# Bypass Fat Production Using Acid Oil, Its Effect on *In Vitro* Rumen Fermentation and Effect of Its Feeding on *In Sacco* DM Disappearance in Sheep

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**ABSTRACT** : Attempts were made in the laboratory to produce bypass fat using acid oil by precipitation and fusion methods. The degree of saponification by both of these methods was above 80 percent. Where heating facilities are not available, precipitation method could be used, otherwise, fusion method of bypass fat production is found to be more convenient, especially for commercial scale operations as handling of large volume of solutions is eliminated.

Bypass fat thus produced was tested *in vitro* for rumen fermentation. Incorporation of acid oil in the incubation medium reduced TVFA conc. from 127.06 to 124.09 mM/l SRL and increased ammonia-N levels from 210.50 to 223 mg/l SRL indicating that the microbial activity was affected on incorporation of acid oil in the incubation medium. However, incorporation of bypass fat in the incubation medium did not significantly affect TVFA conc. as well as ammonia-N levels.

In another experiment, nine rumen fistulated sheep in three groups of three each were fed bypass fat at two different levels. Dry matter disappearance in 24 h from the nylon bags suspended in the rumen of animals under different groups was found to be 47.74±1.10, 47.55±0.21 and 50.74±1.11 in group I (control), group II (fed bypass fat 50 g/day) and group III (fed bypass fat 100 g/day), respectively.

These studies indicated that it is possible to produce bypass fat from acid oils, a by-product of oil refining process, and its feeding did not affect rumen fermentation.
(**Key words**: Acid Oil, Bypass Fat, Rumen Fermentation, TVFA Conc., Ammonia-N)

## INTRODUCTION

Due to constant increase in the prices of oil cakes and other oil supplements in developing countries, their level of incorporation in the ruminants' ration is very low. As a result, energy density of feeds ingested by the high yielding animals is low, affecting milk yield and resulting into substantial body weight losses during first quarter of lactation. Scientists observed milk production response from 2 to 10 percent in cows fed concentrates containing 4 to 7 percent fat than in cows with control containing 1 to 3 percent fat (Lucas and loosli, 1944).

Use of bypass fat (calcium soaps) is widely described in literature for increasing the energy density of feeds in the diet of lactating cows as feeding fat as such above certain level affects fibre digestion in rumen (Scott et al., 1995; Baker et al., 1989 and Bines et al., 1978). Developing countries can hardly afford bypass fat feeding to animals, produced from edible oil, due to economic reasons. In view of this, attempts were made in the laboratory to produce bypass fat from acid oil, a by-product of oil refining process and is available in India at less than one third the price of edible oils.

Bypass fat produced in the laboratory from acid oil was tested for its effect on *in vitro* rumen metabolites and effect of its feeding on nylon bag dry matter disappearance from the rumen of sheep fed bypass fat, the results of these investigations are reported in this article.

## MATERIALS AND METHODS

### Preparation of bypass fat using acid oil:

Attempts were made to produce bypass fat in the laboratory by fusion and precipitation methods. The product thus produced was tested for degree of saponification with the procedure mentioned below.

First of all, total fatty matter was estimated in the product. For this purpose, one g sample was taken to which 30 ml distilled water and 30 ml of 6N HCL was added. Contents were refluxed for one hour. The contents were quantitatively transferred to a separating funnel to which 25 ml petroleum ether was added. The contents

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were shaken for 2 minutes, allowed to settle and aqueous phase was drained off to another funnel. Aqueous phase was reextracted with petroleum ether and the oil fractions were transferred to a preweighed beaker. Excess petroleum ether was evaporated from the beaker and the oil contents were weighed accurately which gave the total fatty matter in the product. The product was extracted as such with petroleum ether and the unsaponifiable oil content were thus measured. Unsaponifiable fatty matter was deducted from the total fatty matter to obtain degree of saponifiable fat.

**Fusion method of bypass fat production:**

Acid oil was directly heated with the calcium oxide/hydroxide to obtain bypass fat. Following attempts were made during these trials:

i) Fifty g acid oil was mixed with 50 g water, contents were stirred for 5 minutes. To this, 15 g CaO was added. A greasy product in the semi-solid state was produced with 20 percent saponification of fat.

ii) Eight g calcium hydroxide was added to 100 g acid oil. Contents were autoclaved at 4 kg per sq. cm. pressure for half an hour. The saponification of fat by this method was upto 30 percent only.

iii) Sixteen g calcium hydroxide was added to 100 g acid oil. Contents were heated to 160 degree celsius for 3 hours. The product formed was hard, crushed to powder form and with 85 percent saponification of fatty matter.

