routine quality control of commercial lipid supplement formulations. The studies reported here arose out of the need to develop simple quality control procedures which would accurately predict the biological effectiveness of lipid supplements prepared from either unsaturated or saturated fats using different sources of protein and different manufacturing technologies (17-20).

Protein Solubility and Microbial Metabolism in vitro

Studies by Ferguson, Hemsley and Reis (21) demonstrated that the insolubility of a protein such as casein is proportional to the concentration of bound formaldehyde over the range from 0 to 2% formaldehyde. In these studies the casein particles were reacted with formaldehyde in an enclosed container for periods of at least four days. In the preparation of protected lipid supplements, however, the formaldehyde is usually added to the emulsion prior to spray or flash drying, and to allow for losses of formaldehyde in the drying process it is necessary to add approximately twice the level (i.e., 4% formaldehyde by weight of protein).

Provided sufficient formaldehyde has been added during preparation, it is unlikely that protein solubility is a contributing factor in the poor biological effectiveness of protected lipid supplements. Table I shows the NH_3-N recovered from incubating formaldehyde-treated or untreated supplements with rumen fluid. The appearance of NH_3 during incubation is an index of the solubility of the proteins (8), and the data of Table I therefore show the

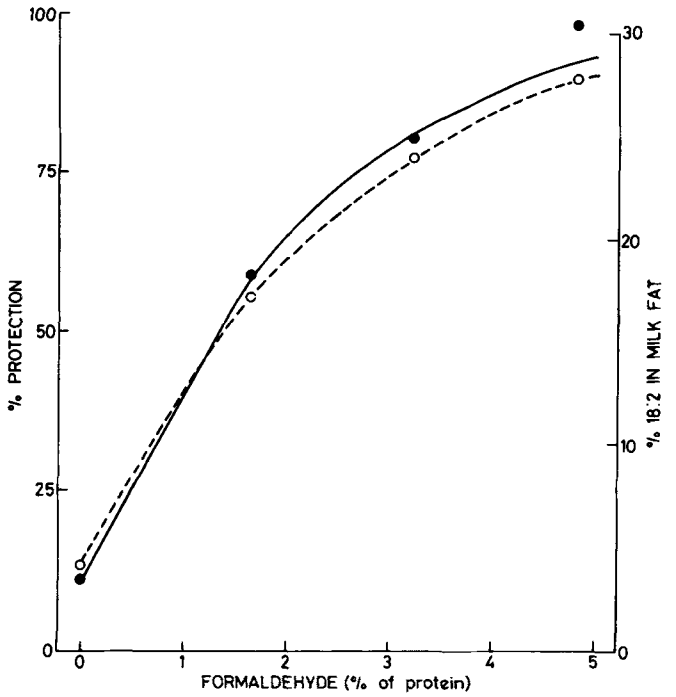

FIG. 1. Effect of formaldehyde level on protection of linoleic acid against ruminal hydrogenation measured in vitro (•) and on the incorporation of the acid in milk fat of goats (○). Protected lipid supplements were prepared by treating sunflower oil/casein (2:1, w/w) emulsions with formaldehyde prior to spray drying. Best fit curves are described by the following equations:

• $y = 100\text{-}88.63\ (\pm\ 5.09)e^{-0.50\ (\pm\ 0.06)\ x}$ r = 0.99
○ $y = 32.99\ (\pm\ 1.59)\ \text{-}28.94\ (\pm\ 1.55)\ e^{-0.37\ (\pm\ 0.04)\ x}$ r = 0.99

complete insolubility of the aldehyde-treated protein during a 20 hr incubation regardless of the in vitro protection of the encapsulated oil. On the other hand, ca. 65% of the untreated protein-N was recovered as NH_3-N. The negative values for NH_3-N production in Table I suggest a net utilization of NH_3 by the microflora rather than a release.

Microbial Lipolysis in vitro

The initial step in the ruminal metabolism of lipids is the hydrolysis of ester bonds, and in the case of TG the products of this hydrolysis are FFA and glycerol. It may be expected then that the extent of TG lipolysis in the rumen would be inversely correlated with the degree of supplement protection.

