A STUDY OF SOME CHARACTERISTICS OF ANTI-BLOAT PASTES RELATING TO THEIR BLOAT CONTROL EFFICACY

A thesis presented in partial fulfilment
of the requirements for the degree of
Master of Veterinary Science at
Massey University

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1977

ABSTRACT

Factors relating to the bloat-controlling efficacy of several anti-bloat pastes were studied.

Firstly, the detergent diffusion rates of various pastes were measured in a rumen model. In comparison with the liquid detergent control, some formulations were found to markedly slow the washout of detergent from the model.

A theoretical paste dosing schedule versus liquid dosing schedule, based on the rumen model calculations, revealed several advantages of the paste formulation over the liquid. For example, the paste maintained a more steady detergent concentration and extended the interval between dosings.

An investigation of the effects of administration to the live animal on the intactness of the paste bolus when delivered at the cardia was undertaken. It was found that the reaction of the animal to the dosing procedure had a strong influence on the degree of intactness of the paste bolus entering the rumen. A difference between animals in this respect was observed.

It was shown that fragmented boli collected at the cardia dissolved more quickly than intact boli when subjected to mild agitation. The consequences of this in relation to persistence of protection for bloat were discussed.

The decay curves of Poloxalene detergent administered to steers in either a liquid or a paste formulation were calculated and compared. As a standard control against which other materials might be compared, the concentration decay curve of a water soluble rumen marker (polyethylene glycol, 4000) was determined in each steer.

It was found that the paste formulation did not slow the washout of detergent from the rumen by more than about two hours. Also, the average concentration dilution rates of detergent and PEG 4000 were found to be similar. However, at high Poloxalene dose rates,

the endogenous water inflow to the rumen was found to increase markedly which in turn influenced the average detergent dilution rate.

The diffusion rate curves of Poloxalene paste either in the rumen model or *in vivo* showed striking similarities. This suggested that the rumen model might be a valuable screening system for new paste formulations.

Several field trials were undertaken to test whether the bloat-controlling efficacy of paste formulations of detergent was better than that of liquids, and to examine the validity of the rumen model findings. Unfortunately, the bloat challenge on each occasion was insufficient to collect the necessary data. However, valuable information regarding the requirements for satisfactory field trials was obtained and are discussed.

In summary, the results of the study showed that:

1) Pastes can be formulated which, relative to similar liquid preparations, will slow the rate of detergent washout from a rumen model.
2) The *in vivo* detergent decay curves of both Poloxalene paste and liquid were similar to those determined *in vitro*. Together with (1) above, this suggests the rumen model might be a useful screening system.
3) Paste boli delivered at the cardia can vary widely in intactness between and within animals and this depends largely on animal reaction to the dosing procedure.
4) Fragmented boli dissolve more quickly than intact boli *in vitro* and this characteristic may be crucial in detergent longevity in the rumen liquor.

Therefore it is concluded that:

1) The rumen model warrants development and testing.
2) An efficient analytical method needs to be developed for detecting detergents in rumen liquor.

This would allow accurate *in vivo* detergent decay curves to be established. Without this, further progress will be slow.

3) Field trials must still be carried out to provide and confirm relationships between *in vitro* results and field efficacy.

ACKNOWLEDGEMENTS

This study was undertaken whilst in receipt of a bursary from Merck, Sharp and Dohme (N.Z.) Ltd. The generosity and assistance from the Company as a whole is gratefully acknowledged. However, I wish to especially thank two of the Company's staff, Mr. Peter Scott for his freely given assistance and Dr. Keith Baker who prepared the paste formulations and tried desperately to develop a suitable analytical method for detergents in rumen liquor.

Special thanks must go to my supervisors, Dr. C.S.W. Reid of the D.S.I.R., Palmerston North, and Dr. B.S. Cooper of the Veterinary Clinical Sciences Department, Massey University. Both men readily gave up their time when needed and their criticisms, interest and encouragement is specially appreciated.

I wish to thank two good friends, Mr. J. Kook and Mrs. A. Russel from the D.S.I.R., Palmerston North, for their interest in the work and their help during field trials.

The generosity of those farmers in the Manawatu who allowed the field trials to be carried out on their properties is also appreciated. In addition, I would like to thank Mr. S. Sim and his staff at the No. 4 Dairy Unit, Ruakura Animal Research Station, Hamilton, for the assistance given in carrying out a field trial on the farm

Thanks also go to Mr. T.W. Law for his photographic assistance and to Mrs. O. Healey of Palmerston North for her excellent preparation of this manuscript.

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INTRODUCTION

The ruminant has a unique digestive tract with a large pre-gastric fermentation chamber. As such, it is particularly prone to bloat and this susceptibility appears especially in the domesticated bovine species.

This often fatal condition can be prevented by the administration of anti-bloating agents. Several methods of administering these materials have been developed; each with the aim of ensuring adequate anti-bloat material is present in the rumen when the animal is most at risk. To be confident that cattle will be protected it is necessary to drench animals with liquid preparations every twelve hours and even then an excessive bloat challenge or unusual individual susceptibility might result in fatalities.

The necessity for repeated dosings at short intervals is that food, water and saliva continually enter the rumen while digesta leaves and this turnover results in dilution of any administered medicines. In addition, chemical breakdown, microbial breakdown or absorption from the rumen, may further reduce the concentration of the material in the rumen liquor.

To improve the persistence of an effective concentration of anti-bloat agent in the rumen liquor, and thereby perhaps extend the necessary dosing interval, some means has to be found of slowing down the washout of the bloat preventive. This might be achieved by incorporating the anti-bloat detergent in a semi-solid base from which it could diffuse into the rumen liquor at a constant rate. Several such depot systems have been under development in recent years and these include the anti-bloat capsule, the anti-bloat gel ring and anti-bloat pastes. The latter are stiff, grease-like materials incorporating anti-bloat detergent and they can be administered to cattle using a specially designed gun.

If any form of depot system, particularly one as simple as a paste formulation, could enable the dosing interval to be extended safely to once every second day

or even once daily, there would be obvious economic and practical advantages to dairy farmers.

The purpose of this study was to investigate some characteristics of experimental anti-bloat pastes which might affect the bloat controlling efficacy of the formulations.

LITERATURE REVIEW

Bloat (Ruminal Tympanites) is the sign of an excessive accumulation of gas in the first two compartments (reticulum and rumen) of the ruminant stomach.

The disorder occurs in all ruminants but is most common in cattle and in this species may have a high mortality. It is particularly common in countries where year-round grazing is practiced such as New Zealand and Australia. In the U.S.A. and Great Britain it occurs most commonly on newly developed highly productive pastures and as a consequence is a deterrent to the development and utilisation of such pastures. It is comparatively uncommon in sheep in these countries.

The disorder occurs in two forms. The less common is secondary bloat which occurs sporadically and is due to extra-gastric factors such as obstruction to the normal eructation of gases due to solid objects lodging in the oesophagus, e.g. apples, turnips.

The other and major cause of bloat is gastric in origin and is termed primary bloat. This occurs in cattle at pasture (pasture bloat, legume bloat) or on dry concentrate feeds (feed-lot bloat) as a result of foaming of the digesta.

The aetiology of the disorder is complex and not fully understood but the environment of the plant, characteristics of the animal and the state of the ruminal microbial population all play a role.

In New Zealand, the incidence of bloat increased from 0.22% of all dairy cows in 1938-43 to 0.74% in 1961-62. It has since fluctuated between 0.30% and 1.20% each spring (Reid, 1973).

Unlike other causes of cattle wastage in which the incidence increases with age, deaths due to bloat occur more commonly in young cattle (Clifford, 1964).

In recent years, the total number of dairy cows has decreased in New Zealand whereas the number of beef animals has increased. Nevertheless, bloat is still a

serious disease of dairy cattle and as yet is not a great problem in beef cattle or in sheep (Clarke and Reid, 1974).

Bloat can be acute or chronic. In acute cases, death is the likely outcome if treatment is not given quickly, whereas in chronic bloat, the chance of death is less likely. However, the feed intakes of chronically bloated cattle are reduced, resulting in lowered milk production in dairy and beef cows and reduced live weight gains, which is particularly important in beef herds (Scott, 1965; Wolfe and Lazenby, 1972).

Bloat in New Zealand occurs predominantly on white and red clover-dominant pastures, particularly when the plants are at the young, lush, rapidly growing stages. The condition has also occurred on subterranean clover, lucerne and lush grass pastures (Johns, 1954; Reid and Johns, 1957; Jones and Lyttleton, 1971).

The Cost of Bloat

A recent estimate of the monetary cost of bloat to the New Zealand dairy industry was $8,000,000 per annum (Clarke and Reid, 1974). In addition there are other costs which cannot be accounted for in terms of money. These include such matters as the mental and physical strain on the farmer, the disturbing effects on farm management, the nullifying effects on production when bloaty pasture cannot be efficiently utilised, the slowing down in pasture improvement for fear of bloat, and the loss of confidence in the industry (Ayre-Smith, 1971).

Known Causes of Bloat

As a result of the normal fermentation processes occurring in the rumen and the acidification of salivary bicarbonate ions in the rumen, there is a large volume of gas produced which has to be removed, principally by eructation and absorption. The rate of gas production varies between 0.20L/min in the fasting animal to 2.0L/min following feeding (Clark and Reid,

1974). The composition of the gas is mainly carbon dioxide (45-70%) and methane (20-30%) with smaller amounts of oxygen, nitrogen, hydrogen and hydrogen sulphide (Washburn and Brody, 1937; Hoernicke, et al., 1964).

Under normal grazing conditions, bubbles of free gas form in the digesta and rise to form free gas pockets in the dorsal regions of the rumen. From here the gas is removed by eructation associated mainly with secondary ruminal contractions (Weiss, 1953; Dougherty et al., 1958; Reid and Cornwall, 1959; Stevens and Sellers, 1959; Dougherty, 1961; Iggo and Leek, 1970).

It is common for foaming of the digesta to occur in the rumen but normally such foam is small in quantity and of low persistence. In bloat, a stable foam develops (Mangan, 1959; Reid, 1960; Jones and Lyttleton, 1969). This particular foam is essentially proteinaceous, of high persistence, and it collects in large volumes so that the gas separation phase is slowed. When the animal attempts to eructate, frothy digesta enters the oesophagus where the bolus induces reflex swallowing. A sequence of events then commences in which the increased intra-ruminal pressure leads to interference with respiration and circulation; these in turn culminating in hypoxic hypoxia, hyperkalaemia and death.

In bloating animals, the small gas bubbles rising through the digesta fail to coalesce probably because of the physico-chemical nature of the surface active materials at the gas-liquid interphase and the increased viscosity of the rumen contents. Soluble plant proteins appear to be a major component of the surface active complex (Mangan, 1959; Jones and Lyttleton, 1969; Laby and Weenink, 1966; Jones and Lyttleton, 1973; Laby, 1975) and are considered to be the main plant factor primarily responsible for the stabilization of the foam.

Other plant factors undoubtedly play some role as surfactants in the foaming of ruminal digesta but in most cases a relationship between the concentration of

these substances in the plant and the onset of bloat has not been firmly established. Such secondary surface-active substances include saponins, pectins, plant carbohydrates and lipids (McWilliam, 1973; Clarke and Reid, 1974).

Tannins, which are special types of phenolic compounds of plant origin, are strong protein precipitants and some plant species contain high concentrations of condensed tannins. These plants do not cause bloat (Jones, et al. 1970; Reid et al. 1974).

Susceptibility to Bloat

Cattle can vary widely in their susceptibility to bloat. The range extends from the highly susceptible (HS) animal which bloats readily and frequently when conditions favour foam formation, to the almost resistant animal (low susceptibility, LS) which rarely if ever bloats (Clarke and Reid, 1970). The similarity in bloating behaviour of identical twin cattle suggests a genetic basis for susceptibility. Other evidence of a genetic influence includes differences in susceptibility between cattle breeds and the occurrence of bloat-prone families within a breed (Knapp, et al., 1943; Johns, 1954; Reid, et al., 1972; Reid, et al., 1974: McIntosh and Cockrem, 1977).

The mechanisms by which genetic predisposition is translated into an individual have not yet been fully defined but their final site of effect appears to be within the rumen itself. This proposition is supported by the fact that bloat susceptibilities are temporarily exchanged when the rumen contents of an HS and LS animal are interchanged by way of rumen fistulae (Clark and Reid, 1970).

An obvious difference between individuals may exist in the character of saliva and at present work is being directed at the role saliva might play in bloat. Such factors as composition and secretion rate may be important. Saliva contributes bicarbonate and phosphate ions which help buffer pH changes in the rumen. No

difference has been found though in the buffering capacity between the rumen contents of HS and LS animals, either before or after feeding (Mendel and Boda, 1961). Acidification of bicarbonate ions is an important source of CO_2 gas in the rumen and HS animals have been found to secrete a greater amount (not volume) of bicarbonate ions than LS animals (Mendel and Boda, 1961).

Saliva is now known to exhibit both foaming and anti-foaming properties. The mucoprotein secreted in bovine saliva is responsible for the thick nature of mucous saliva and is a major factor of the soluble foaming constituents (Lyttleton, 1960; Jones and Lyttleton, 1973). In contrast, salivary mucin has been found to exhibit antifoaming properties (Van Horn and Bartley, 1961). However, the full role of saliva in bloat has yet to be determined.

No firm relationships have been established between bloat susceptibility and other factors such as rumen fluid pH, the rate of fermentation or the rate of fluid turnover in the rumen (Mendel and Boda, 1961).

An important factor in the development of bloat appears to be the composition and the state of the rumen contents before feeding (Laby and Weenink, 1966; Howarth, 1975). The prefeeding rumen contents from LS animals have more coarse fibrous residues whereas the contents of HS animals prior to feeding consist of finely divided particulate matter. These differences in consistency seem to relate to differences in feed intakes (Clark and Reid 1970; Clark and Reid, 1974).

The role of the rumen's microbial population in the generation of bloat is unclear. However, the bursting of holotrich protozoa during feeding can add easily foamed protozoal protein to that derived from plants (Clark, 1965a; 1965b). This extra material may tip the balance towards overt bloat as the addition could raise the protein concentration in the rumen fluid to a threshold, critical for foam formation.

One,2 - dimethyl - 5 - nitroimidazole (dimetridazole) is an anti-protozoal agent which has been used to

remove the holotrich ciliates from the rumen (Clark, 1966). It was found that after defaunation bloat still occurred and it was concluded that although holotrichs are not necessary for bloat, the incidence and severity may be increased in their presence (Clark and Reid, 1974).

Differences in the rumen content fermentation rates between HS and LS animals have not been demonstrated conclusively (Clark and Hungate, 1971). Also, excessive gas production itself is not proven as a cause of bloat (Hungate, et al., 1955) and overproduction of acid is unlikely because of the neutralising effect of the protein and polyuronides in legumes (Hungate, 1965).

Although excessive gas production is not proven as a cause of bloat, the production of gas must be sufficient to cause foaming of the digesta. Soluble carbohydrates are the important substrates of microbial fermentation and as such their persistence and extent of exposure to sugar fermenting organisms in the rumen must be sufficient to allow them to be metabolised and produce sufficient gas to cause foaming (Clark and Reid, 1974). If substrate is in excess, holotrich ciliates may burst following excess starch storage as mentioned above. The release of their cell contents can contribute to both increased fermentation and foaming of the rumen contents. This is the basis of the protozoal catastrophy theory of Leng (1973).

The only major bacterial difference so far demonstrated in HS and LS cattle is the higher number of mucinolytic bacteria demonstrated in HS animals (Mishra, et al., 1968). This may result in an increased removal of salivary mucin, the possible anti-foaming component of saliva.

Until the complex interactions of plant, animal and microbial factors leading to the formation of a stable foam in the rumen and resulting in bloat are more fully understood, the problem has to be controlled, and where possible prevented, by empirical means.

Control of Bloat

Several methods of control and prevention of

bloat have been devised and are practiced with varying degrees of success. The method adopted in any one situation is dependent upon such things as:

1) Pasture composition
2) The intensity and prevalence of bloat
3) The facilities, which the farmer has, to deal with the problem
4) The economic constraints on therapy
5) The stock management and grazing practices of the farmer.

As bloat is most commonly associated with the ingestion of rapidly growing legumes on apparently all soil types and under various fertiliser regimes, a basic approach to the problem is to improve pasture management. Here, the aim is to dilute the intake of legumes by increasing the growth of the associated grasses in the pasture and then ensuring that the increased grass production is eaten at the same time as the lush legume growth. (Hancock, 1953, 1954; Sears, 1953). The introduction of short rotation ryegrass with its earlier spring growth and the proper use of electric fences to control grazing were major steps forward in achieving control.

Other systems of reducing the clover intake of animals have been used such as allowing the herd access to a roughage, for example, hay, prior to grazing pasture (Cole, et al., 1945) and on-off grazing schedules whereby the herd is allowed to graze for short periods interspersed with periods off the pasture.

However, it is not always possible to completely control bloat by the grazing management; there may be no safe pasture on the farm, or conserved feeds may have been exhausted. In such cases, short term preventive measures have to be used. These are based on the administration of a number of different materials collectively called anti-bloat agents. These range from the anti-microbial compounds, penicillin and dimetridazole, through the anti-foaming oils (e.g. paraffin) to the surface active non-ionic materials such

as the "Pluronics" (Wyandotte Chemical Corporation, Wyandotte, Michigan).

The anti-microbial compounds are not used routinely as bloat preventives. Their action is short lived and resistance develops quickly (Moore, et al., 1957; Clark, et al., 1969).

The anti-foaming oils have a relatively short-lived effectiveness also (Reid and Johns, 1957) but resistance does not develop. Paraffin oil is very widely used as a bloat preventive and is administered to cattle in several different ways.

The synthetic surfactants (non-ionic detergents) lower the surface tension of the bloat foam but how they prevent bloat is not fully established. Smaller doses protect animals for a longer period of time than do the anti-foaming oils. This is thought to be related to the fact that a large proportion of the administered dose is adsorbed onto the solids in the rumen contents and from here slowly released back into the fluid fraction. Another hypothesis is that the synthetic surfactants may activate dietary fats (naturally occurring anti-foaming agents) by releasing them from sequestration (Clark and Reid, 1974; Laby 1975). This is supported by the fact that Laby has found detergency and wetting power of anti-bloat detergents to correlate better in bloat prophylaxis than do foaming properties (Laby, 1975).

The concentration of any medicament in the rumen continually declines because of one or more of the following: breakdown by micro-organisms, absorption from the rumen, adsorption onto particulate matter, and washout because of the water flow through the rumen. Water enters the rumen in feed, drinking water, saliva and by secretion across the rumen wall.

The salivary inflow has been estimated at 25 to 90L. per 24 hours in cattle (Kay, 1966). The total water flow through the rumen in 24 hours can vary widely but Hyden (1961) gave estimates of 150 to 170L. This can represent an hourly flow rate of 8 to 30% of fluid volume per hour (Corbett, et al., 1959). These figures

can vary according to diet and whether or not the cows are lactating. Reid (pers.comm.)has found similar water turnover rates to those mentioned.

The relative importance of those factors contributing to the dilution of materials in the rumen can vary depending on the material. For example, adsorption onto particulate matter plays a more important part in the dilution of the non-ionic detergents than it does in the dilution of the anti-foaming oils.

Therefore to maintain an effective concentration of anti-bloat agent in the rumen, the animal must be dosed or have access to the preventive regularly.

There are a number of ways of administering anti-bloat agents. The aim of all methods is to get enough medicament into the rumen to reach a concentration that will prevent foaming and to ensure the concentration does not go below the effective level over the period when the animals are placed at risk. However, the methods vary widely in the degree to which they succeed.