**Precipitation method of bypass fat production:**

As shown in table 1, various levels of sodium hydroxide and calcium chloride were tried to obtain optimum yield and degree of saponification. Fifty g acid oil was added in 500 ml. water and mixed. To this 100 ml of 10 percent sodium hydroxide solution was added. The contents were heated to boiling. While hot, 100 ml. 30 percent calcium chloride solution was added which resulted into precipitation of calcium soaps (by pass fat), which was washed with water, dried and crushed.

**Table 1.** Preparation of by-pass fat using precipitation method

| Quantity of acid oil (g) | Sodium hydroxide (g) | Calcium chloride (g) | Total output (g) | Degree of saponification (%) |
|---|---|---|---|---|
| 50 | 12 | 40 | 55 | 65 |
| 50 | 7.5 | 15 | 58 | 60 |
| 50 | 7.5 | 20 | 55 | 65 |
| 50 | 10 | 20 | 60 | 70 |
| 50 | 10 | 30 | 58 | 80 |

***In vitro* effect of acid oil or bypass fat on rumen metabolites:**

Forty ml. McDougal's (1948) buffer was taken in a conical flask to which 10 ml rumen liquor was added from a healthy sheep. To this, different substances were added as per the details given in table 2. After 6 hours of incubation, one drop of 6N sulphuric acid was added to stop microbial activity. Each treatment was tested in three replicates. The incubation medium was tested for ammonia-N and TVFA conc. as follows:

**Table 2.** *In vitro* ammonia-N (mg/l SRL) and TVFA conc. (mM/l SRL) using oaten chaff with acid oil or bypass fat as substrate (6 h incubation)

| Sl. No. | Particular | Ammonia-N (mg/l SRL) | TVFA conc. (mM/l SRL) |
|---|---|---|---|
| 1. | Rumen fluid `0' hr. | 196.25 | 100.57 |
| 2. | Rumen fluid `6' hr. | 230.50 | 111.48 |
| 3. | Rumen fluid + 2 g oaten chaff | 210.50 | 127.06 |
| 4. | As in `3' + 0.5 g acid oil | 227.50 | 130.24 |
| 5. | As in `3' + 0.5 g bypass fat | 211.25 | 143.41 |
| 6. | As in `3' + 1.0 g acid oil | 223.00 | 124.09 |
| 7. | As in `3' + 1.0 g bypass fat | 210.00 | 144.55 |

**Ammonia-N**: One ml. rumen liquor was taken from the incubation medium and distilled in Kjeldhal's apparatus for nitrogen. The nitrogen without digestion of rumen liquor represented ammonia-N fraction.

**TVFA conc.**: Total volatile fatty acids (TVFA) conc. was determined by using Markham's distillation method.

***In sacco* nylon bag DM degradability:**

Nine rumen fistulated Merino sheep were divided into three groups of three each with average body weight of approx. 38 kg. Animals in all the groups were offered 700 g oaten and lucerne chaff in the ratio of 70:30, alongwith 15 g mineral mixture. Animals in group I with basal diet alone served as control, whereas, animals in group II and III were fed bypass fat @50 and 100 g per animal per day.

After four weeks of feeding, nylon bags with 50 micron pore size in duplicate, containing approx. 3 g oaten chaff (2-3 mm size), were suspended in the rumen of animals for 24 hours, under three different dietary treatments. After 24 hours, the bags were taken out, washed gently unless clear water started flowing from them. These bag were dried in an oven at 60 degree celcius, to a constant weight. DM disappearance from bags under three different dietary treatments was measured.

## RESULTS AND DISCUSSION

### Manufacturing of bypass fat using acid oil:

Attempts were made to manufacture bypass fat using acid oil. Acid oil is a by-product of oil refining process which contains 60 to 70 percent free fatty acids, 15 to 20 percent triglycerides and 10 to 15 percent sterols. Acid oil is available in India usually at one third the price of edible oil and its main use is in manufacturing crude soap. For cost reasons, acid oil was considered as a basal material for production of bypass fat.