Two procedures were developed for measuring the extent of ruminal lipolysis in vitro, one employing TLC and the other GLC. The TLC method provides a qualitative assessment of the degree of TG hydrolysis during incubation with rumen fluid. Figure 3 shows that the TG of unprotected tallow supplement was almost completely converted to FFA during a 20 hr rumen incubation, whereas the TG of the protected tallow supplements was largely unaffected by the ruminal microflora. The TLC technique may thus be useful in evaluating the protection of saturated fat supplements, but the usefulness of this technique would be greatly enhanced if it were quantified by densitometry measurments.

A GLC method was developed for quantifying the degree of in vitro ruminal lipolysis of TG. Here an internal standard (17:0) was added to each rumen reaction mixture, and the dilution of the relative proportion of 17:0 in the FFA fraction was used to calculate the extent of TG lipolysis and hence the degree of supplement protection.

There was a significant correlation (r = .96, n = 6) between the resistance to ruminal lipolysis and the resistance of the same samples to ruminal hydrogenation (Fig. 4). The

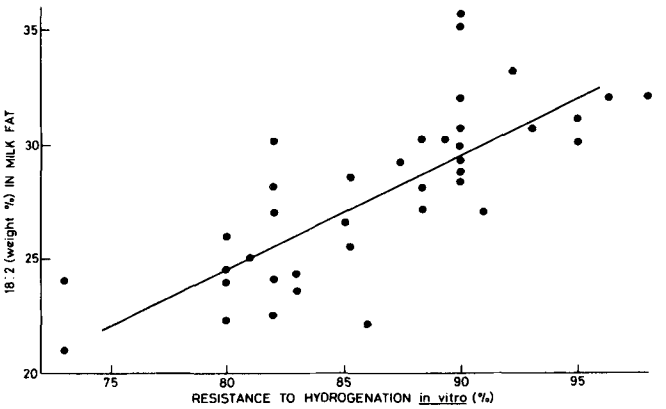

FIG. 2. The relationship between the in vitro and in vivo biological effectiveness of formaldehyde-treated sunflower seed supplements. Supplements were prepared from sunflower seeds and were dried using a pneumatic drier as described in the text. The resistance to hydrogenation in vitro was measured using rumen fluid as descirbed in the text. The in vivo (milk % 18:2) response was determined by feeding the supplements to lactating goats for periods of at least 2-3 days and measuring the fatty acid composition of the milk fat. The ration for the goats was supplement (400 g), chopped alfalfa hay (400 g) and crushed oats (400 g).
$y = 0.54\ (\pm\ 0.07)\ x\ \text{-}\ 18.57\ (\pm\ 6.03)$ r = 0.79

TABLE I

Ruminal NH_3-N Production in vitro[a]

Supplement Type	Net NH_3-N production (mg)	% in vitro protection of oil
Formaldehyde-treated		
Spray dried casein/oil	-1.0	95.0
Flash dried casein/oil	-0.9	91.4
Flash dried seed	-0.7	86.0
	-1.0	88.0
	-1.1	76.2
	-1.1	70.2
	-1.0	67.7
Untreated		
Spray dried casein/oil	+7.8	4.5
Spray dried casein	8.2	---

[a]Supplements (containing 75 mg protein)were prepared and incubated with rumen fluid for 20 hr as described in the text.

data of Figure 4 were derived from supplements containing polyunsaturated fatty acids, but the method is equally applicable in the testing of supplements which do not contain polyunsaturated fatty acids, e.g., tallow, coconut and palm oil.

Pancreatic Lipolysis in vitro

The previous in vitro methods for testing the biological effectiveness of lipid supplements depended upon incubations with rumen contents. The difficulties of collecting rumen fluid and the long incubation times necessary prompted us to examine the possibility of using commercial lipase preparations for the biological evaluation of protected lipid supplements.

Figure 5 shows the observed hydrolysis of lipid supplements incubated with pancreatic lipase for varying time periods. It was apparent that the untreated oil supplements were extensively hydrolysed during a 3 hr incubation with pancreatic lipase, but this hydrolysis was considerably reduced with formaldehyde treated preparations.

A number of protected polyunsaturated lipid supplements were tested for lipolysis with pancreatic lipase and for hydrogenation with rumen fluid. There was a significant correlation (r = .95, n = 18) between the results derived from the two methods after a 2 hr incubation (Fig. 6).