The methods that have been used include: drenching, pasture spraying, flank application of anti-bloat agent, water-trough treatment and the use of anti-bloat lick blocks.

The most reliable method of legume bloat control is to drench all cows with anti-bloat material twice daily. The majority of cattle readily come to accept this régime (Flynn, 1965; Scott, 1965; Reid, 1974). Oral drenching is a time consuming and costly chore for which medicinal paraffin oil and the non-ionic detergents are most often used.

The pasture may be sprayed with an anti-foaming oil prior to grazing. This method ensures that dangerous pasture when eaten will be accompanied by anti-bloat material. It is also one of the more reliable methods provided cattle do not have access to unsprayed pasture (Reid,1955; Reid,1958; Johns,1963; Flynn,1965).

For this method, mixtures of emulsified tallow and paraffin oil are commonly used. There are two disadvantages of this method; one is the cost of spraying equipment, and

the other is the limitations of spraying on ground other than flat land.

Application of bloat preventives to the animal's flank with a paint brush, squeeze bottle or with automatic applicators, is a widely practiced method (Reid, 1973). However, under severe bloat challenges the method is liable to frequent failures because the success of the method depends on the animal licking off sufficient medicament to reach and maintain an effective concentration in the rumen (Flynn, 1965; Scott, 1965; Flynn, 1976a). Similar criticisms can be levelled at the practice of adding bloat preventives to roller drums in troughs containing molasses as an attractant. This is a common practice in some of Australia's beef cattle grazing regions (Langlands and Holmes, 1975).

A further method depending on animal behaviour for its success is the use of lick blocks incorporating the anti-bloat agent. To be effective, all animals must lick a sufficient amount to provide protection; obviously, block palatability is all-important (Barr and Day, 1977).

The addition of oils to feed supplements such as meals, has had limited success (Bartley and Meyer, 1967; Scott, 1965). In New Zealand animals will ignore or refuse such supplements.

An alternative method is to add the bloat preventive to the drinking troughs to which the animals have access throughout the day or night. But again, the method relies for its success on animal behaviour, this time on the drinking habits of individual animals. The amounts of agent ingested can be variable between and within animals from day to day (Phillips, 1968a; Phillips, 1968b; Langlands and Holmes, 1975). Water sources other than the trough water, e.g. ponds, drains must be fenced off. Failures occur in wet weather and on very succulent pastures when water drinking is low.

Medicaments Used for Bloat Control

Materials such as paraffin oil and tallow have

been used either in the raw or emulsified state for many years. They do not adversely effect the ruminal microflora or the animal's production. They are believed to inhibit foaming and to breakdown bloat foam if it has formed; freeing trapped gases (Reid and Johns, 1957). They are relatively inexpensive and widely used (Reid, 1974).

The most effective anti-bloat agents currently in use are the non-ionic detergents. They are widely used in New Zealand, Australia, Canada and the U.S.A. The materials most commonly used are the polyoxypropylene-polyoxyethylene block copolymers, "Pluronics" L62 and L64 (Wyandotte Chemical Corporation, Wyandotte, Michigan). Also used is nonyl-phenol ethoxylate, "Marlophen 89" (Chemische Werke Hüls). More recently the alcohol ethoxylate series of detergents (I.C.I.A.N.Z.) were found to be biodegradable, unlike the Pluronics, and also more palatable to cattle (Laby, 1973; Howarth, 1975).

The major advantages of the synthetic surfactants over the anti-foaming oils are:

1) A smaller dose requirement
2) A longer persistence of activity.

These two favourable properties suggest that the compounds have a higher potency and their mode of action differs from that of the anti-foaming oils (Clarke and Reid, 1974).

Recent Developments in Bloat Prevention

Systems based on the administration of water-soluble "inert" substances such as detergents are theoretically inefficient. The rate of loss of inert water soluble substances from the rumen is exponential (Hyden, 1961). It follows that the amount of medicament that must be given at one dosing to ensure an effective quantity still remains in the rumen at the time of next dosing, will increase exponentially as the time between dosings increases. This wasteful situation could be avoided if a "depot" of medicament were to be introduced into the rumen and there released the medicament at the

same rate at which it is lost from the rumen.

Several systems based on this idea have been studied overseas (Laby, 1975). These include the administration of detergents in gel form, plastic capsules containing surfactant in gels, and of gel rings.

Gel drenching involves the dosing of a stiff grease-like material incorporating surfactant and from which the surfactant slowly diffuses (Laby, 1973; Flynn, 1976b). Field trials have indicated that gels might reduce the frequency of dosing to once a day.

Plastic capsules have major problems of administration but have been found to be effective for up to three weeks in experimental trials (Laby, 1973; Woodruffe et al., 1972).

The anti-bloat gel ring is a low cost adaptation of the capsule. These compressible gel rings stiffen in the rumen and thus prevent loss by regurgitation (Laby 1973; Woodruffe, et. al., 1972).

The depot idea is still being explored in terms of both materials and physical systems. Apart from more efficient use of the active ingredient, a major goal is to reduce the period between successive dosings. If successful, such a system would provide the cattle farmer with an economic and p ractical means of defence against the bloat problem.

CHAPTER 1

An investigation of the in vitro diffusion rate of detergents from anti-bloat pastes.

INTRODUCTION

When a bovine animal is drenched with a liquid anti-bloat detergent, the maximum detergent concentration in the rumen is reached soon after drenching. A sufficient amount must be given so that the detergent concentration in the rumen will remain above the minimum effective detergent concentration necessary to prevent bloat, long enough to provide protection over the critical periods. From the point of maximum detergent concentration in the rumen, there is a decline in concentration until it falls below the minimum effective concentration and protection against bloat is no longer afforded.

To reduce detergent losses by the "washout" process, it is necessary to protect or sequester the detergent in a "depot" in the rumen. Such a "depot" could take the form of a stiff paste incorporating the anti-bloat detergent. A slow release of the detergent from the paste would continue to replenish the detergent washed out from the rumen. This would be analogous to the use of the cobalt bullet to control disease associated with cobalt deficiency in cattle and sheep (Andrews, 1971).

The duration of protection against bloat would then be dependent on how long the detergent concentration could be maintained above the minimum effective level.

The work to be reported here concerns investigations of the efficacy of some experimental anti-bloat pastes formulated in Australia.

The first section involved an *in vitro* study of the performance of the pastes under controlled conditions in a rumen model.

This section had four objectives:

1. To determine whether there were any differences in detergent diffusion rate from the various paste formulations.
2. To discover if any of the differences could be related to differences in composition and method of preparation of the pastes.
3. To select one paste on the basis of its detergent releasing characteristics and to propose a theoretical dosing schedule that might be effective in the live animal.
4. To compare persistence in the rumen model of detergents released from pastes, with a liquid formulation of the same detergents.

EXPERIMENTAL DESIGN

Using a rumen model which allowed control of temperature, agitation and water turnover rate, the rate of diffusion of detergent from several anti-bloat paste formulations was studied. These were compared with the decay rate of a liquid formulation of the same detergents.

MATERIALS AND METHODS

Materials

Six anti-bloat pastes were formulated by Merck, Sharp and Dohme (Aust.) Ltd. as shown in Table I. 2.

Preparation of the Pastes

The two pastes, KB_3/66A and 32A, were prepared by dispersing the gelling agents (Ethylcellulose, KB_3/66A; and Volclay 325, KB_3/32A) in their molten Terics first and then in the case of KB_3/32A, adding the Antifoam A, followed by the water.

The other three pastes containing Teric detergents, KB_3/32B, 34A and 34B were prepared by dispersing the gelling agent (Volclay 325) in hot water first and then adding the Antifoam A, followed by their molten Terics.

In the case of $KB_6/16$, the method of preparation was similar to $KB_3/66A$: that is, the ethylcellulose was dispersed in the molten Poloxalene first.

Physical Characteristics of the Teric Pastes

The melting points and the consistency values obtained by MSD chemists are shown in Tables I. 3 and I. 4.

Rumen Model

The rumen model consisted of a measuring cylinder containing 600 ml of water kept at 39.5°C in a water bath.

Agitation was provided by a brass disc 6.5cm in diameter with four 1.0cm diameter holes in it, connected by a brass rod and string to a motor driven crank. This caused the disc to rise and fall 9cm, forty times a minute in the cylinder.

The samples of paste were held in small 20 gauge stainless steel baskets (2cm x 2cm) which were attached by clips to the brass rod 1.5cm above the brass disc (Fig. I.1).

Turnover of water in the rumen model was provided by a peristaltic pump arranged to deliver water to the cylinder and to remove fluid containing diffused detergent away from the cylinder at the same rate. The turnover rate could be adjusted by changing the bore size of the pump tubing.

The method of sampling was as follows: The fluid removed from the cylinder (the effluent) was collected in a beaker in timed periods and the volume collected per time period was noted. An aliquot of this volume was taken and the detergent concentration was determined using an appropriate in vitro assay method for Teric or Poloxalene detergents. In this way curves describing the release of detergent from each of the pastes were determined.

Four units were set up allowing four materials to be examined simultaneously (Fig. I. 2).

Experimental Programme

The experiment was divided into three separate runs, A, B and C. The groups of formulations, the water turnover rates and the durations of each run are shown in Table I. 1.

TABLE I.1

The experimental variables of runs A, B and C

Run	Formulations Tested	Starting Wt. of Detergent[a] mg.	Water Exchange Rate ml.h^{-1}	%h^{-1}	Duration of Run h
A	KB_3/32A	700	55	92	11.50
	32B	"	"	"	12.00
	34A	"	"	"	"
	34B	"	"	"	"
	66A	"	"	"	"
	Liquid Teric[b]	"	"	"	"
B	KB_3/32A	1400	115	192	26
	32B	"	"	"	34
	34A	"	"	"	"
	Liquid Teric	"	"	"	22
C	KB_6/16	700	72	12	8
	Liquid Poloxalene[c]	"	"	"	"

a The size and shape of the paste samples placed in the mesh baskets was kept the same within runs so that the surface area of paste exposed for dissolution remained approximately the same.

b The liquid Teric control formulation had the same proportion of Terics 12A3 and 12A23 (1.5:1) as in the pastes but contained no gelling agent or water. It did contain Antifoam A at 2% w/w.

c The liquid Poloxalene control formulation was 100% liquid Poloxalene detergent

Analytical methods

1. In vitro assay of Teric detergents

Apparatus : 250 ml stoppered Erlenmeyer flasks
10 ml pipettes
A small bench centrifuge
25 ml measuring cylinders
A spectrophotometer capable of reading at 620nm

Reagents : Sodium chloride, R - grade NaCl
Chloroform
Ammonium cobaltothiocyanate reagent - made up by dissolving 280g of cobaltous nitrate hexahydrate and 620g of ammonium thiocyanate in water and making the volume up to exactly I.CL.

Procedure : i) An aliquot of the effluent from the cylinder, containing 0-10mg of Teric 12A3 + 12A23, or 0-6mg of Teric N8 + GN15, was pipetted into an Erlenmeyer flask and the volume made up to 50 ml with water.

ii) 17g of NaCl was added and dissolved by shaking.

iii) 15 ml of ammonium cobaltothiocyanate reagent was added and the flask shaken vigorously for 5 minutes and again after 15 minutes.

iv) 10 ml of chloroform was added and the flask shaken vigorously for 5 minutes and allowed to stand for 5 minutes.

v) The aqueous layer was removed by aspiration and any water cloud in the organic layer was removed by centrifugation up to 2000 rpm for 2 minutes.

vi) The organic layer was transferred to a 10 ml spectrophotometer tube and the absorbance was read at 620nm against a solvent blank

vii) A standard calibration graph was prepared by assaying known concentrations of Teric 12A3 + 12A23 and Teric N8 + GN15 as described above.

All detergent concentrations were expressed as mg of detergent per 10ml of effluent.

NB Because of the non-specificity of the in vitro detergent assay methods, for both Teric and Poloxalene detergents, care was taken to avoid contaminating glassware etc. with laboratory

detergents. All glassware etc. was rinsed in hot water, a sulphuric-hydrochloric acid wash, ethanol and distilled water prior to use.

To ensure that all of the detergent-cobaltothiocyanate complex was being extracted in the first chloroform wash, the aqueous layer was periodically washed again with 10 ml of chloroform and steps iv) to vii) repeated.

Tetrachloroethane was sometimes substituted for chloroform but new calibration graphs had to be prepared.

New calibration graphs were also prepared when a new batch of ammonium cobaltothiocyanate reagent was prepared.

To settle any water cloud in the organic layer, a pinch of anhydrous sodium sulphate may be added instead of using a centrifuge.

2. <u>In vitro assay of Poloxalene detergent</u>

Apparatus : 50 ml stoppered centrifuge tubes
Automatic test tube shaker
A spectrophotometer capable of reading at 620nm
Small bench centrifuge

Reagents : Ethanol
1,2 Dichloroethane
Ammonium cobaltothiocyanate reagent (as for the Teric detergent assay)

Procedure :

i) An aliquot of effluent, of not more than 10 ml and containing 0 - 7mg of Poloxalene was pipetted into a 50 ml centrifuge tube. When necessary, the volume was made up to 10 ml with distilled water.

ii) 2 ml of ethanol was added and mixed well.

iii) 5 ml of ammonium cobaltothiocyanate reagent was added, mixed well, and the volume allowed to stand for 15 minutes.

iv) 10 ml of 1, 2, dichloroethane was added and the mixture shaken vigorously for 10 minutes with the automatic tube shaker and then allowed to stand for 10 minutes.

v) The aqueous layer was removed by aspiration.

vi) Any water cloud in the organic layer was settled by centrifuging up to 2000 rpm for 2 minutes.

vii) The organic layer was transferred to a 10 ml spectrophotometer tube and the absorbance was read at 620 nm against a solvent blank.

viii) A standard calibration graph was prepared by assaying known concentrations of Poloxalene detergent.

All concentrations were expressed as mg of Poloxalene per 10 ml of effluent.

Standard Calibration Graphs

The absorbance values obtained for the standard concentrations of Teric detergents and for Poloxalene detergent are shown in Table I. 5 and plotted in Fig. I.3.

Fig. I.3 shows that the calibration curves were linear up to 10 mg, 6 mg and 7 mg of detergent per 10 ml of solvent, for Terics 12A3 + 12A23, Terics N8 + GN15 and Poloxalene, respectively.

The Terics N8 + GN15, because they are chemically different from the Terics 12A3 + 12A23, gave higher absorbance values for given concentrations of detergent.

The Poloxalene detergent assay method gave repeatable results for given detergent concentrations up to 7 mg Poloxalene per 10 ml of water.

Calculation of detergent removed from the rumen model and expressed as a percentage of the starting weight of detergent

The weight of detergent removed from the rumen model in a given time interval is equal to the concentration of detergent found in the aliquot of effluent from that time interval multiplied by the total volume of effluent collected in the same time interval. By summing the amounts of detergent recovered in successive time intervals, a cumulative curve of detergent washout could be constructed, expressed in terms of the percentage of detergent lost from the rumen model.

The percentage of detergent recovered from the rumen model after time "t" was obtained by dividing the weight of detergent lost from the rumen model after time "t"

by the starting weight of detergent and multiplying by 100.

Thus, if the volume of effluent collected after 1 hour was 55 ml and the detergent concentration was found to be 2.70 mg/10 ml, then the weight of detergent lost from the rumen model after 1 hour was $\frac{2.70mg}{10\ ml} \times 55\ ml = 14.85mg.$ If the starting weight of detergent was 700 mg then 14.85mg/700 mg x 100/1 = 2.12% of the starting weight of detergent was removed from the rumen model in the first hour.

If the detergent concentration in the volume of effluent collected between the first and second hours was 8.10 mg/10 ml and the volume was again 55 ml, then $\frac{8.10mg}{10ml} \times 55\ ml = 44.55\ mg$ of detergent was removed between hours 1 and 2 of the run.

The total detergent lost after two hours was:-

$$14.85mg + 44.55mg = 59.40mg$$

This represents a percentage of $\frac{59.40mg}{700mg} \times 100 = 8.48\%$ of the starting weight of detergent had been removed after 2 hours of the run.

RESULTS

The results obtained are recorded in Table I. 6 along with the calculated percentages of detergent recovered with time. They are plotted as the detergent concentration in the effluent (mg/10ml) versus elapsed time (Figs. I. 4, I. 5, and I. 6 for runs A, B and C respectively) as well as the percentage of detergent recovered versus elapsed time (Fig. I. 7, runs A and B; Fig. I. 8 run C).

Differences in the diffusion rate of detergents from the different paste formulations are clearly evident in figures I. 4 - I. 8.

In run A (Fig. I. 4), two of the paste formulations, KB_3/32A and 66A, showed a fast rate of detergent release and reached a peak detergent concentration in the effluent after approximately four hours. Thereafter the concentration of both began to decline.

The two pastes KB_3/34A and 34B had similar intermediate rates of detergent release with KB_3/34A being slightly the quicker. These two pastes had almost parallel curves after 4 hours had elapsed and both were still approaching their peak detergent concentrations when the run ended.

The paste KB_3/32B showed the slowest rate of detergent release in run A. The diffusion rate curve of KB_3/32B was similar in shape to KB_3/34A and 34B. KB_3/32B was also still approaching its peak detergent concentration at the end of the run.

The liquid detergent control curve appeared to decay in an exponential manner. The KB_3/32A detergent diffusion curve intersected the liquid control decay curve after 2¾ hours of the run had elapsed. The curves of KB_3/32B, 34A and 34B did not intersect the liquid detergent decay curve at all.

The detergent diffusion curve of KB_3/66A did not intersect the liquid detergent (Teric 12A3 + 12A23) decay curve either. However, because KB_3/66A contained two chemically different Teric detergents, its detergent diffusion rate curve is not strictly comparable with the control decay curve.

The rates of detergent release in run B are shown in Fig. I. 5 for the three pastes containing Terics 12A3 and 12A23 detergents only. Again KB_3/32A showed a rapid release rate, KB_3/34A an intermediate rate and KB_3/32B a slow rate. However, the extended duration of run B enabled the relationship between the paste curves and the liquid detergent control decay curve to be more clearly seen. All three pastes increased the persistence of detergent in the rumen model in comparison with the rapid washout of the liquid control.

A comparison of the detergent diffusion rate curve of the Poloxalene paste KB_6/16 with the decay curve of the liquid Poloxalene control shows that this particular paste formulation behaved very much like a liquid (Fig. I. 6). The liquid control decay curve and the paste detergent

diffusion curve intersected after about 2½ hours. This was also the time when the maximum detergent concentration from the paste was reached in the rumen model. Consequently the detergent concentration decay of both the liquid control and the paste was very similar after 2½ hours of run C had elapsed.

Plotting the percentage of detergent recovered against elapsed time emphasises the detergent persistence provided by the paste formulations (Fig. I. 7). In run A after 12 hours had elapsed, about 78% of the initial weight of liquid detergent had been washed out of the rumen model. This compared with 76% of detergent released from KB_3/32A, about 40% for both KB_3/34A and 34B and only about 22% for KB_3/32B.

In run B, the differences became more apparent because of the greater duration of the run. Almost all of the liquid detergent and detergent released from KB_3/32A had been washed from the rumen model after 23 hours had elapsed. This compared with 80% for KB_3/34A and about 52% for KB_3/32B.

The failure of the Poloxalene paste, KB_6/16, to increase the persistence of Poloxalene in the rumen model is indicated in Fig. I. 8. This shows that 55% of the liquid Poloxalene control and 53% of detergent from the paste had been recovered after 8 hours of the run had elapsed.