Heating acid oil with calcium oxide/hydroxide for 100 to 125 degree celcius for half an hour to one hour resulted into production of greasy product with only 25 to 30 percent saponification of fatty acids. These methods were attempted initially for economic reasons to know if it was possible to achieve high degree of saponification by heating the mixture of acid oil and calcium oxide at comparatively lower temperature and from half an hour to one hour. As these attempts didn't yield satisfactory results, acid oil was heated with calcium hydroxide at higher temperature and for longer duration (160 degree celcius for 3 hours). The degree of saponification was more than 85 percent.

At places, where there is no facility of heating at high temperature, precipitation method of manufacturing bypass fat was tried (Kulkarni, 1978). Various levels of sodium hydroxide and calcium chloride were tried (table 1) for obtaining optimum yield and with higher degree of saponification. When 10 g sodium hydroxide was used to form sodium soaps with 50 g acid oil and 30 g calcium chloride was used to replace sodium, bypass fat yield was 58 g and degree of saponification was upto 80 percent.

However, the disadvantage with this method seems to be the difficulty faced in handling high volumes for production of small quantity of bypass fat and disposal of alkali solutions is a problem from pollution point of view.

### *In vitro* TVFA conc. and ammonia-N levels in strained rumen liquor (SRL) using acid oil or bypass fat:

In this experiment, ammonia-N and TVFA conc. was estimated in SRL after 6 hours of incubation as per the treatments mentioned in table 2. As shown in table 2, ammonia-N level in SRL was high when acid oil was added in the incubation medium. This indicated that the acid oil hampered efficient utilization of ammonia-N for microbial protein synthesis. Whereas, when the acid oil was incorporated in the form of bypass fat, the microbial activity in the incubation medium remained unaffected.

TVFA conc. in SRL was lower on incorporation of acid oil, whereas, it remained unchanged on incorporation of bypass fat. This again confirmed that the acid oil was interfering with the microbial activity in the incubation medium while the bypass fat of acid oil origin did not affect microbial activity. This experiment indicated that the bapass fat of acid oil origin was inert in the incubation medium and did not affect microbial activity or the fibre digestion.

It has been reported that the addition of fat to ruminant diets depresses fibre digestibility in both cattle and sheep (Ward et al., 1957 and Brooks et al., 1954). Devendra and Lewis (1974) summarized four theories to explain this effect: (i) Physical coating of fibre to prevent microbial attack; (ii) modification of the rumen microbial population from possible toxic effects of fat on certain micro-organisms; (iii) inhibition of microbial activity from surface active effects of fatty acids on cell membranes; (iv) reduced cation availability from formation of insoluble complexes with long chain fatty acids.

This has also been reported that the development of a process for encapsulation of lipids in a formaldehyde treated protein allows the feeding of large quantities of lipids to ruminants without adversely affecting the rumen fermentation functions (Scott and Hills, 1975). Klusmeyer et al. (1991) fed four Holstein rumen fistulated cows with varying level of protected fat alongwith forage based diet. They have reported that the inclusion of protected fat (bypass fat) in the diet did not affect volatile fatty acid production, ammonia-N and solids dilution rate in the rumen. Thus, bypass fat can be used for increasing the energy density of conventional diets, without affecting rumen fermentation.

### Dry matter disappearance from nylon bags suspended in the rumen of sheep fed bypass fat at two different levels:

Dry matter disappearance from the bags suspended in the rumen of animals under different dietary treatments was estimated which was $47.74 \pm 0.21$, $47.55 \pm 0.21$ and $50.74 \pm 1.11$ in groups I, II and III respectively which were non-significantly different from each other (table 3). These observations once again confirmed that the supplementation of bypass fat in the diet of ruminants does not seem to be affecting rumen fermentation.

Table 3. Effect of feeding bypass fat to sheep at two different levels on nylon bag DM disappearance

| Treat-ment | Feed intake (g) | | Bypass fat intake (g) | % DM disappearance from nylon bags |
|---|---|---|---|---|
| | Oat hay | Lucerne hay | | |
| Control | 490 | 210 | nil | *47.74±1.10 |
| T1 | 490 | 210 | 50 | *47.55±0.21 |
| T2 | 490 | 210 | 100 | *50.74±1.11 |

* Each figure in average of six observations.

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