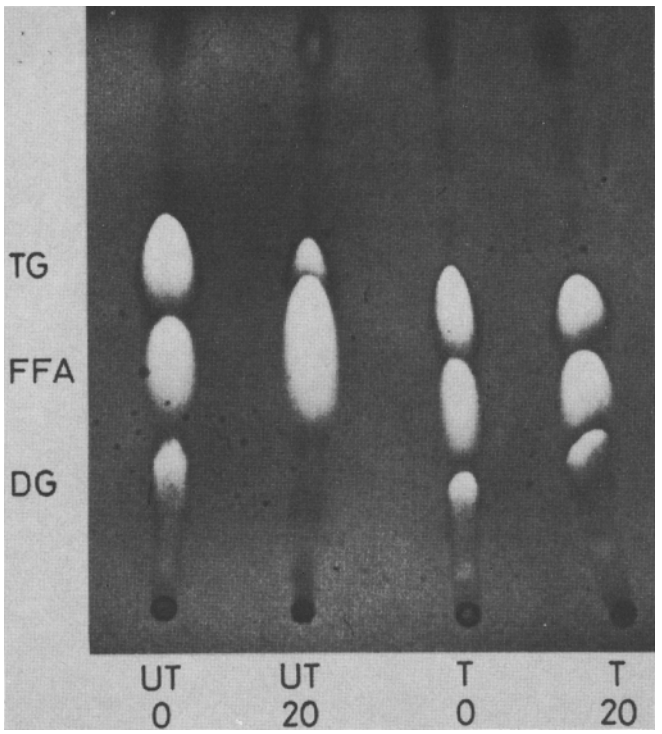

FIG. 3. Ruminal lipolysis of triacylglycerols. Spray dried tallow/casein (2:1, w/w) supplements were incubated with rumen fluid and the extracted lipids were separated by TLC as described in the text. TG - triacylglycerol; FFA - free fatty acid; DG - diglyceride; UT - untreated; T - treated with formaldehyde; O - zero time (unincubated) control; 20-20 hr incubated sample.

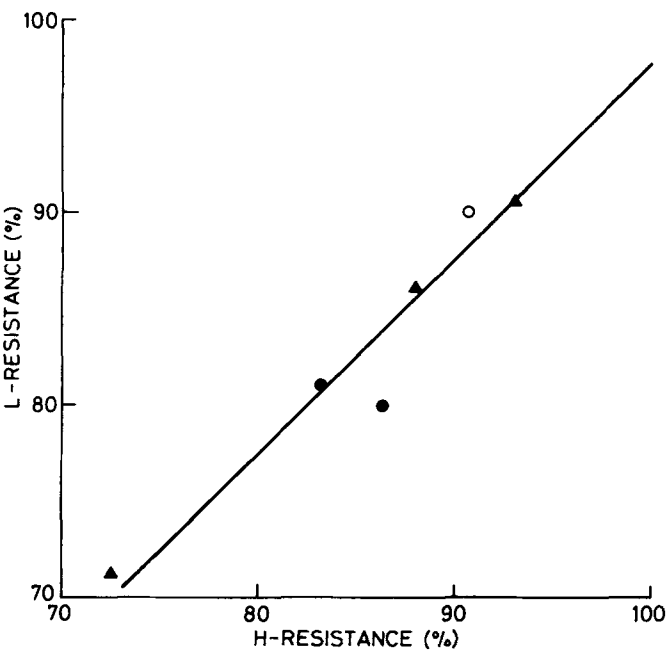

FIG. 4. Relationship between resistance to ruminal lipolysis (L - resistance) and the resistance to ruminal hydrogenation (H - resistance). Formaldehyde-treated supplements were prepared from sunflower oil/casein emulsions either spray dried (○) or flash dried (●) or from sunflower seeds (flash dried) (▲). Ruminal incubations were carried out for 20 hr as described in the text. 17:0 was added as an internal standard to calculate L – resistance as described in the text.
$y = 1.02 (\pm 0.14)x - 4.59 (\pm 12.23)$ $r = 0.96$

The observed hydrolysis of untreated supplements was incomplete after 3 hr (Fig. 5) partly due to the stereospecificity of the pancreatic lipase for the 1 and 3 positions