DISCUSSION

The method

Analytical limitations - The analytical method used for the Teric 12A3 - 12A23 paste formulations describes only the release of Teric 12A23 from the paste. This is because Teric 12A23 has a high degree of ethoxylation (23 moles of ethylene oxide per mole of alcohol) and as such is very water soluble. Teric 12A3 on the other hand has a low degree of ethoxylation and is therefore water insoluble. However, it is assumed that the curve describing the release of 12A23 describes the release of

both detergents. As far as dispersion rate (diffusion) is concerned, this is considered a valid assumption.

The use of the two Terics in combination could be important in the rumen. Here, preferential adsorption of either Teric onto the particulate matter in rumen liquor could occur thus imbalancing the ratio of the two detergents in the fluid fraction. Therefore, analysis of detergents in rumen fluid would need to include both the particulate and fluid portions.

Adsorption of detergents onto particulate matter may also serve to increase the persistence of the detergents in the rumen by slowly releasing the bound material as the concentration in the fluid falls.

Rumen model - No attempt was made to allow for the possible effects of abrasive action of rumen solids or rumen motility on the dissolution of the paste. Such effects are likely to be important in increasing the surface area of paste exposed to rumen fluid.

The water turnover rate in the rumen of milking cows is 0.15 - 0.27 h^{-1} and 0.1 - 0.2 h^{-1} in dry cattle grazing on ryegrass-clover pastures. (Reid, pers. comm.). Hence, in the experiment, the water turnover rates used in the three runs tended to the low end of the range.

Distilled water was used as the exchange fluid and the effects of rumen fluid pH, particulate matter, rumen absorption and micro-organisms on the paste was not investigated. These factors would need to be considered if the results are to be interpreted in terms of the live cow.

The results

The liquid detergent control decay curves in each run were used to compare the persistence of detergents released from the different paste formulations and were therefore a means of measuring the relative effectiveness of each paste formulation as a "depot system".

In each run, the liquid decay curves were found to be nearly exponential as would be expected because of the constant water turnover rate. Plotted on semi-log paper, the decrease in detergent concentration in the effluent with elapsed time is nearly linear as shown in Fig. I. 9.

This represents the liquid detergent control used in run B. The rate was found to be 0.15 h^{-1}.

However, when the liquid detergent decay rate in run B, 0.15 h^{-1}, is compared with the water turnover rate of approximately 0.19 h^{-1} in run B, a 0.04 difference is seen. This is possibly a reflection of the limitations of the analytical method

The liquid Poloxalene decay rate in run C and the water turnover rate were both approximately 0.12 h^{-1}.

The differences found in the rate of detergent release from the different paste formulations appeared to relate to the paste's composition and its method of preparation. Of the Teric containing pastes, KB_3/32A showed the fastest detergent release rate followed by KB_3/66A, 34A, 34B and 32B (Fig. I. 4). KB_3/32A and 66A were prepared by dispersing the gelling agent in the molten Terics first.

The other three pastes, KB_3/32B, 34A and 34B were prepared by dispersing the gelling agent in hot water prior to adding the molten Terics. The differences in the detergent release rates of these three pastes may be explained in terms of the amount of gelling agent each paste contained. The slowest detergent releasing paste, KB_3/32B, contained 1.33 times more gelling agent (Volclay 325) than the next slowest, KB_3/34B and 2.0 times more than the next slowest, KB_3/34A. KB_3/34B contained 1.5 times more gelling agent than KB_3/34A (see Table I. 2).

The composition of the pastes KB_3/32A and KB_3/32B was the same but a difference in the method of preparation of each resulted in a big difference in their rates of detergent release.

KB_6/16 was prepared in a similar way to KB_3/66A and KB_3/32A, and this paste also had a rapid rate of detergent release (Fig. I. 6).

Therefore, those pastes which were prepared by dispersing the gelling agent in the molten detergent first, had faster detergent release rates than those prepared by dispersing the gelling agent in hot water prior to adding the detergents.

For the Teric containing pastes, there appeared to be correlations between the physical properties of each (Tables I.3 and I.4) and its ranking in detergent release rate. The softer pastes (higher penetrometer readings at 40°C) had the higher melting points and faster detergent release rates, i.e. KB_3/32A and 66A. Conversely, the stiffer pastes (lower penetrometer readings at 40°C) had the lower melting points and slower detergent release rates.

Thus three points emerge from the discussion of the Teric pastes so far,

1. The composition of the paste appears to influence the detergent release rate.
2. The method of preparation used for the pastes also appears to influence the detergent release rate.
3. The consistency and melting point of the pastes seems to be related to the detergent release rate in vitro.

Interpretation in terms of a field situation has been attempted by comparing the theoretical results of dosing a paste once daily with dosing a liquid twice daily.

The results obtained in run B of the rumen model experiment have been used to obtain detergent concentrations up to 24 hrs. (Fig. 1.5).

The paste KB_3/32B was selected because of its slow detergent release rate and was compared with a liquid control of the same detergents.

The assumptions madein establishing the curves were;

1. The minimum effective detergent concentration necessary to prevent bloat was that concentration reached by the liquid control after 12 hours had elapsed in run B, (Fig. I. 5). This concentration was approximately 3mg of detergent per 10 ml of effluent.
2. The liquid detergent concentration would continue to decay at a rate of 0.15 h^{-1}.
3. The paste KB_3/32B was fully dissolved at 24 hours post dosing and the detergent concentration

commenced to decay exponentially at 0.15 h^{-1} from 24 to 44 hours post dosing. After 44 hours the detergent concentration from each paste dose was considered negligible.

The concentration values of detergent released from the paste $KB_3/32B$ over the first 24 hours were obtained from Fig. I. 5 and were calculated from Fig. I. 9 thereafter up to 44 hours post-dosing.

The calculated detergent concentration changes over a 66 hour period of time for the liquid detergent and over an 80 hour period of time for the paste $KB_3/32B$, are given in Tables I. 7 and I. 8 respectively. Both are plotted against elapsed time in Fig. I. 10.

From Fig. I.10 several advantages of a 24 hourly paste dosing programme over a 12 hourly liquid dosing programme are evident.

1. The frequency of paste dosing necessary to maintain a detergent concentration above the assumed minimum was half that required for the liquid. Correspondingly, the amounts of detergent required to provide protection were less for paste than for liquid.
2. The paste maintained an even detergent concentration level over the 80 hour period whereas the liquid detergent showed large concentration changes associated with a large detergent input every 12 hours.

A disadvantage of the paste was that the minimum effective detergent concentration level was not reached until 12 hours after the primary dose. This could be overcome by accompanying the primary dose with a single liquid dose or by increasing the primary paste dose 2 to 3 fold. It was difficult to interpret the rumen model findings relative to the in vivo situation because of unknown factors associated with the live animal.

Despite this, the rumen model did serve to demonstrate distinct advantages of some paste formulations over others, and over the liquid control formulations.

SUMMARY

1. An experiment was designed using a rumen model to demonstrate differences in the rate of detergent release from several different anti-bloat paste formulations under controlled conditions.

2. Some formulations were shown to delay the washout of detergent from the rumen model when compared with washout of the corresponding liquid control.
3. The differences in the rate of detergent release of different paste types appeared to be related to the paste's composition and method of preparation as well as to the paste's physical properties of consistency and melting point.
4. A theoretical drenching programme based on the results obtained in the experiment showed advantages of the paste formulation, $KB_3/32B$, over the liquid control detergent.
5. Several important factors relating to the live animal were mentioned and it is necessary to consider these factors carefully before extrapolating the *in vitro* results to the *in vivo* situation.
6. The conclusion is that pastes can be formulated with differing detergent release rates. However, until similar curves can be accurately established *in vivo* it is difficult to say how closely the rumen model relates to the live animal situation.

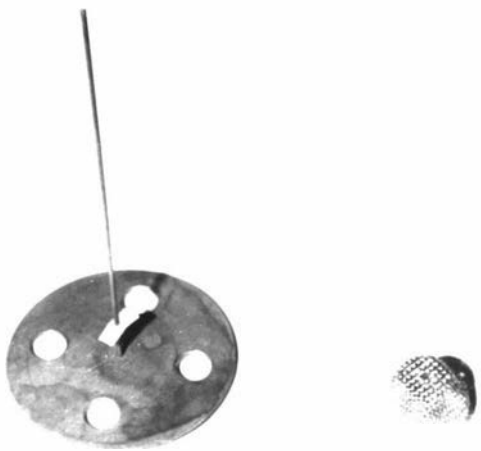


Fig. I. 1 A brass disc used to provide agitation in the rumen model and a mesh container used to hold the paste sample.

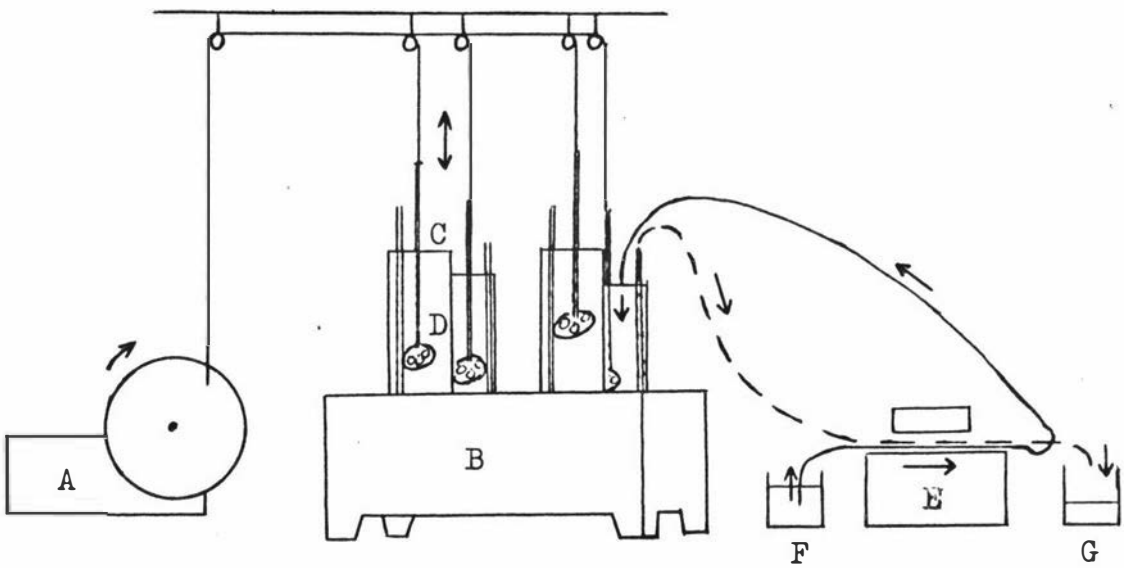


Fig. I. 2 The model rumen

A = motor driven crank
B = water bath
C = cylinder
D = agitation disc with attached basket
E = peristaltic pump
F = water supply beaker
G = effluent collecting beaker

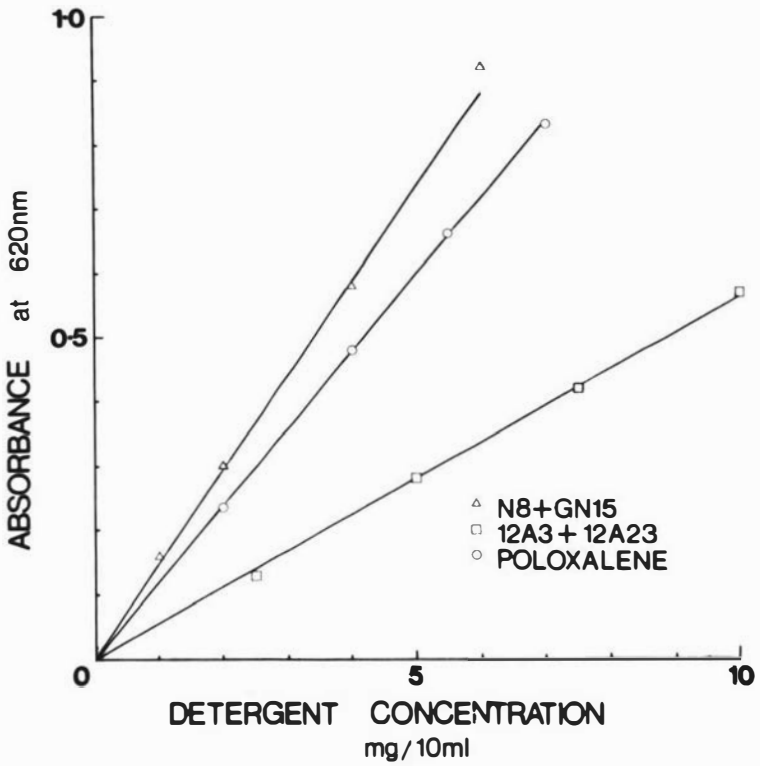


Fig. I. 3 Calibration graphs used to determine detergent concentrations.

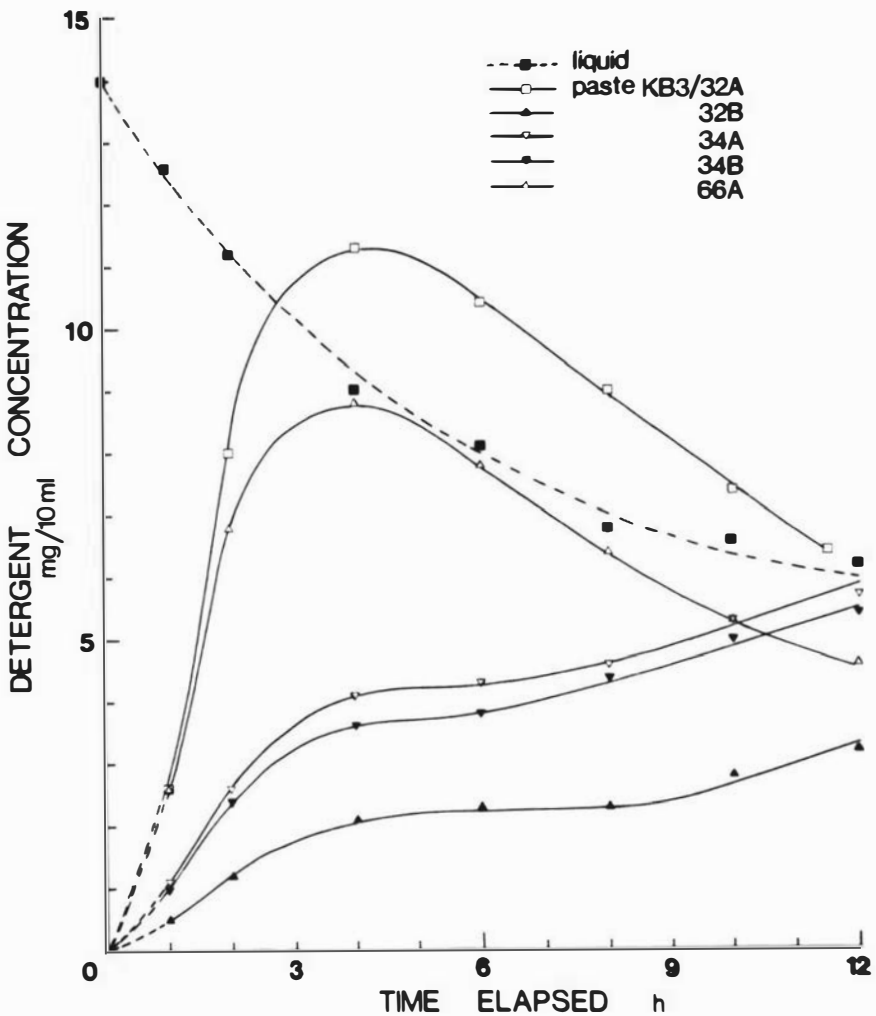


Fig. I. 4 The detergent concentration versus elapsed time for the 5 paste formulations and the liquid detergent control in run A.

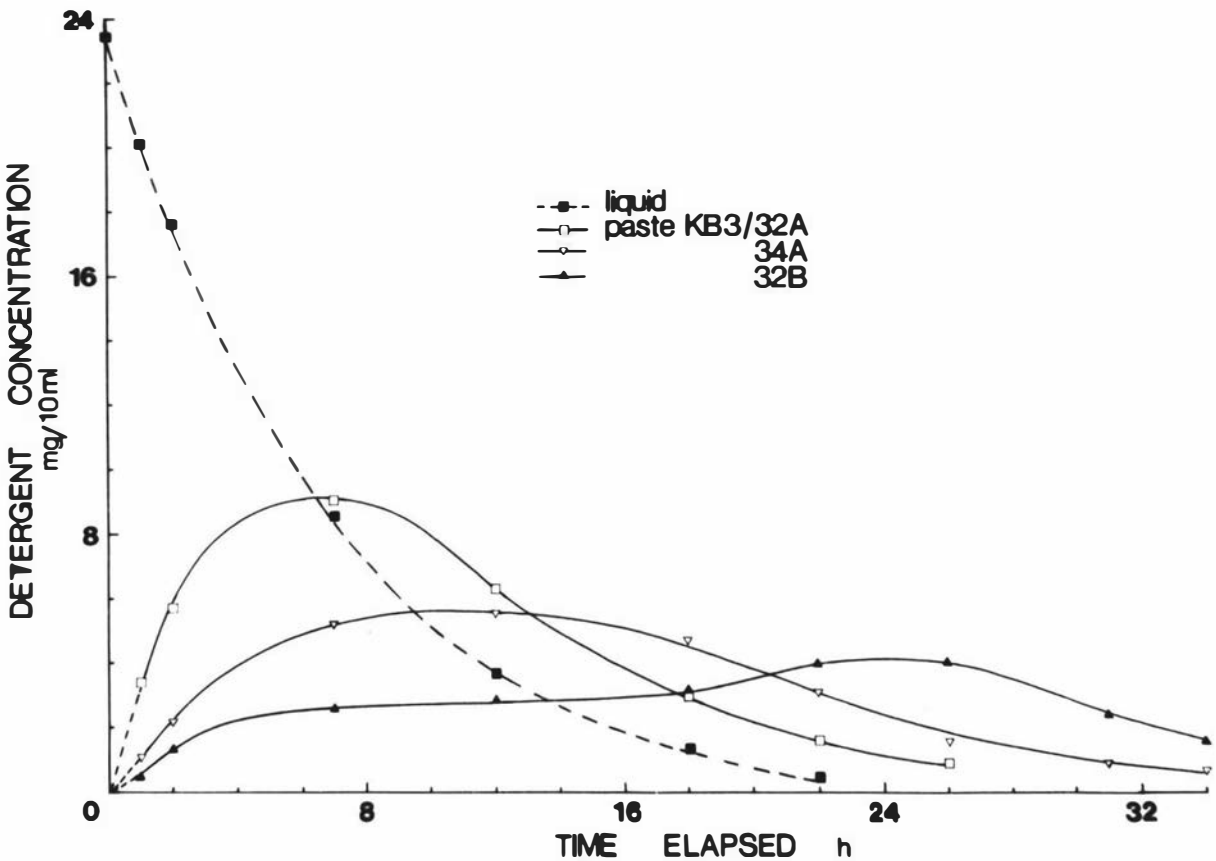


Fig. I. 5 The detergent concentration versus elapsed time for the 3 paste formulations and the liquid detergent control in run B.

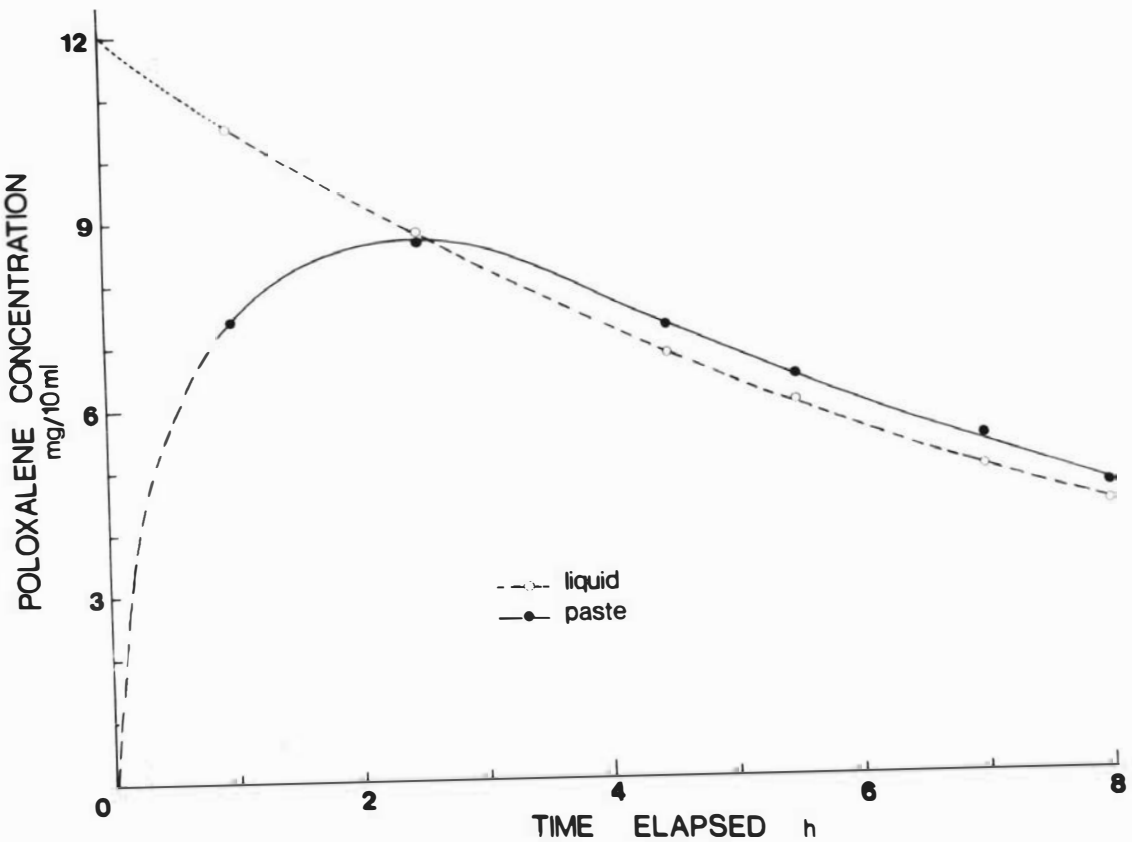


Fig. I. 6 The Poloxalene concentration versus elapsed time for the paste formulation $KB_6/16$ and the liquid Poloxalene control in run C.

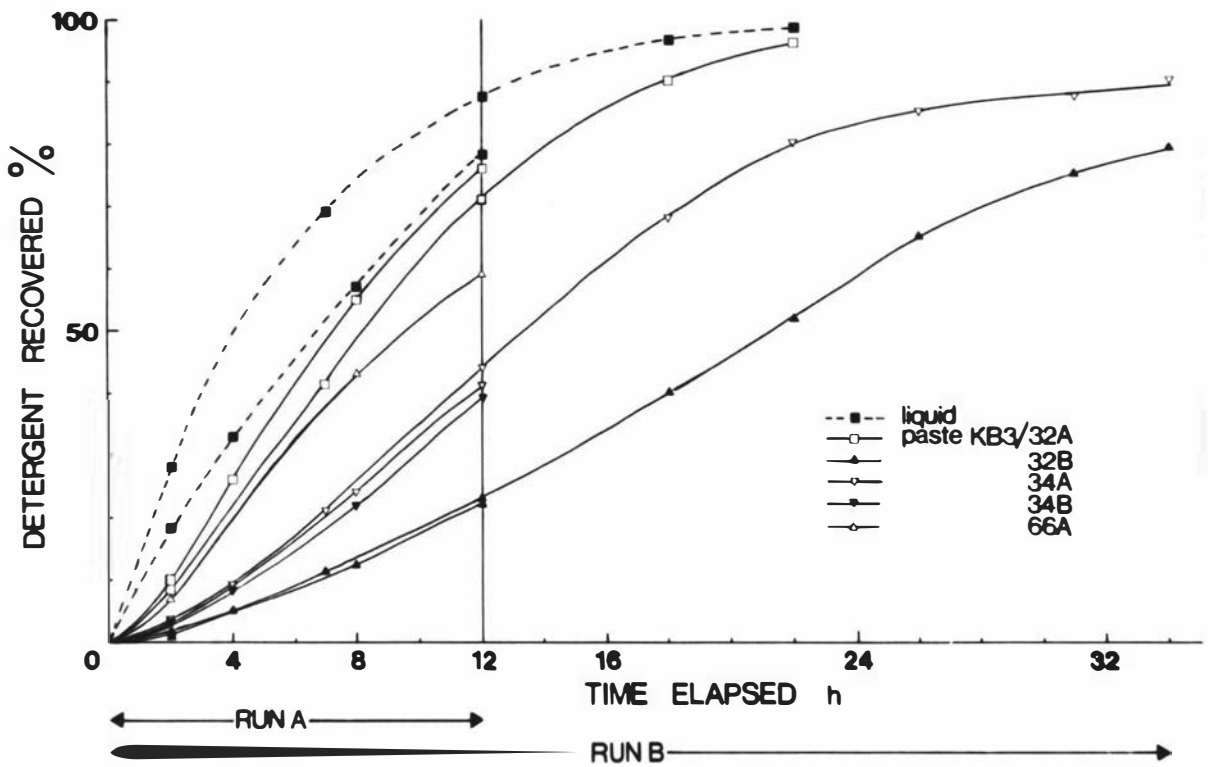


Fig. I. 7 The percentage of detergent recovered versus elapsed time for runs A and B.

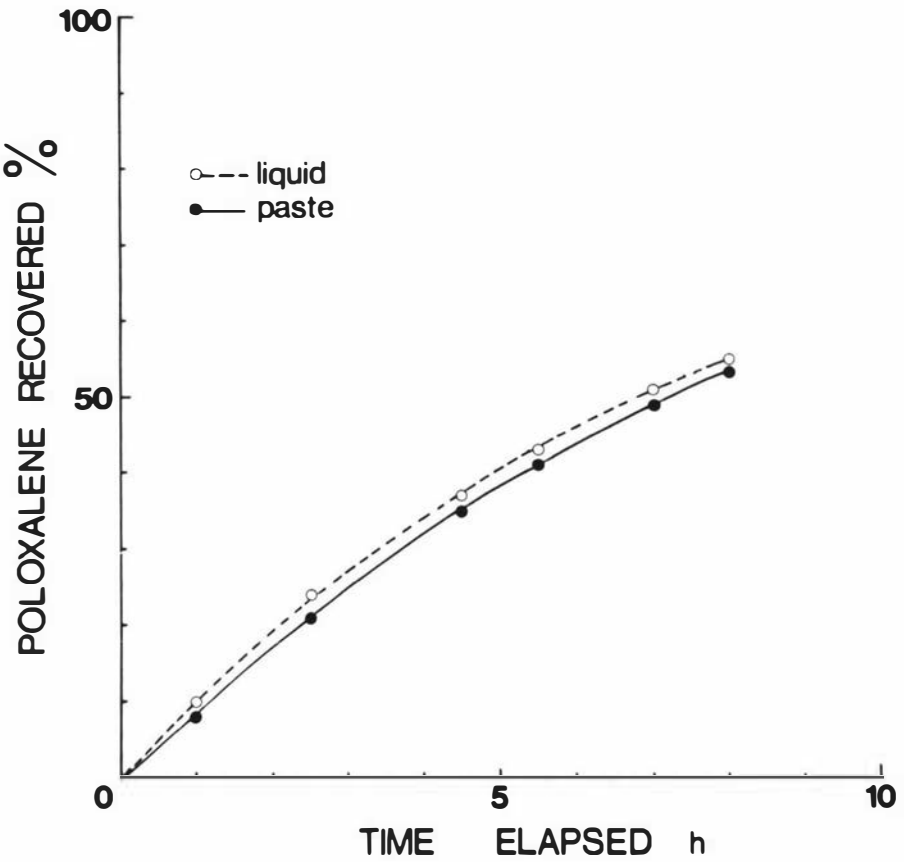


Fig. I. 8 The percentage of poloxalene recovered versus elapsed time for run C.

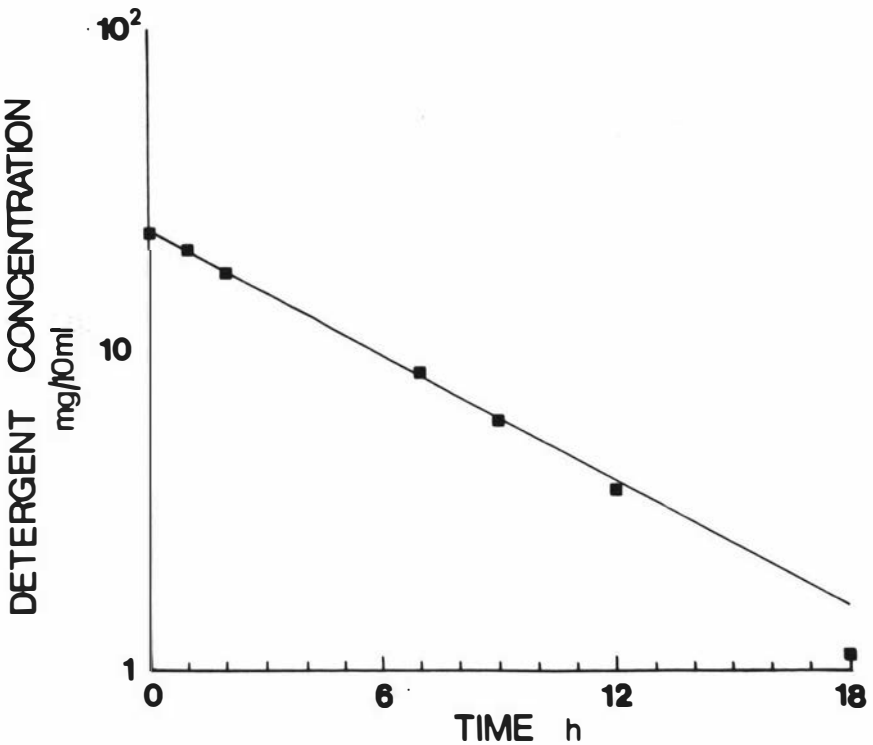


Fig. I. 9 The detergent concentration versus elapsed time, for the liquid detergent in run B, plotted on semi-log paper. Detergent decay rate = 0.15 h^{-1}.

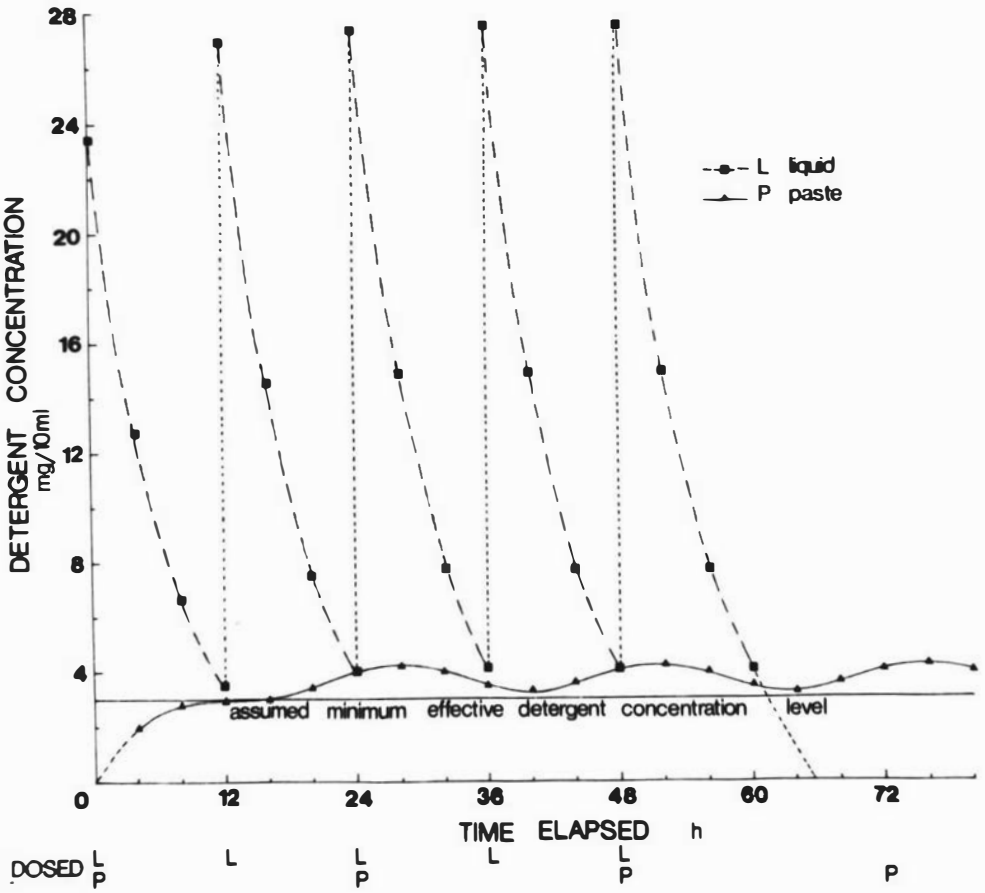


Fig. I. 10 The detergent concentration changes based on a 12 hourly liquid and 24 hourly paste dosing schedule with a continuous detergent decay rate of $0.15h^{-1}$

TABLE I. 2

The composition of the anti-bloat pastes tested in the rumen model.

Paste Code	Detergents % w/w		Antifoam A[a] % w/w	Gelling Agent % w/w	Remainder % w/w
	Teric[b]			Volclay 325[c]	Water
	12A3	12A23			
KB_3/32A	40.80	27.20	2.00	5.00	25.00
32B	40.80	27.20	"	5.00	"
34A	42.30	28.20	"	2.50	"
34B	41.60	27.65	"	3.75	"
	N8[d]	GN15[d]		Ethylcellulose	
KB_3/66A	85.50	10.00		4.50	
	Poloxalene[e]				Cyclomid
KB_6/16	92.50		0.50	4.00	3.00

a Antifoam A is a Dow-Corning product and is an anti-foaming agent

b Teric is a trade name of ICI ANZ

Teric 12A3 and Teric 12A23 are lauryl alcohol condensed with 3 and 23 moles of ethylene oxide per mole of alcohol respectively.

c Volclay 325 is a bentonite clay used commonly as a gelling agent.

d Teric N8 and Teric GN15 are nonyl phenol condensed with 8 and 15 moles of ethylene oxide per mole of phenol respectively.

e Poloxalene is a Smith Kline and French product. It is a polyoxypropylene - polyoxyethylene polymer in the ratio of 67% : 33% with an average total mol. wt. of about 3000.

TABLE I. 3

The melting points of the Teric pastes[a].

Paste Code	Melting Point °C.
KB_3/32A	51 - fairly distinct
32B	42 - distinct
34A	41 - "
34B	42 - "
66A	45 - not distinct

a These results were obtained by MSD chemists.

TABLE I. 4

Consistency of the five Teric pastes at various temperatures[a].

Paste Code	Temp °C.	Consistency (mm)[b]
KB_3/32A	RT	306
	4	217
	40	240
KB_3/32B	RT	186
	4	186
	40	204
KB_3/34A	RT	205
	4	215
	40	210
KB_3/34B	RT	200
	4	180
	40	215
KB_3/66A	RT	248
	4	157
	40	270

a These results were obtained by MSD chemists.

b A "Arthur H. Thomas Co." Penetrometer was used for consistency measurement

TABLE I. 5.

The absorbance values of the standard Teric and Poloxalene detergent concentrations used to establish the calibration graphs.

Detergents	Concentration (mg/10ml solvent)	Absorbance at 620 nm
Terics 12A3 + 12A23	2.50	0.13
	5.00	0.28
	7.50	0.42
	10.00	0.57
Terics N8 + GN15	1.00	0.16
	2.00	0.30
	4.00	0.58
	6.00	0.92
Poloxalene	2.00	0.24
	4.00	0.48
	5.50	0.66
	7.00	0.83

TABLE I. 6

The detergent concentrations in the effluent and the percentage of detergent recovered with elapsed time for runs A, B and C.

Run	Formulat.	Time h.	Effluent collect. ml.	Absorb. at 620 nm.	Deterg. Concent. mg/10ml of effluent	mg Deterg. collected per vol. of effluent		Detergent recov. %
A	Liq.Teric	0	0	.39/5ml	14.00			0
		1	54	.35/5ml	12.60	68	68	10
		2	55	.31/5ml	11.20	62	130	18
		4	115	.50	9.00	104	234	33
		6	112	.45	8.10	91	325	46
		8	109	.38	6.80	74	399	57
		10	110	.37	6.60	75	474	68
		12	110	.34	6.20	70	544	78
	$KB_3/32A$	1	55	.15	2.60	14	14	2
		2	56	.45	8.00	45	59	8
		4	110	.62	11.30	124	183	26
		6	112	.57	10.40	116	299	43
		8	112	.50	9.00	101	400	55
		10	110	.41	7.40	81	481	66
		11.5	83	.36	6.40	53	534	74
	$KB_3/32B$	1	56	.03	.50	3	3	.5
		2	55	.07	1.20	7	10	1
		4	112	.12	2.10	24	34	5
		6	115	.13	2.30	26	60	9
		8	114	.13	2.30	26	86	12
		10	113	.16	2.80	32	118	17
		12	112	.17	3.20	36	154	22

TABLE I. 6 (cont'd.)

A	KB_3/34A	1	55	.07	1.20	7	7	1
		2	54	.15	2.60	14	21	3
		4	110	.23	4.10	45	66	9
		6	110	.24	4.30	47	113	16
		8	112	.26	4.60	52	165	24
		10	113	.29	5.30	60	225	32
		12	114	.32	5.70	65	290	41
	KB_3/34B	1	55	.06	1.00	5	5	1
		2	54	.13	2.40	13	18	3
		4	115	.20	3.60	41	59	8
		6	112	.21	3.86	43	102	15
		8	112	.24	4.40	48	151	22
		10	114	.28	5.00	57	208	30
		12	115	.30	5.40	62	270	39
	KB_3/66A	1	54	.39	2.60	14	14	2
		2	53	.50/5ml	6.80	36	50	7
		4	109	.65/5ml	8.70	95	145	21
		6	106	.58/5ml	7.80	84	229	33
		8	112	.48/5ml	6.40	73	302	42
		10	110	.79	5.30	58	360	51
		12	110	.69	4.60	51	411	59
B	Liq.Teric	0	600	.65/5ml	23.40			
		1	93	.56/5ml	20.10	187	187	13
		2	115	.49/5ml	17.60	202	389	28
		3.5	162	.40/5ml	14.60	237	626	45
		7	393	.47	8.50	334	960	69
		9	235	.33	6.00	141	1101	79
		12	340	.20	3.60	122	1223	87
		14.75	315	.13	2.30	72	1295	92
		18	370	.07	1.30	48	1343	96
		19.75	210	.04	.80	17	1360	97
		2.2	254	.03	.50	13	1373	98

TABLE I. 6 (cont'd.)