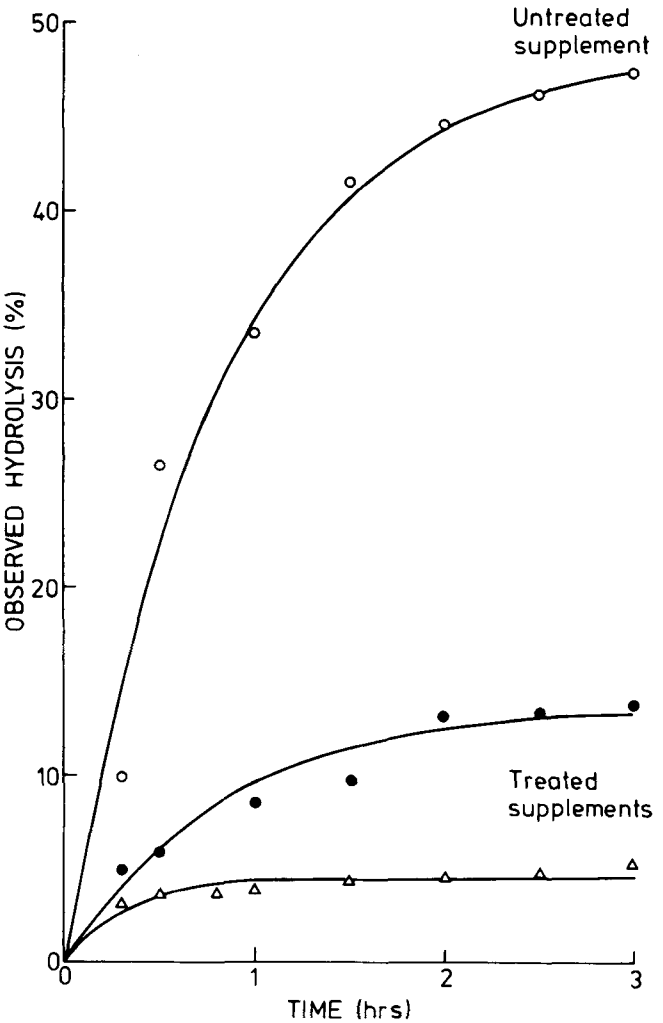

FIG. 5. Pancreatic lipolysis of triacylglycerol supplements. Formaldehyde-treated supplements were incubated with pancreatic lipase as described in the text. The untreated supplement (○) was spray dried sunflower oil/casein (2:1; w/w). The treated supplements were flash dried sunflower oil/casein (2/1; w/w) (△) or flash dried sunflower seed kernel (●) prepared as described in the text. All relationships were constrained to pass through the origin and are represented by the following equations:

Untreated (○) $y = 48.57 (\pm 2.39)[1-e^{-1.22(\pm 0.18)x}]$ $r = 0.99$
Treated (△) $y = 4.58 (\pm 0.22)[1-e^{-3.08(\pm 0.70)x}]$ $r = 0.96$
Treated (●) $y = 13.69 (\pm 1.07)[1-e^{-1.20(\pm 0.28)x}]$ $r = 0.97$

of triacylglycerol molecules. Consequently, as untreated supplements have an observed hydrolysis of 44% after 2 hrs, which corresponds to a resistance of 56%, the range of protection values obtained with this method must be limited to those supplements having protection levels greater than 65-70% in vitro, otherwise falsely high results will be obtained. This limitation will not affect the practical application of this procedure, as in our experience supplements with 20 hr in vitro protection values of less than 85% are generally unsuitable for feeding to ruminants due to large amounts of free fat present in the rumen or excessive hydrogenation resulting in poor efficiencies of utilization.

It is important to note that all of the in vitro methods for evaluating biological effectiveness were directly correlated with each other, and the correlation was on a 1:1 basis (Figs. 4 and 6).

Ruminal Hydrogenation in vitro

The extent of in vitro protection was calculated from the GLC analysis of the fatty acids extracted from abomasal digesta. The method is similar to that of Hogan et al.