B	$KB_3/32A$	1	96	.19	3.40	33	33	2
		2	115	.32	5.70	66	99	7
		3.5	163	.43	7.70	125	224	16
		7	395	.50	9.00	355	579	41
		9	225	.50	9.00	202	781	56
		12	350	.35	6.30	220	1001	71
		14.75	312	.26	4.70	147	1148	82
		18	365	.17	3.00	109	1257	90
		19.75	216	.13	2.40	52	1309	93
		22	250	.09	1.60	40	1349	96
		26	450	.05	.90	40	1389	99
	$KB_3/32B$	1	100	.03	.50	5	5	.50
		2	117	.67	1.30	15	20	1.50
		3.5	162	.10	1.80	29	49	3.50
		7	395	.15	2.60	103	152	11
		9	240	.16	2.90	70	222	16
		12	340	.16	2.90	99	321	23
		14.75	317	.17	3.10	98	419	30
		18	370	.17	3.10	141	560	40
		14.75	220	.18	3.30	73	633	45
		22	245	.22	4.00	98	731	52
		26	455	.22	4.00	182	913	65
		31	565	.13	2.40	136	1049	75
		34	335	.09	1.60	54	1103	79
	$KB_3/34A$	1	96	.06	1.10	11	11	1
		2	115	.12	2.20	25	36	3
		3.5	163	.19	3.40	55	91	6
		7	395	.29	5.20	205	296	21
		9	225	.30	5.50	124	420	30
		12	350	.30	5.50	192	612	44
		14.75	312	.29	5.20	162	774	55
		18	365	.26	4.70	172	946	68
		19.75	205	.25	4.50	92	1038	74
		22	248	.17	3.10	71	1115	80
		26	453	.09	1.60	12	1187	85
		31	573	.05	.90	52	1239	88
		34	335	.04	.70	23	1262	90

TABLE I. 6 (cont'd)

C	Polox. Liquid	1	68	.50/4ml	10.50	71	71	10
		2.5	108	.52/5ml	8.80	99	166	24
		4.5	140	.40/5ml	6.80	95	261	37
		5.5	72	.31/ "	6.00	43	304	43
		7	105	.29/ "	4.90	51	355	51
		8	72	.25/ "	4.30	31	386	55
	$KB_6/16$	1	76	.44/5ml	7.40	56	56	8
		2.5	106	.51/ "	8.60	91	147	21
		4.5	134	.43/ "	7.20	96	243	35
		5.5	73	.38/ "	6.40	47	290	41
		7	101	.32/ "	5.40	54	344	49
		8	72	.27/ "	4.60	33	377	54

TABLE I. 7

The calculated detergent concentration changes with time based on a 12 hourly liquid dosing schedule (Liquid decay rate = 0.15^{-1}).

Time h	Total detergent concentration (mg/10ml) Liquid
0*	23.4
4	12.7
8	6.6
12*	3.5 + 23.4=26.9
16	14.5
20	7.6
24*	4.0 + 23.4=27.4
28	14.9
32	7.7
36*	4.1 + 23.4=27.5
40	14.9
44	7.7
48*	4.1 + 23.4=27.5
52	14.9
56	7.7
60*	4.10

TABLE I. 8

The calculated detergent concentration changes with time based on a 24 hourly paste dosing schedule (Liquid decay rate = $0.15h^{-1}$)

Time h	Total detergent concentration (mg/10ml) Paste (KB_3/32B)
0*	0
4	2.0
8	2.8
12	2.9
16	3.0
20	3.4
24*	4.0 + 0 =4.0
28	2.2 + 2.0=4.2
32	1.2 + 2.8=4.0
36	0.6 + 2.9=3.5
40	0.3 + 3.0=3.3
44	0.2 + 3.4+3.6
48*	- + 4.0=4.0 + 0 =4.0
52	2.2 + 2.0=4.2
56	1.2 + 2.8=4.0
60	0.6 + 2.9=3.5
64	0.3 + 3.0=3.3
68	0.2 + 3.4=3.6
72*	- + 4.0=4.0 + 0 = 4.0
76	2.2 + 2.0 = 4.2
80	1.2 + 2.8 = 4.0

CHAPTER 2

An investigation of the effects of administration on the physical state of administered paste boli delivered at the cardia.

INTRODUCTION

The release rate of active materials from anti-bloat pastes into the surrounding fluid will be dependent on two factors:-

1) The paste's composition and method of preparation as shown in Chapter 1.
2) The surface area of paste exposed for dissolution.

The second factor could be largely influenced by the degree of physical separation of the paste which might occur during administration. Also, the degree of agitation and attrition suffered by the paste bolus in the rumen is likely to be important.

The possibility that some pastes might survive the biting, chewing and swallowing at administration better than others was suggested by the different physical characteristics and formulations of the different pastes (Tables I. 2, I. 3, and I. 4).

A systematic investigation of the effects of administration on the physical state of the paste bolus was therefore undertaken.

This study had three objectives:-

1. To determine the effects of administration on the physical state of the paste bolus delivered at the cardia.
2. To determine to what extent the animal reaction to administration affected the condition of the paste bolus at the cardia.
3. To observe the resistance of the swallowed paste bolus to mild agitation in the saliva collected with the bolus.

EXPERIMENTAL DESIGN

The rumen contents were bailed from two steers with rumen fistulae. Each was dosed up to six times, on different days, with one of five different paste formulations. On each occasion the reaction of the steer to the dosing procedure was noted and graded (animal reaction grade). The swallowed paste dose was collected at the cardia in a beaker (250ml) along with the accompanying saliva. Each collected paste dose was transferred to a petri dish, photographed and graded for intactness (intactness grade). All samples were then transferred to containers and placed in a water bath kept at 39°C.

Half of the samples were left undisturbed for $2\frac{1}{2}$ hours.

Half of the samples were periodically agitated over $2\frac{1}{2}$ hours.

All of the samples were then transferred to a petri dish, re-photographed and their physical state was compared with that immediately after collection. This allowed a measurement of the resistance of individual paste formulations to break down.

Correlation of reaction grade with paste bolus intactness grade gave some indication of the physical stability of the paste formulation and the effects of animal behaviour at dosing on the intactness of the bolus at the cardia.

MATERIALS AND METHODS

Materials

Five pastes were tested. Four were the Teric 12A3 - 12A23 combinations, KB_3/32A, 32B, 34A, 34B, and the fifth was a Poloxalene paste. The Poloxalene paste differed from KB_6/16 (Table I. 2) in that it contained 91% Poloxalene and 9% ethylcellulose as the gelling agent.

For details of the formulations and physical properties of the four Teric pastes, see Tables I. 2, I. 3, and I. 4. All pastes were delivered from a cartridge by a paste dosing gun (Fig. II. 1).

Animals

Two 3 year old Jersey steers with rumen fistulae and weighing approximately 400 kg each were chosen at random for the experiment from a group of six.

The animals, 'GREY' and '353' were used to handling, but neither had had experience of the Merck, Sharp and Dohme paste dosing gun.

Dosing method

After emptying the rumens of each steer (Reid, 1965,) an assistant delivered the paste dose.

The technique used here was to deliver the dose on to the back of the tongue as this most often resulted in immediate swallowing.

Approximately 3 minutes were allowed between successive doses.

Reaction grade system

The animals reaction to each dosing was graded according to the following classification:-

A - Immediate swallowing of the paste dose without chewing

B - One to two chews of the paste, some tongue rolling before swallowing within 5 seconds.

C - A period of 5 to 10 seconds of chewing the paste, tongue rolling and head tossing before swallowing.

D - Prolonged chewing of the paste, froth appearing at the lips and muzzle and occasionally pieces dropped from the mouth.

Sample collection method

The operator's right arm was extended through the rumen fistual and the cardia was located. A small pliable plastic beaker was held firmly over the opening just as the assistant delivered the paste dose to the animal. The beaker was held in this position until the animal had finished swallowing and had settled down again. The beaker was then withdrawn from the rumen and the volume of saliva collected on each occasion immediately adjusted to a standard amount (60 mls).

Photography

A white cardboard background was prepared showing the paste type, animal identification, dose order number, time elapsed since collection and a centimetre scale.

A petri dish with the sample was placed in the centre of the card. Photographs were taken vertically using an Asahi Pentax camera (lens 1:2, 55mm) and flash mounted on a tripod above the card

Ilford FP4, 135 black and white film was used.

Agitation method

Capped pottles containing the paste sample and saliva were placed in a trough attached to a horizontally oscillating arm driven by an electric motor. The throw was 10 cm and the rate of oscillation was 40 per minute.

The samples were agitated for 60 secs. every ½ hour over a 2½ hour period. Samples were returned to the water bath after agitation.

Paste bolus intactness grading system

The intactness of the paste bolus sample was graded according to the classification in Fig.II.2. Typical examples appear above each grade description.

RESULTS

From the number of experiments carried out there appeared to be no significant difference between the paste formulations in their ability to withstand the physical effects of administration. (No statistical analysis was carried out because of the small experimental numbers involved).

This is reflected in the totals recorded for each reaction grade (Table II.1) and intactness grade (Table II. 2), according to each paste type.

There was however, an obvious animal effect.

The influence of the individual animal

Tables II. 1 and II. 2 show that for all pastes combined there was a difference in the total reaction and intactness grades recorded by each animal.

Steer Grey scored reaction grades of mainly A and B and intactness grades of E, F and G. Steer 353 however, scored mainly B and C reaction grades and F and G intactness grades.

The difference in reaction and intactness grades recorded for each steer is emphasised in Fig. II. 3. This shows the percentage that each reaction grade and intactness grade represents of the total number of tests carried out in each steer.

Figure II. 3 shows that steer 353 scored a higher percentage of C and D reaction grades and G and H intactness grades than did steer GREY.

Correlation of dosing grades with intactness grades

A trend towards increased paste fragmentation with increased animal reaction to dosing was observed. This is demonstrated in Table II. 3 which shows a comparison of the reaction grade with its corresponding intactness grade for each individual administration.

This table also shows a trend towards increased animal reaction with successive dosings for each paste formulation. This effect was more marked in steer 353 which scored more C and D reaction grades and G and H intactness grades than did steer Grey as dosing was repeated. The total number of grades A - D; E - H, scored in successive dosings is shown in Table II. 4.

Effects of agitation on the swallowed paste bolus

Samples of swallowed paste boli of grades E and F when left undisturbed at 39°C for 2½ hours changed only slowly. However, samples of grades G and H which were already fragmented on collection appeared to break down more quickly (Fig. II. 4).

No differences attributable to paste formulation were seen.

Agitation increased the rate of dissolution of the paste samples. However, intact boli withstood agitation better than fragmented boli. This is clearly shown in Fig. II. 5 which compares the effect of agitation on samples of the two extremes.

DISCUSSION

In these experiments the overriding factor determining the degree of physical disruption of the paste bolus before arrival at the cardia, was the reaction of the animal to the dosing procedure. A difference between animals in this respect was shown (Table II. 1, II. 2; Fig. II. 3). Steer 353 reacted more adversely to dosing than did steer Grey and consequently scored more G and H intactness grades.

The disruptive effects caused by passage down the oesophagus are difficult to measure but are likely to be minor compared with that caused by chewing and rolling the paste bolus against the teeth, and cheek and tongue papillae.

Two factors could influence paste dosing acceptance. Firstly, taste may be important and the acceptance of dosing based on taste could vary between animals

Secondly, animal temperament is likely to influence animal reaction to dosing and temperament also varies between animals. Steer 353 was more easily upset than steer Grey, (Table II. 4).

On a herd basis, these two factors could result in a wide range in the degree of intactness of paste boli delivered to the rumen.

The mild agitation treatment was adequate to demonstrate that fragmented paste boli dissolved more quickly than intact boli (Fig. II. 5). This seems reasonable in view of the greatly increased surface area exposed for dissolution with fragmented boli.

A critical fact emerges if one attempts to interpret the results of the experiment in terms of the field situation. It is, that there can be a large variation in the degree of disruption of the paste dose during administration both within and between animals. Consequently, for any given paste formulation the surface area of the swallowed bolus exposed for dissolution in rumen fluid can vary widely.

This fact becomes of critical importance if the persistence of detergent in the rumen for any given paste dose is determined by the degree of intactness of the swallowed bolus on entering the rumen.

To answer this question it is necessary to discover the fate of the swallowed paste bolus in the rumen. Visual observation is impractical. The ideal method would be to establish the detergent decay curves in rumen liquor for intact and fragmented paste boli deposited in the rumens of cattle with rumen fistulae.

If the detergent decay curves of both types of boli were similar then it could be interpreted that animal reaction to paste administration was not an important factor influencing the persistence of detergent in the rumen. Also, that it was likely rumen motility quickly caused fragmentation of the intact paste bolus.

If the two types of boli resulted in markedly different decay curves, then animal reaction to paste administration becomes a vital aspect of the efficacy of anti-bloat pastes. It could also be interpreted that rumen motility played no significant role in the further breakdown of the paste bolus.

An attempt was made to answer some of the above questions by establishing in vivo detergent decay curves of Poloxalene liquid and paste using steers with rumen fistulae. That work is reported in the following Chapter.

SUMMARY

1. Five anti-bloat paste formulations were each administered by mouth up to six times to two Jersey steers having rumen fistulae.

2. Animal reaction to each dosing was noted and graded.

3. Each dose of paste was collected as it was delivered to the rumen. The collected paste was graded for intactness immediately and after artificial agitiation.

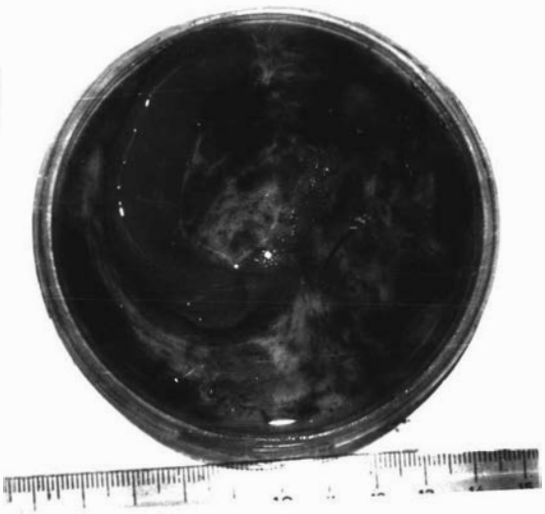
4. The degree of intactness of the boli delivered through the cardia varied widely. The dominant factor found affecting intactness was the animal reaction to the dosing procedure. The paste formulation had no obvious effect.

5. Evidence of a difference between animals in animal reaction to dosing was obtained.

6. Boli fragmented after administration, dissolved more quickly than intact boli, whether left undisturbed or mildly agitated.

7. It is concluded that the efficacy of the pastes as a field treatment could be seriously reduced by animal reaction to administration and that a further limitation could be imposed by the susceptibility of the pastes to agitation by rumen movement.

Fig. II. 1 Paste dosing gun with a mounted cartridge.



E. (KB_3/34A, Steer GREY) One to two large elongated segments, no fragments.

F. (KB_3/34B, Steer 353) Three to four smaller segments with occasional fragments.

G. (KB_3/34A, Steer 353) Several small segments and many broken up into fragments.

H. (KB_3/32B, Steer 353) No segments, many fragments and quite frothy upon collection.

Fig. II. 2 Typical examples of the bolus intactness grades classification.

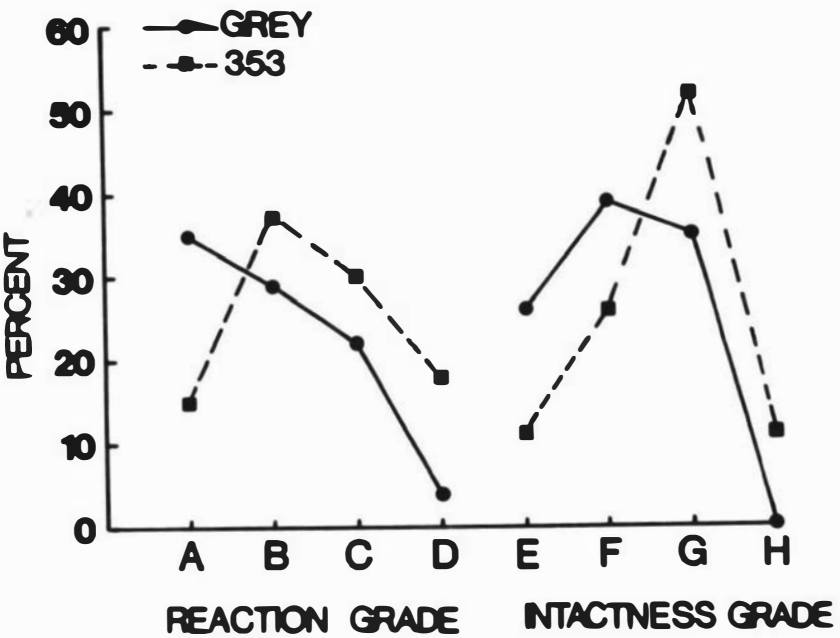
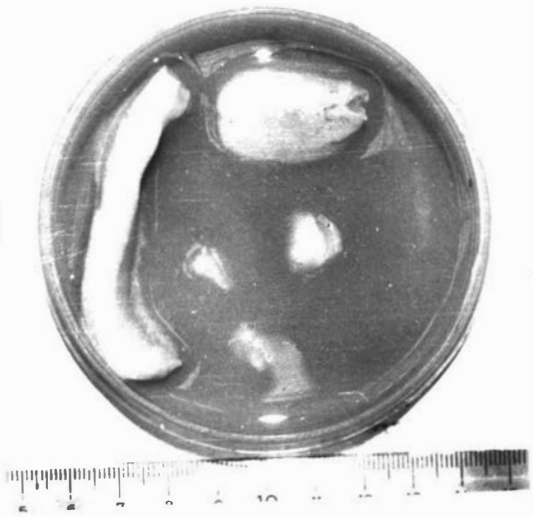
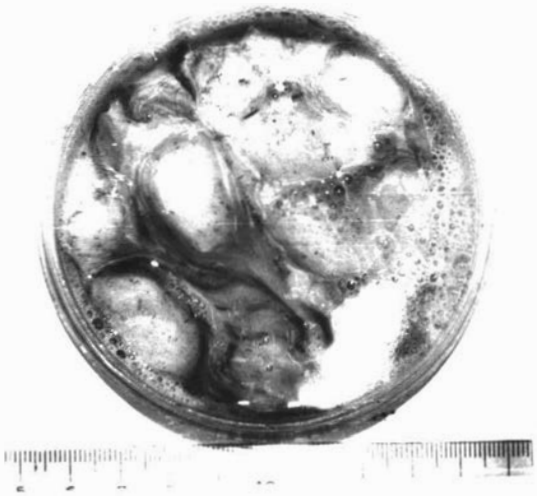


Fig. II. 3 The percentage that each reaction grade and intactness grade represents of the total number of tests carried out in each steer.

1a. (Polox. Steer 353) Sample of intact boli 15 min. after collection.



1b. The same sample $2\frac{1}{2}$ hours later. (no agitation)



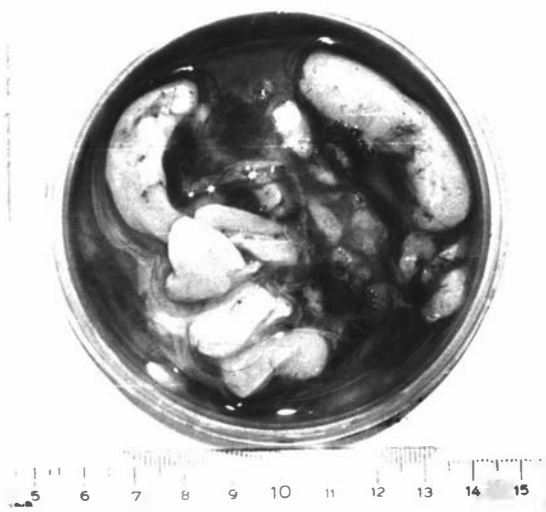
2a. (Polox. Steer GREY) Sample of fragmented boli 15 min. after collection.

2b. The same sample $2\frac{1}{2}$ hours later. (no agitation)

Fig. II. 4 Samples of intact and fragmented paste boli not subjected to agitation.

1a. (Polox. Steer GREY) Sample of intact boli 15 min. after collection.

1b. The same sample 2½ hours later (agitation).



2a. (Polox. Steer GREY) Sample of fragmented boli 15 min. after collection.

2b. The same sample 2½ hours later (agitation)

Fig. II. 5 The effect of agitation on samples of intact and fragmented paste boli. TREATMENT: 60 secs. agitation every ½ hour for 2½ hours.

TABLE II. 1

Reaction grade according to paste type

Paste Type	Steer GREY					Steer 353					Both Steers				
	Total Tests	Totals for each react. grade				Total Tests	Totals for each react. grade				Total Tests	Totals for each react. grade			
		A	B	C	D		A	B	C	D		A	B	C	D
KB_3/32A	6		3	3		5		3	1	1	11		6	4	1
32B	3	1	2			6		2	2	2	9	1	4	2	2
34A	4	2	1	1		5	1	2	1	1	9	3	3	2	1
34B	5	3	1		1	6	1	2	2	1	11	4	3	2	2
Polox. (SKF)	5	2	2	1		5	2	1	2		10	4	3	3	
Summed Totals	23	8	9	5	1	27	4	10	8	5	50	12	19	13	6

TABLE II. 2

Intactness grade according to paste type

Paste Type	Steer GREY					Steer 353					Both Steers				
	Total Tests	Totals for each intact. grade				Total Tests	Totals for each intact. grade				Total Tests	Totals for each intact. grade			
		E	F	G	H		E	F	G	H		E	F	G	H
KB_3/32A	6		2	4		5		2	3		11		4	7	
32B	3	1	2			6	1	2	2	1	9	2	4	2	1
34A	4	2	1	1		5	1		3	1	9	3	1	4	1
34B	5	1	1	3		6	1	1	3	1	11	2	2	6	1
Polox. (SKF)	5	2	3			5		2	3		10	2	5	3	
Summed Totals	23	6	9	8		27	3	7	14	3	50	9	16	22	3

TABLE II. 3

Reaction grade and corresponding intactness grade according to dosing order number for each paste formulations.

Paste Type	Dosing order number	Steer GREY						Steer 353					
		1	2	3	4	5	6	1	2	3	4	5	6
$KB_3/32A$	React. grade	B	B	C	C	B	C	B	C	D	B	B	
	Intact. "	G	F	G	G	G	F	H	G	G	F	F	
32B		A	B	B				C	C	D	C	B	B
		E	F	F				G	G	H	F	E	F
34A		B	A	C	A			A	B	B	C	D	
		G	E	F	E			E	G	G	G	H	
34B		A	A	D	B	A		A	B	C	D	C	D
		F	E	G	G	G		E	F	G	G	G	H
Polox. (SKF)		B	C	A	A	B		B	A	A	C	C	
		F	F	E	E	F		G	F	F	G	G	

TABLE II. 4

Total reaction and intactness grades scored by each steer according to dosing order number (all paste formulations combined).

Grade	Steer GREY						Steer 353					
	Grade totals according to dosing order number						Grade totals according to dosing order number					
	1	2	3	4	5	6	1	2	3	4	5	6
React. A	2	2	1	2	1		2	1	1			
B	3	2	1	1	2		2	2	1	2	2	1
C		1	2	1		1		2	1	3	2	
D			1				1		2		1	1
Intact. E	1	2	1	2			2				1	
F	2	3	2		1	1	1	2	1	1	1	1
G	2		2	2	2		2	3	3	4	2	
H			1						1		1	1

CHAPTER III A comparison of the Poloxalene decay curves in free rumen liquid following administration of liquid and paste formulations of the detergent to steers.

INTRODUCTION

The concept of incorporating anti-bloat detergents in paste-like materials is to try to improve the efficacy by slowing the washout of detergent from the rumen. In Chapter I it was shown that different paste formulations had different rates of detergent release when studied in a rumen model. In Chapter II, it was shown that these same pastes could suffer considerable physical disruption during dosing and that the degree of intactness of the paste bolus on arrival in the rumen could vary widely. Broken-up boli were shown to dissolve more quickly than intact boli when mildly agitated and it was proposed that this could be critical when paste boli were subjected to rumen motility. Therefore, the effects of disruption may outweigh the gains of any paste formulation.

From the results of the rumen model experiments the most promising paste formulation appeared to be $KB_3/32B$. However, it was not possible to study this formulation in vivo because of lack of a suitable assay method. A non-specific method was available for determining Poloxalene detergent in rumen fluid. Poloxalene paste was therefore used as a model and an experiment was designed with three objectives.

1) To establish and compare the detergent concentration decay curves of Poloxalene given as either liquid or paste.
2) To compare these detergent decay curves with the concentration decay curve of the standard rumen marker, polyethylene glycol 4000 (PEG), established in each of the steers.

3) To compare the detergent decay curves found *in vivo* with those obtained in the rumen model.

EXPERIMENTAL DESIGN

Three treatments, Poloxalene paste (P.P.), Poloxalene liquid (P.L.), and PEG were administered to three steers housed indoors and fed freshly cut pasture. On any one experimental day, one steer received P.P., one P.L. and the other PEG. The three treatments were administered to the three steers on a number of occasions divided into two runs, run X and run Y.

Treatments were given prior to the morning feeding and observations carried out over the following 24 hours.

The general procedure was as follows. The rumen contents were emptied out of each steer into separate insulated drums, weighed, mixed and sampled for estimation of the dry matter percentage. The method used was that according to Reid (1965).

The rumen contents of the two steers receiving the Poloxalene treatments were returned to the rumen and each animal dosed. PEG was not administered to the animal but was simply mixed into the rumen contents of the animal receiving this treatment. This was carried out after the dry matter samples had been taken. Further samples were taken after mixing for estimation of the zero time PEG concentration. The rumen contents were then returned to the steer.

The three animals were then fed fresh cut pasture (clover - ryegrass, 40:60) *ad lib*. Water was also available *ad lib*. Records of the feed and water intakes were kept.

Each steer was then periodically sampled over 24 hours for detergent or PEG analysis.

At the end of 24 hours, the rumen contents were again emptied from each steer, weighed, mixed and sampled for estimation of the dry matter percentage and "agent" (Poloxalene or PEG) concentration.

MATERIALS AND METHODS

Materials

The paste and liquid Poloxalene formulations studied were those described in Tables I.2 and I.3.

PEG is a highly polymerised water soluble marker which is inert and unabsorbed in the rumen. It has been commonly used to study water turnover in the rumen (Hyden, 1955).

Animals

The three steers, A, B and C were Jerseys, were three years old, had rumen fistulae and weighed approximately 400 Kg each.

They were bought into the cattle accommodation several days prior to the experiment to accustom them to the experimental environment.

Administration of the treatments

PEG : 200g of PEG was dissolved in 1.0L of water and mixed into the rumen contents as described.

Poloxalene liquid : Doses, ranging from 20, 50, 60, 125 to 150g of active detergent, were administered in a total volume of 500 ml from a long neck bottle.

Poloxalene paste : Doses up to 60g of active detergent were administered by the paste dosing gun (Fig. II. 1). Doses from 60g up to 150g were expressed from a modified paste cartridge close to the cardia by extending an arm through the rumen fistula.

Sampling method

When sampling from each steer, several sub-samples were taken from various regions of the rumen. The fluid was expressed into a 1.0L beaker and the solid material returned to the rumen. The fluid (sample) was then mixed and about 100g poured into a pottle which was then capped. The remainder was returned to the animal.

The samples were labelled and placed in an ice

bath.

Analytical methods

(a) PEG : PEG in the rumen liquor was determined by a modification of the turbidimetric method of Hyden as described by Ulyatt (1964).

(b) Poloxalene detergent : It became apparent during the first run, run X, that the method of detergent analysis being used was not efficiently recovering Poloxalene from the rumen fluid. An investigation of the method was carried out and resulted in an improved method which was used in run Y.

In particular, it was found that the recovery of Poloxalene was highly sensitive to the degree of salt saturation attained and this in turn depended largely on the degree of agitation used during the salting out process.

Hence, two methods of Poloxalene analysis were used : method X in run X and method Y in run Y.

In method X, the reagents Ferric chloride and Ammonium thiocyanate were made up in distilled water. In method Y, they were made up in saturated saline. Also, whereas shaking (agitation) was done by hand in method X, an automatic flask shaker was used in method Y.

Reagents
1. Sodium chloride R-grade NaCl.
2. 0.33M Barium Hydroxide stored at 4°C
3. 1.00M Zinc sulphate " " "
4. 60% w/w Ferric chloride
5. 57% w/w Ammonium thiocyanate
6. 1, 2, Dichloroethane

Apparatus
250ml stoppered Erlenmeyer flasks
50ml centrifuge tubes
Ultra-speed centrifuge (Sorval SS3).
3,5,10,15 and 20ml pipettes.
Automatic flask shaker
Spectrophotometer capable of reading at 515 nm.

Procedure

1. The sample of rumen fluid (100g) was centrifuged at 16,000 rpm for 10 mins. to remove particulate matter. The supernatant layer was decanted off and cooled to 0^{o}c in an ice bath.
2. 50ml of the cold clarified rumen fluid was measured into a beaker and 10ml of cold barium hydroxide was added with stirring. 3ml of cold zinc sulphate was added in the same fashion to make a final volume of 63ml. The mixture was then centrifuged at 16,000 rpm for 10 mins.
3. Up to 15ml of the supernatant layer was poured into a measuring cylinder. Depending on the Poloxalene concentration, this volume was diluted with water to obtain a final concentration within the range 25 to 75 ug/ml.
4. 10ml of the final solution (250 to 750ug of Poloxalene) was placed in an Erlenmeyer flask and made to a volume of 70mls : in method X with distilled water, in method Y with saturated saline.
5. 3ml of Ferric chloride was added and the flask briefly shaken.
6. 15ml of ammonium thiocyanate was added and the flask briefly shaken.
7. NaCl was added and dissolved by shaking. In method X, 30g of NaCl was added, in method Y, only 4g (because agents already made up in saturated saline).
8. 20ml of 1, 2, Dichloroethane was added and the flask vigorously shaken. In method X, shaking was done by hand for 5 mins., in method Y, the flask was shaken for 30 mins. on an automatic flask shaker.
9. Following shaking the flask was allowed to stand for 5 mins. The aqueous layer was then removed by aspiration and discarded. The organic layer was transferred to a 50ml centrifuge tube and spun at up to 2000 rpm for 2 mins. to settle and water cloud.
10. The organic layer was then transferred to a 10ml

spectrophotometer tube and its absorbance read at 515nm against a Poloxalene-free rumen liquor blank. The Poloxalene concentration was then determined by reference to a calibration graph. The concentration in the original sample was calculated after making allowances for dilutions occurring during sample preparation and expressed as ug of Poloxalene per ml of rumen liquor.

Standard calibration graphs

Calibration graphs for Poloxalene detergent and PEG were prepared by assaying known concentration of the two materials in rumen fluid.

The absorbance values obtained for the standard concentrations are shown in Table III. 1 and plotted in Figs. III. 1 and III. 2.

Calculations

(a) PEG concentration : Depending on the expected PEG concentration in the rumen fluid, the assay procedure involved two successive dilutions. These had to be accounted for when calculating the PEG concentration in ug per ml of undiluted rumen liquor.

(b) Percentage of PEG and liquid Poloxalene recovered at zero time : This calculation could be done only for the liquid Poloxalene and PEG treatments in each steer.

The zero time PEG concentration in rumen fluid was obtained by analysis of the zero time rumen liquor sample.

The zero time liquid Poloxalene concentration was obtained by plotting the detergent decay curves on semi-log paper and extrapolating the curve back to zero time (Figs. III. 3, III. 4, III. 5, III. 6, III. 7, and III. 8).

The zero time PEG and liquid Poloxalene concentrations were then multiplied by the rumen water volume calculated at zero time (see following) to give the total amount of agent recovered. This amount was divided by the dose of agent and multiplied by 100 to give the percentage recovered at zero time.

(c) Rumen dry matter percentage and rumen water volume : The dry matter percentage (D.M.%) of the rumen contents was determined by weighing samples obtained at bailing before (wet weight) and after drying for 48 hours in an oven (97°C; fan operated), (dry weight). The figure taken was the average of the samples.

The rumen water volume was then obtained by multiplying the total weight of the rumen contents removed at bailing by (100 - D.M.%). The volumes obtained required corrections for water which was added when PEG or liquid Poloxalene was the treatment, i.e. 1.0L and 500 ml respectively to obtain the corrected total rumen water volume at zero time.

(d) Total water inflow and outflow from the rumen: The water flux was calculated by methods proposed by Reid (1965). If the rumen water volume at the start and end of each experimental run (V_o and V_T respectively) is known, and the agent concentration at those times (Co and C_T respectively) is known, then the net water inflow to the rumen over the time period T can be calculated using the formula below:-

$$\text{Inflow } (L.h^{-1}) = \frac{\frac{Vo - V_T}{T} \times \log (Co/C_T)}{\log (Vo/V_T)}$$

$$= \frac{\frac{Vo - V_T}{T} \times (\log Co - \log C_T)}{(\log Vo - \log V_T)}$$

Vo = Rumen water volume at zero time
V_T = " " " " time T
C_o = Agent concentration at zero time
C_T = " " " time T
T = Time period of run (hours)

$$\text{Outflow } (L.h^{-1}) = \text{Inflow t } \frac{(Vo - V_T)}{T}$$

This calculation was carried out for all PEG treatments in runs X and Y and for the liquid Poloxalene treatments in run Y.

The total water inflow to the rumen is made up of endogenous water (mainly water secreted with saliva and influx across the rumen epithelium), and exogenous water (drinking water and water associated with feed).

(e) Exogenous water inflow to the rumen : For every fresh cutting of pasture fed to the animals, the dry matter percentage was estimated from two samples (200g each) in the manner described for rumen D.M.%.

Using the feed D.M.% and the recorded feed intakes, the amount of water each steer ingested in the feed was calculated. To this was added drinking water consumed. The rate of water intake was then given by the equation below:-

$$\text{Exogenous water intake rate } (L.h^{-1}) = \frac{\text{Feed intake x(100-Feed D.M.\%) + water drunk}}{\text{elapsed time}}$$

(f) Endogenous water inflow to the rumen : This was calculated by subtracting the exogenous water inflow from the calculated total water inflow to the rumen. This could be done for all PEG treatments in runs X and Y and for the liquid Poloxalene treatments in run Y.

(g) Average dilution rate of PEG and of liquid Poloxalene : The gradient of the curve relating agent concentration (ug/ml) with time (h), plotted on semi-log paper, is at any time negative (Figs. III. 3, III. 4, III. 5, III. 6, III. 7 and III. 8). By fitting a straight line by eye to the points relating concentration with time, an average agent dilution rate was obtained. The average dilution rate, D, was calculated from the formula:-

$$D = \frac{\log_e (C_1/C_2)}{t_2 - t_1}$$

C_1 = agent concentration at time t_1

C_2 = " " " " t_2

t_1 and t_2 = time in hours

(h) Calculation of D from the half life : It is convenient to read off the half times which is when $C_1/C_2 = 2$ from the dilution curves. Hence D equals:-

$$D = \frac{\log_e (C_1/C_2)}{t_2 - t_1} = \frac{\log_e 2}{t_{\frac{1}{2}}}$$

$t_{\frac{1}{2}}$ = time taken for agent concentration at C_1 to decay to half its value (C_2).

RESULTS

The initial dose rate of Poloxalene was 20g. However, this apparently disappeared from the rumen at a very fast rate and it could not be detected in rumen fluid after 12 hours. The dose rate was successively increased to 60g and 150g in the hope of extending the period in which the detergent could be detected but this did not occur.

An inspection of the results in run X revealed that the theoretical recoveries of Poloxalene were poor (9 - 26%). This suggested that the method might be at fault and following an investigation it was improved. In the second run, run Y, treatments were carried out with 50g and 125g doses and the recoveries of detergent were increased to 60 - 70%.

The results obtained in both runs are given for each steer as agent concentration versus elapsed time in Table III. 2. They are also plotted in Figs. III. 3, III. 4, III. 5, III. 6, III. 7 and III. 8.

No obvious differences were noted between the detergent decay curves of Poloxalene paste (P.P.) or liquid (P.L.) in any of the steers in run X. However, using the more sensitive Poloxalene assay in run Y, an initial build up period was evident in which the detergent concentration increased in the rumen as it was released from the paste (Figs. III. 4, III. 6 and III. 8).

The Poloxalene paste formulation did not appear to slow the washout of detergent from the rumen by more than 1½ to 2 hours in comparison with the liquid Poloxalene.

Recovery of PEG and P.L. at zero time, expressed as a percentage of the dose administered, is shown in Table III. 3.

Table III. 3 shows the marked improvement of recovery of Poloxalene in run Y following use of the more sensitive Poloxalene assay method.

The recovery of PEG was consistently high in both runs.

The average agent dilution rates determined for all experiments are given in Table III. 4, along with each steer's exogenous water intake over the first 10-12 hours of the run.

The average agent dilution rate is plotted against the exogenous water intake for each treatment during the first 10 - 12 hours after administration in Fig. III. 9.

Generally, a trend of increasing dilution rate with increasing hourly exogenous water intake was seen (Fig. III. 9).

Calculation of the total water inflow to the rumen showed a marked increase for Poloxalene. However, the exogenous water intakes for PEG and Poloxalene were similar in run Y. Therefore, the increase in the total water inflow in association with Poloxalene treatments was due to an increase in the endogenous water inflow.

The endogenous water inflow to the rumen and the hourly feed dry matter intakes of each steer over 24 hours are shown in Table III. 5 for all PEG treatments in runs X and Y and for the Poloxalene treatments in run Y. The hourly total water inflow is also shown.

The data in Table III. 5 is plotted in Fig. III. 10 as endogenous water inflow versus feed dry matter intake.

Fig. III. 10 shows a marked increase in the endogenous water inflow rate when the treatment was 125g of liquid Poloxalene (upper two Poloxalene symbols, Fig. III. 10). The lower Poloxalene symbol in Fig. III. 10 represents the 50g liquid Poloxalene dose to steer C. In this case, the endogenous water inflow is similar to the highest rate of inflow following administration of PEG.

DISCUSSION

The Method

Analytical limitations : The method was basically unsatisfactory for several reasons:-

1. It was non-specific
2. It was highly dependent on a salting out process which was difficult to standardise.
3. The concentration range over which the method could be used with any confidence was limited.
4. Interferences occurred when handling volumes of greater than 15ml of undiluted rumen fluid. A red scum formed which discoloured the organic layer making it impossible to obtain useful absorbance readings.
5. It could not be used to analyse the rumen particulate matter for detergent.

A full determination of the distribution of the detergent would require better methods than were available during this investigation.

The improved Poloxalene analytical method Y recovered 60-70% of the Poloxalene dose at zero time (Table III. 3). The remaining 30-40% could have been:

1) Binding to sites on the particulate matter and therefore removed during the initial centrifugation of the sample.
2) Lost during the protein precipitation step of the analytical method.
3) Present in the rumen fluid fraction but not detected because the degree of sensitivity of the analytical method was not high enough.

If for example 30% of the Poloxalene dose was bound to particulate matter, it could be an important factor affecting detergent concentration in rumen liquid.

Absence of satisfactory chemical methods of detergent analysis in rumen fluid limits further investigation of the kind described here.

Some of these problems might well be overcome by using isotopically labelled detergents.