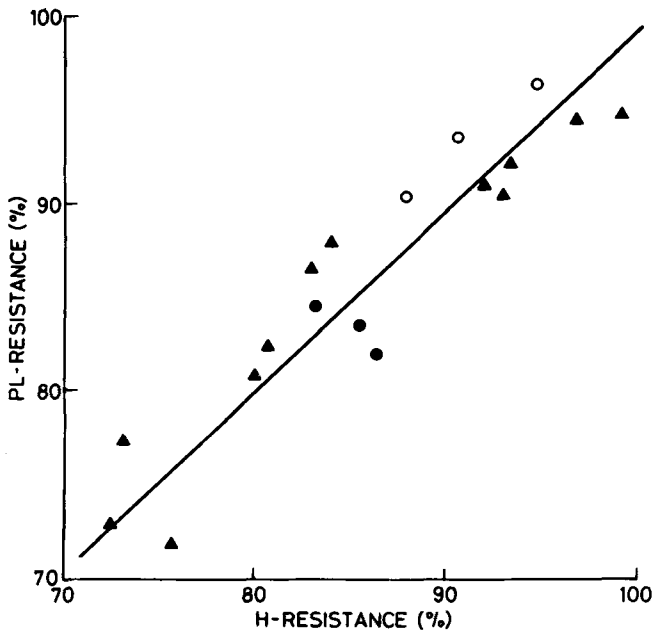

FIG. 6. Relationship between the resistance to pancreatic lipolysis (PL resistance) and the resistance to ruminal hydrogenation (H - resistance). Formaldehyde-treated supplements were incubated with pancreatic lipase for 2 hr and with rumen fluid for 20 hr. The supplements were prepared from sunflower oil/casein emulsions either by spray drying (○) or by flash drying (●) or by flash drying of sunflower seed emulsions (▲).
y = 1.09 (± 0.09x - 8.35 (± 7.73) r = 0.95

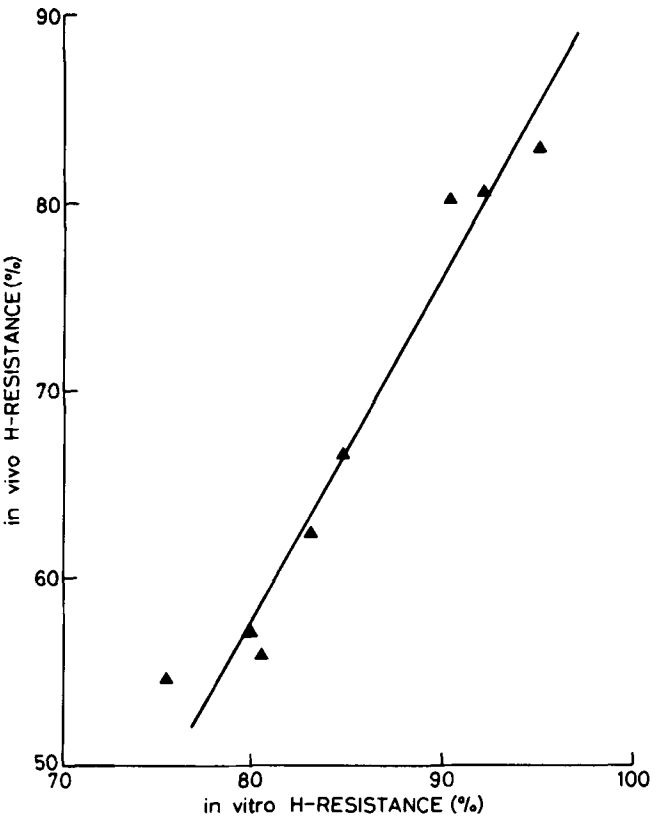

FIG. 7. Relationship between in vitro and in vivo resistance to ruminal hydrogenation. Supplements were all formaldehyde treated and contained sunflower oil. The in vivo H – resistance was measured by feeding and sampling from the abomasum as described in the text.
y = 1.80 (± 0.16x - 85.23 (± 13.89) r = 0.98

(11) and can be applied only to those supplements which contain relatively large amounts of polyunsaturated fatty acids.

When samples of abomasal contents were collected at various times after supplementation, it was found that the 18:2 content of the digesta remained constant after 8 hr post feeding. Two samples were taken at 8 and 24 hr post feeding, the mean 18:2 value was measured, and the in vivo resistance to hydrogenation determined. There was a significant correlation (r = .98, n = 8) with the corresponding in vitro resistance values (Fig. 7). It was apparent that the value for in vivo resistance was less than the corresponding value for in vitro resistance. The differences were greatest at lower in vitro levels, as the relationship between the two procedures was not 1:1 over the range of values determined here. These differences probably reflect the more efficient ruminal metabolism in the animal than in the in vitro system; other factors such as mastication could also be important.

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