Results

Despite the deficiencies of the Poloxalene assay methods, all the Poloxalene decay curves showed a similar pattern within each run (Figs. III. 3, III. 4, III. 5, III. 6, III. 7 and III. 8). Neither the Poloxalene paste nor liquid appeared to decay at a slower rate than the PEG. No systematic variation in the decay curves other than what could normally be expected due to day to day variation within an animal, was observed.

The improved sensitivity of Poloxalene assay method Y showed a period of concentration build-up in the rumen for detergent released from the paste (Figs. III. 4, III. 6, and III. 8). This period may leave animals at the risk of bloat until an effective detergent concentration is reached in the rumen.

Fig. III. 4, and III. 8 show that the decay of detergent released from the paste in the rumen of steers A and C, lagged behind the decay of the liquid Poloxalene detergent by 1½ to 2 hours. This was not the case in Steer B (Fig. III. 6). The apparent faster rate of washout for detergent released from paste in steer B, run Y, could be related to the manner of administration of the

paste to this animal. Whereas in steers A and C the larger doses (125g) were deposited at the cardia in a single block, the 50g given to B was administered by mouth using the paste dosing gun. The gun had to be operated several times to deliver that dose resulting in a series of small boli reaching the rumen. This broken up dose would present a greater surface area per unit weight of paste to the rumen fluid and would be expected to allow faster diffusion of detergent.

Generally, the average agent dilution rate was found to increase as the rate of exogenous water intake increased (Fig. III. 9). The average dilution rate of all treatments in run Y were higher than in run X (Table III. 4) and correspondingly the water intakes associated with the feed were higher. This may be explained by the fact that the pasture fed to the steers in run X had been kept free of grazing for some months over the winter (i.e. Autumn saved pasture). By contrast, the pasture fed to the animals in run Y was 4 weeks old re-growth and appeared to be more palatable and the animals ate more.

Many of the concentration decay curves in Figs. III. 3, III. 4, III. 5, III. 6, III. 7 and III. 8 showed a more rapid decay (dilution rate) during the first 6 hours of the run than at any other time. It was also the period when the steers ate hungrily.

As the rate of dry matter intake increased, there was generally an increase in the endogenous water inflow to the rumen for each steer (Table III. 5, Fig. III. 10) probably due to an associated increase in the salivary flow.

The most noticeable feature was that the endogenous water inflow increased by more than 50% when the treatment was 125g of Poloxalene liquid. This increase did not appear to occur when the treatment was 50g of Poloxalene liquid suggesting that above a certain dose of Poloxalene, the endogenous water inflow to the rumen is influenced. The effect of this would be to increase the average dilution rate of the liquid Poloxalene.

Although the same calculations cannot be done for the Poloxalene paste in run Y, there is no reason to suppose that the same events did not occur. This possibility is supported by the fact that the average dilution rates of the Poloxalene paste and liquid in run Y were very similar for the three steers (Table III. 4).

The standard dose of Poloxalene for bloat control in grazing cattle is 10-20g and it would seem unlikely that the effects on endogenous water inflow seen here with high doses would occur in the field.

In these experiments it was found that the:-

1) Poloxalene paste formulation, $KB_6/16$, did not slow the washout of detergent from the rumen.
2) Dilution rates of Poloxalene paste, liquid and PEG were similar within each steer in run Y.
3) Dilution rates of high doses of Poloxalene liquid and probably paste, were influenced by a large increase in the endogenous water inflow to the rumen.

These results have a direct bearing on the validity of the rumen model as a testing system. Comparison of the in vivo Poloxalene detergent decay curves in run Y (Fig. III. 4, III. 6 and III. 8) with the in vitro Poloxalene decay curves (Fig. I. 6) shows an obvious similarity in shape. That is, in both in vivo and in vitro, the paste failed to slow the washout of detergent. Although this evidence is very limited, it would suggest that the rumen model could well be a useful screening system. However, the rumen model is limited to the extent that if the role of particulate matter and endogenous water inflow to the rumen are important in detergent dilution in the rumen, then the model won't point this up.

Nevertheless, at this stage, it would seem that the in vitro system warrants further study and development.

Summary

1. The detergent decay curves of a Poloxalene paste and liquid, and the concentration decay curves of PEG 4000 were established in three steers with rumen fistulae.

2. The paste formulation was found to slow the washout of detergent from the rumen by only 1½ to 2 hours over the liquid Poloxalene control.

3. High doses of Poloxalene liquid were found to markedly increase the endogenous water inflow to the rumen and thereby increase the average detergent dilution rate. The same appeared likely to occur with large doses of Poloxalene paste.

4. The in vivo detergent decay curves established in run Y of the experiment were similar in shape to those obtained for the same materials in the rumen model experiments described in Chapter I. However, no firm conclusions can be drawn as to how closely the rumen model conditions correlated with those in vivo.

5. The conclusion is that the Poloxalene paste formulation $KB_6/16$ is unlikely to offer any advantages over the liquid formulation in terms of reducing the dosing frequency.

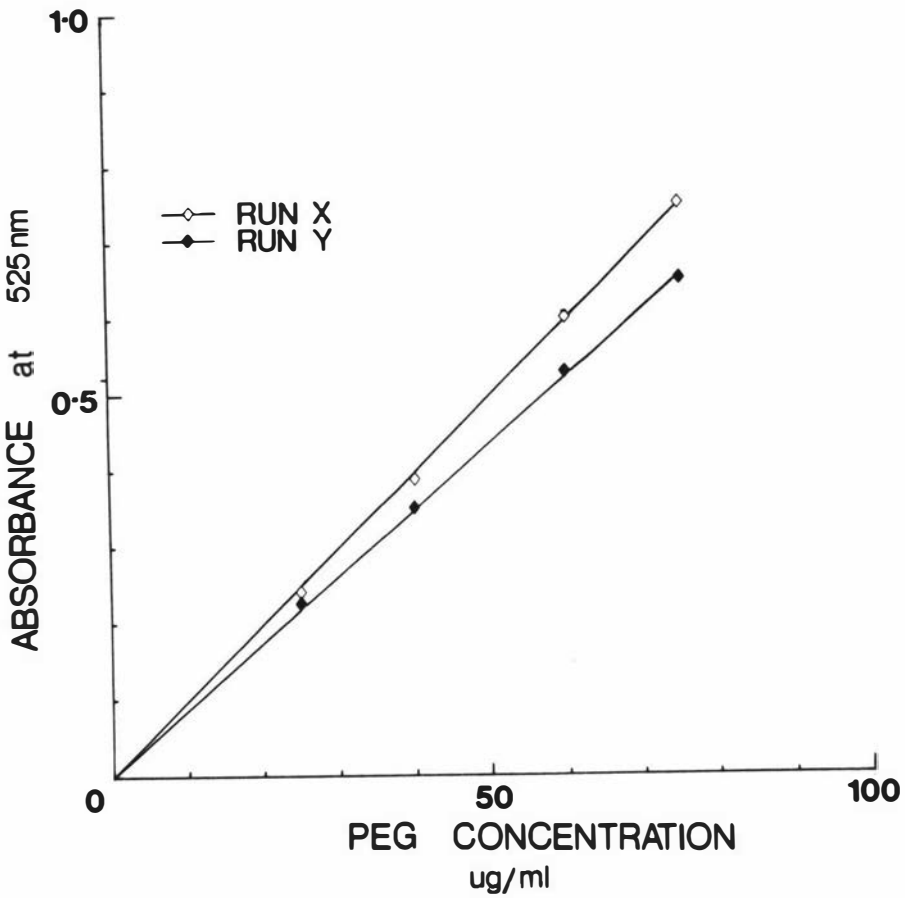


Fig. III. 1 Calibration graphs used to determine PEG concentrations in rumen fluid.

Note the difference betweeen runs X and Y.

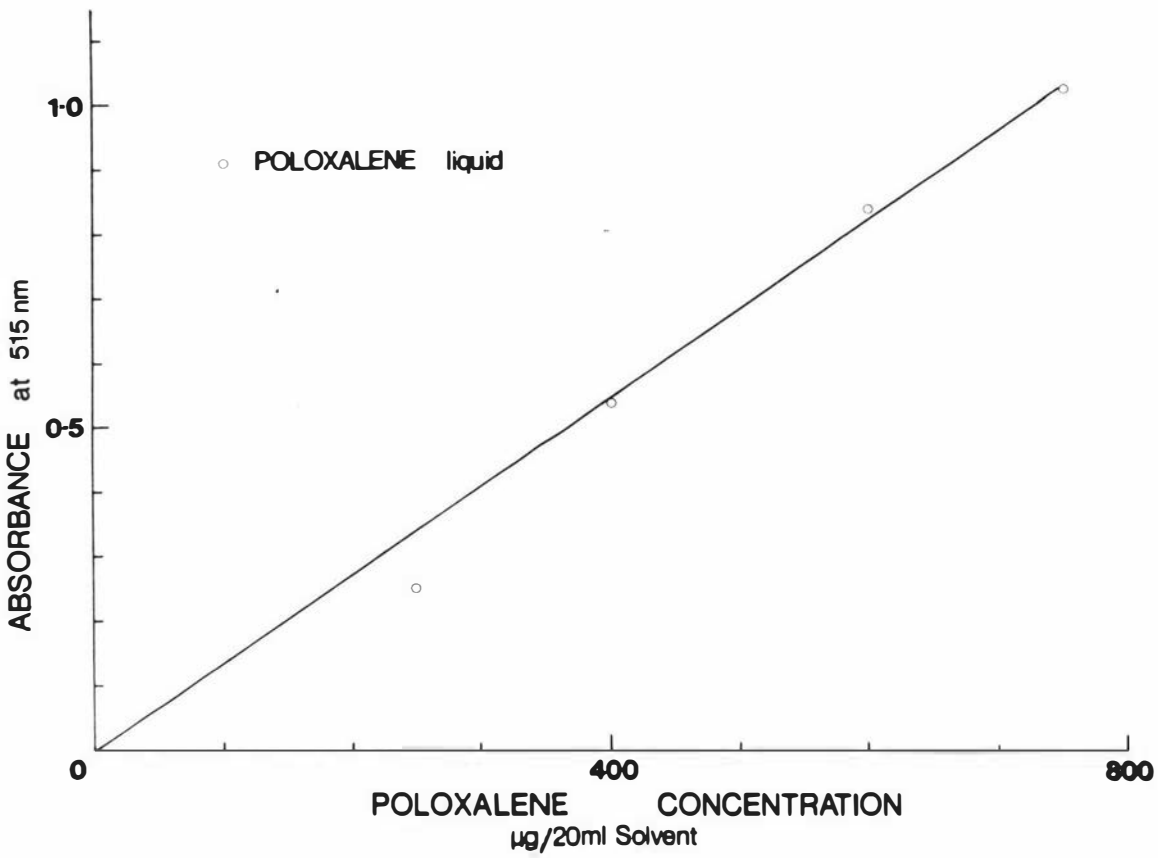


Fig. III. 2 Calibration graph used to determine the Poloxalene concentration in rumen fluid in both runs.

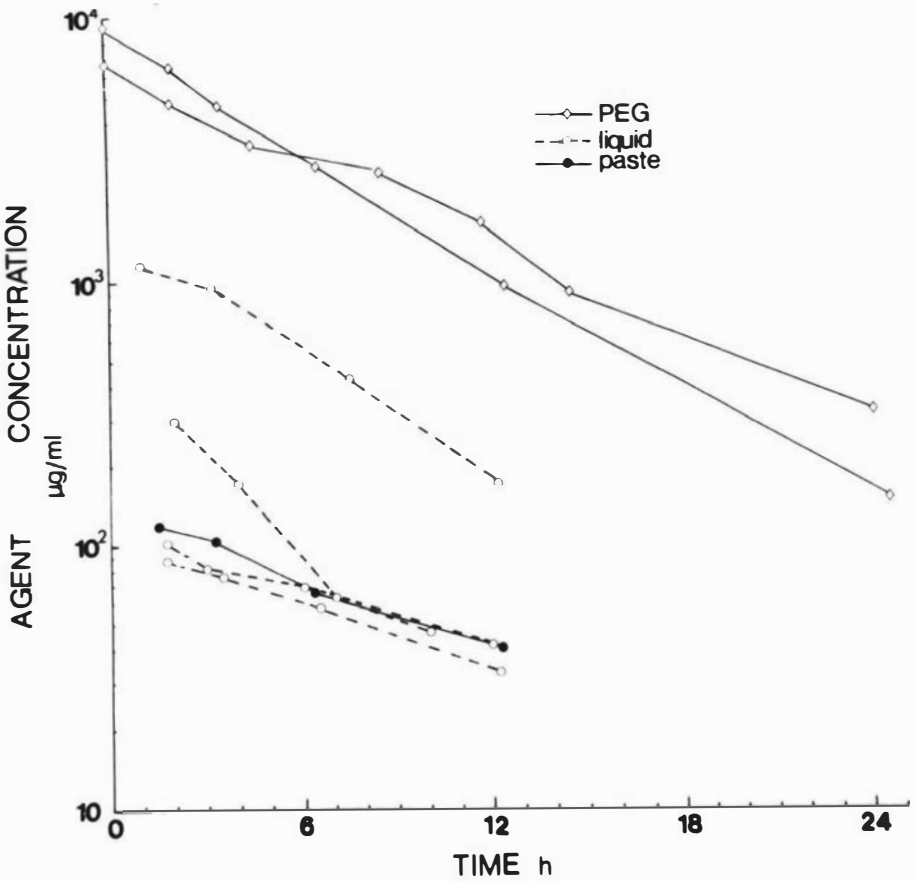


Fig. III. 3 Agent concentration versus elapsed time for steer A in run X.

Doses: PEG = 200g
P.L.= 20, 60, 150g
P.P.= 20g

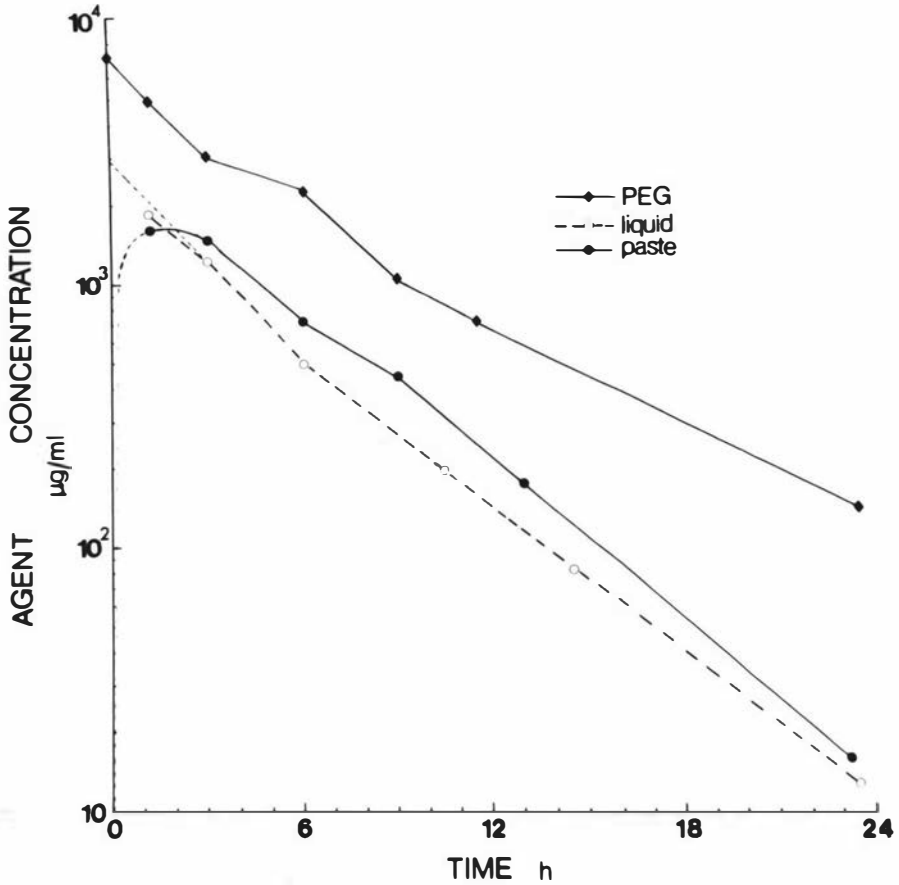


Fig. III. 4 Agent concentration versus elapsed time for steer A, run Y.

Doses: PEG = 200g
P.L.= 125g
P.P.= 125g

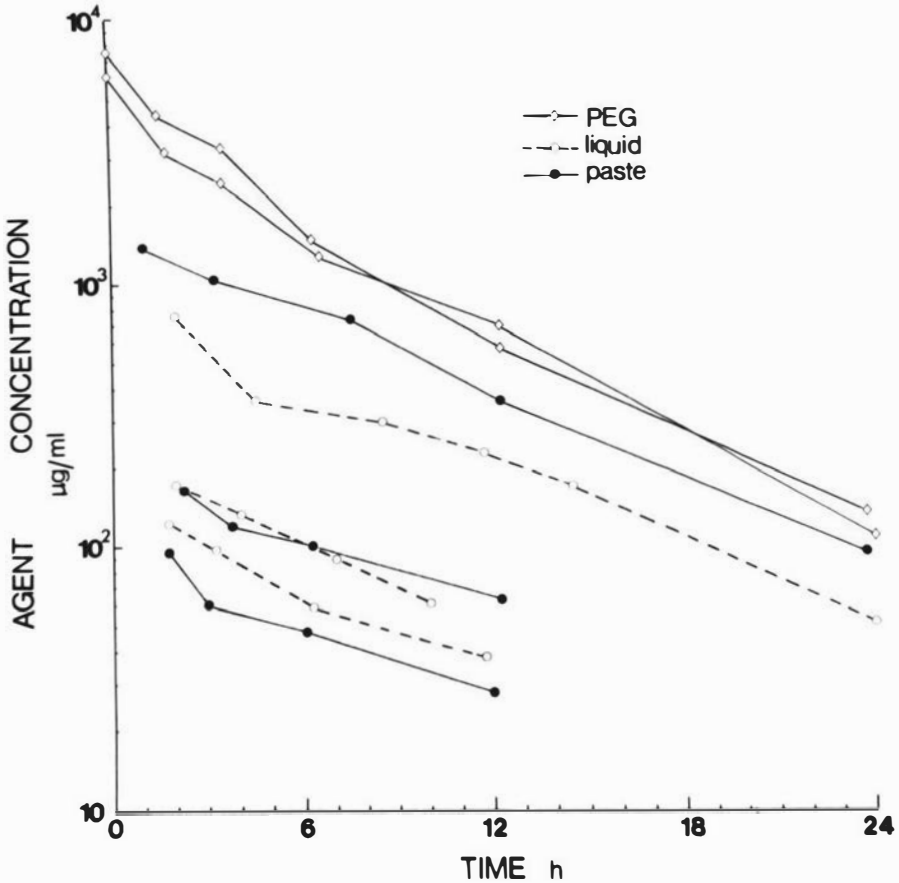


Fig. III. 5 Agent concentration versus elapsed time for Steer B, run X.

Doses: PEG = 200g
P.L.= 20, 60, 150g
P.P.= 20, 60, 150g

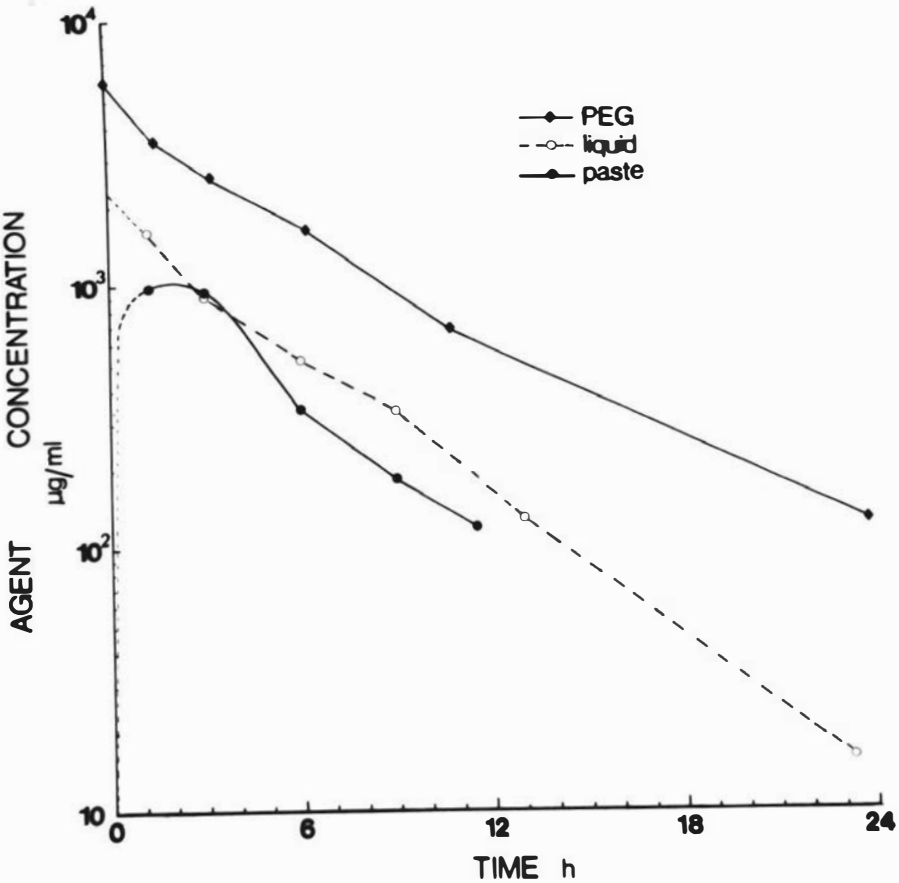


Fig. III. 6 Agent concentration versus elapsed time for Steer B, run Y.

Doses: PEG = 200g
P.L.= 125g
P.P.= 50g

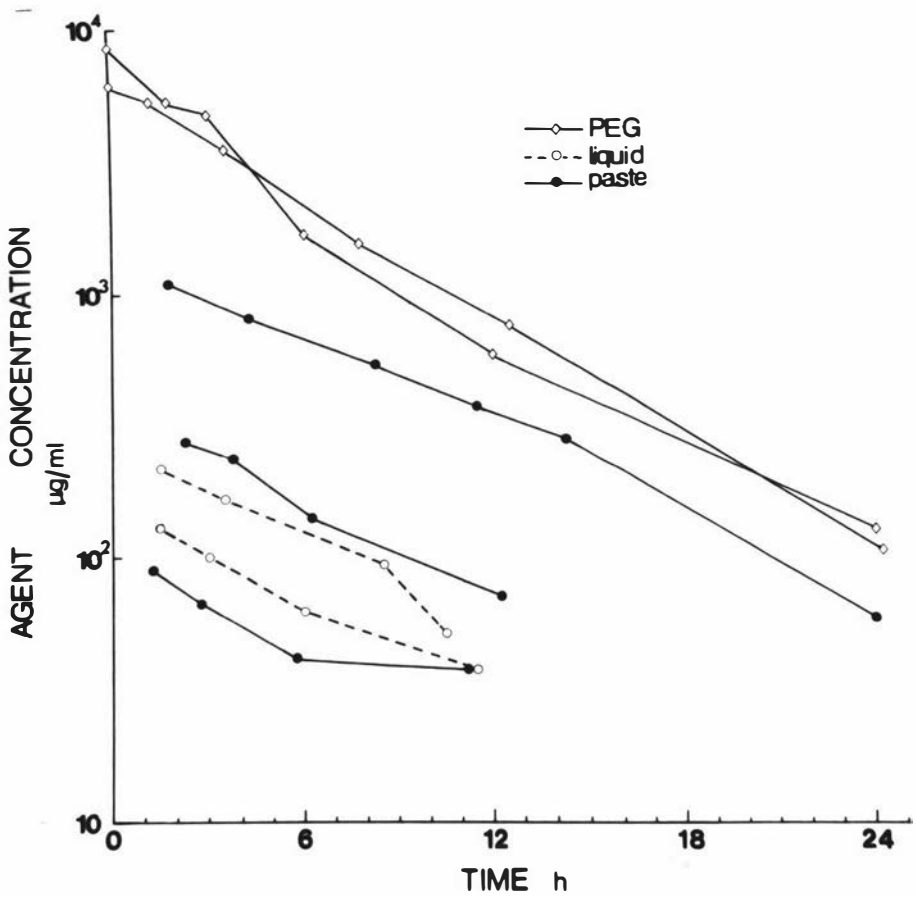


Fig. III. 7 Agent concentration versus elapsed time for steer C, run X.

Doses: PEG = 200g
P.L.= 20, 60g
P.P.= 20, 60, 150g

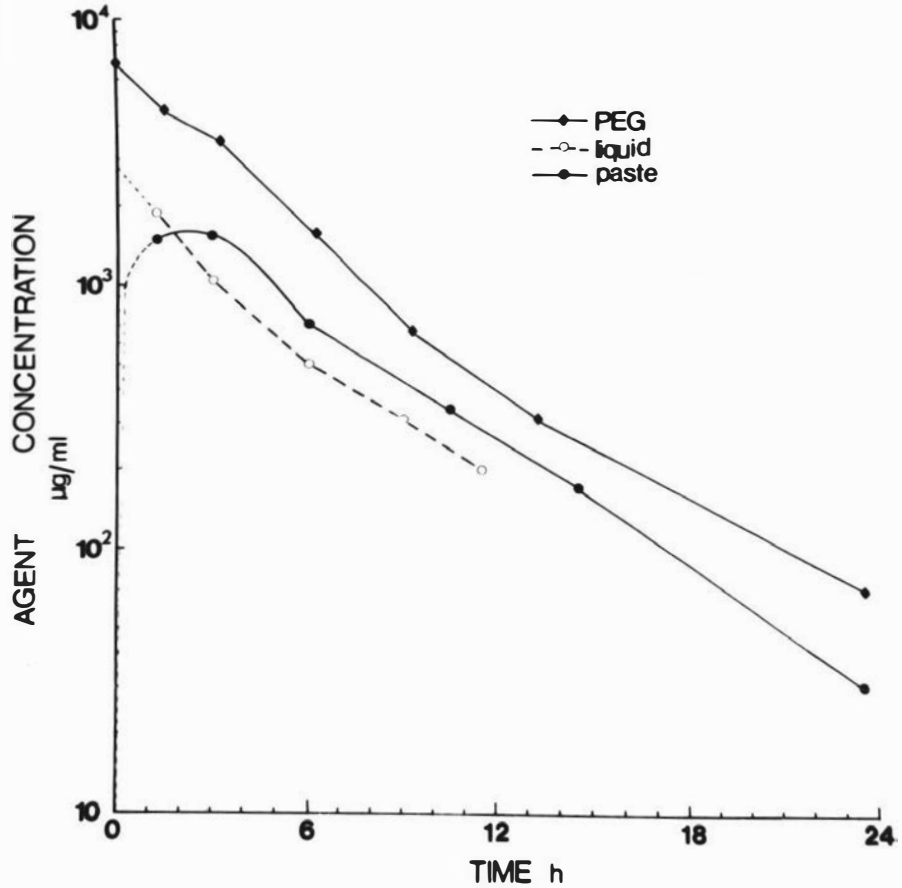


Fig. III. 8 Agent concentration versus elapsed time for steer C, run Y.

Doses: PEG = 200g
P.L.= 50g
P.P.= 125g

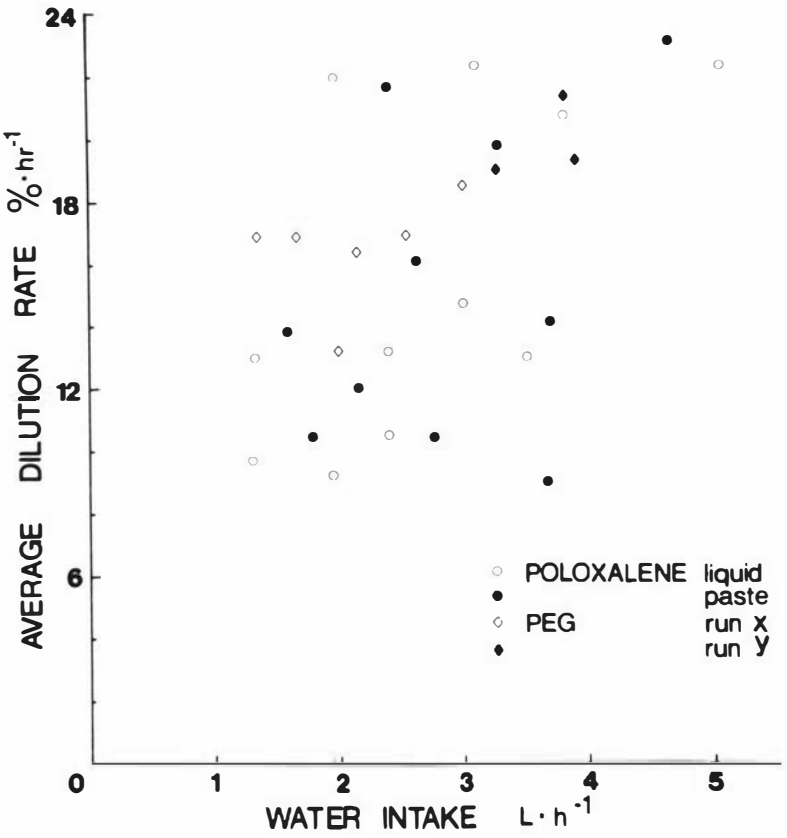


Fig. III. 9 Average dilution rate versus exogenous water intake over the first 10-12 hours of each treatment.

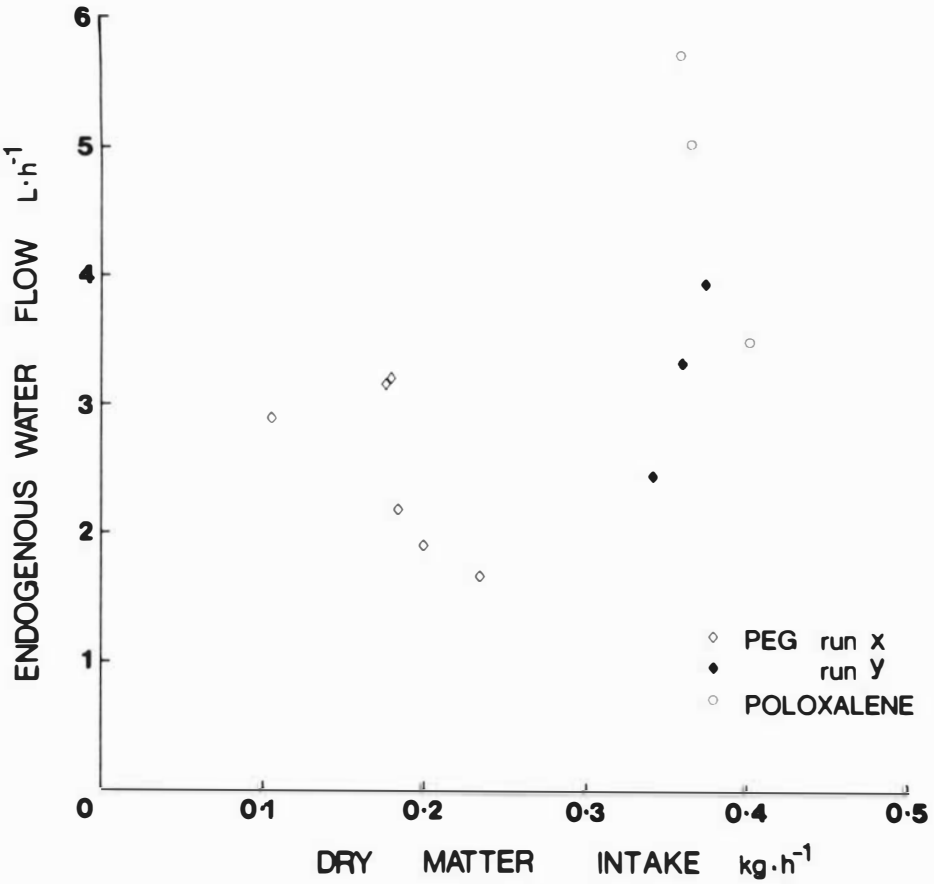


Fig. III. 10 Endogenous water inflow versus the hourly feed D.M. intake in each steer during all the PEG treatments and during the liquid Poloxalene treatments in run Y.

TABLE III. 1

The absorbance values of standard PEG and Poloxalene concentration.

ug PEG	Absorbance at 525mu	
	Run X	Run Y[a]
75	0.75	0.65
60	0.60	0.53
40	0.39	0.35
25	0.24	0.23
ug Poloxalene	**Absorbance at 515mu**	
750	1.00	
600	0.84	
400	0.54	
150	0.25	

a In the assay procedure for PEG used in run Y, a new batch of Trichloroacetate-Barium chloride reagent was used. This could account for the small differences in the absorbance values obtained between run X and run Y.

Calibration graphs for these data are shown in Figs. III. 1 and III. 2.

TABLE III. 2

Concentration of agent at different times after treatment.

P.L. = Poloxalene liquid, P.P. = Poloxalene paste
PEG = Polyethylene glycol 4000.

Steer	Run	Treatment	Elapsed time (h) / Agent concentration (ug.ml)	
A	X	P.P. 20g	1.5 3.3 6.3 12.3	(t)
			120 105 68 41	(c)
		P.L. 20g	1.75 3.0 6.0 12.0	(t)
			103 82 69 42	(c)
		P.L. 20g	1.75 3.5 6.5 12.25	(t)
			87 76 58 33	(c)
		P.L. 60g	2.0 4.0 7.0 10.0	(t)
			299 172 63 47	(c)
		P.L. 150g	1.0 3.25 7.5 12.25	(t)
			1160 962 431 172	(c)
		PEG 200g	0 2.0 3.5 6.5 12.5 24.5	(t)
			9120,6540,4680,2760,975,150,	(c)
		PEG 200g	0 2.0 4.5 8.5 11.75 14.5 240	(t)
			6720,4800,3350,2592,1680,918, 325	(c)
	Y	PEG 200g	0 1.25 3.0 6.0 9.0 11.5 23.5	(t)
			7100,4900,3050,2260,1032,732,144	(c)
		P.L. 125g	1.25 3.0 6.0 10.5 14.5 23.5	(t)
			1865,1129,499,199,83,13	(c)
		P.P. 125g	1.25 3.0 6.0 9.0 13.0 23.0	(t)
			1625,1463,726,446,175,16	(c)
B	X	P.P. 20g	1.75 3.0 6.0 12.0	(t)
			95 61 47 128	(c)
		P.P. 60g	2.25 3.75 6.25 12.25	(t)
			164 122 102 63	(c)
		P.P. 150g	1.0 3.25 7.5 12.25 23.75	(t)
			1374 1046 743 365 96	(c)
		P.L. 20g	1.75 3.25 6.25 11.75	(t)
			123 98 59 38	(c)
		P.L. 60g	2.0 4.0 7.0 10.0	(t)
			171 135 90 61	(c)
		P.L. 150g	2.0 4.5 8.5 11.75 14.5 240	(t)
			768 361 300 227 171 52	(c)
		PEG 200g	0 1.5 6.25 12.25 23.75	(t)
			7620,4410,1590,570,137	(c)

TABLE III. 2 (cont'd.)

B	X	PEG 200g	0 1.75 3.5 6.5 12.25 24.0 (t) 6180,3200,2440,1400,700,110, (c)
	Y	PEG 200g	0 1.5 3.25 6.25 10.75 23.75 (t) 5900,3500,2575,1640,680,128 (c)
		P.L. 125g	1.25 3.0 6.0 9.0 13.0 23.25 (t) 1607,921,522,340,132,14 (c)
	a.	P.P. 50g	1.25 3.0 6.0 9.0 11.5 23.5 (t) 975, 930, 340, 182, 122 (c)
C	X	P.P. 20g	1.25 2.75 5.75 11.25 (t) 90 67 42 38 (c)
		P.P. 60g	2.25 3.75 6.25 12.25 (t) 284 240 142 71 (c)
		P.P. 150g	1.75 4.25 8.25 11.5 14.25 24.0 (t) 1108 818 545 384 286 59 (c)
		P.L. 20g	1.5 3.0 6.0 11.5 (t) 131 101 63 38 (c)
		P.L. 60g	1.5 3.5 8.5 10.5 (t) 220 167 95 52 (c)
		PEG 200g	0 1.75 3.0 6.0 12.0 24.0 (t) 8490,5445,4792,1740,600,130 (c)
		PEG 200g	0 1.25 3.5 7.75 12.5 24.25 (t) 6180,5409,3618,1620,780,108 (c)
	Y	PEG 200g	0 1.5 3.25 6.25 9.25 13.25 23.5 (t) 6800,4500,3550,1600,680,320,72 (c)
	b.	P.L. 50g	1.25 3.0 6.0 9.0 11.5 (t) 1922, 1040, 510, 217, 202 (c)
		P.P. 125g	1.25 3.0 6.0 10.5 14.5 23.5 (t) 1492,1562, 714, 340, 175, 31 (c)

a. The concentration values obtained for steer B, run Y, for the 50g paste dose, were multiplied by 2.5 to be comparable with the 125g paste doses given to steers A and C in run Y.

b. The concentration values obtained for steer C, run Y, for the 50g Poloxalene liquid dose, were multiplied by 2.5 to be comparable with the 125g liquid doses given to steers A and B in run Y.

TABLE III. 3

Percentage of PEG and Poloxalene liquid (P.L.) recovered at zero time.

Run	Steer	Treatment		Percent Recovered
X	A	P.L.	20g	15.20
		P.L.	20g	13.50
		P.L.	60g	18.17
		P.L.	150g	22.55
		PEG	200g	99.83
		PEG	200g	97.42
	B	P.L.	20g	16.25
		P.L.	60g	9.49
		P.L.	150g	26.30
		PEG	200g	105.64
		PEG	200g	95.79
	C	P.L.	20g	22.98
		P.L.	60g	14.43
		PEG	200g	101.57
		PEG	200g	96.02
Y	A	P.L.	125g	65.81
		PEG	200g	101.63
	B	P.L.	125g	71.28
		PEG	200g	108.26
	C	P.L.	50g	60.77
		PEG	200g	112.98

TABLE III. 4

Average agent dilution rate and the exogenous water intake of each steer during the first 10-12 hours following each treatment.

Run	Steer	Treatment	Average Dilution rate % h^{-1}	Exogenous water intake L. h^{-1}
X	A	P.P. 20g	10.42	2.80
		P.L. 20g	10.50	2.40
		P.L. 20g	9.24	1.95
		P.L. 60g	22.00	1.97
		P.L. 150g	16.50	2.43
		PEG 200g	16.96	1.34
		PEG 200g	13.20	2.00
	B	P.P. 20g	13.86	1.81
		P.P. 60g	8.94	3.65
		P.P. 150g	10.42	1.78
		P.L. 20g	13.07	1.33
		P.L. 60g	13.20	2.43
		P.L. 150g	9.76	1.36
		PEG 200g	16.90	1.67
		PEG 200g	16.37	2.15
	C	P.P. 20g	16.12	2.61
		P.P. 60g	14.15	3.72
		P.P. 150g	12.05	2.16
		P.L. 20g	13.07	3.48
		P.L. 60g	14.75	3.05
		PEG 200g	18.48	3.00
		PEG 200g	16.96	2.54
Y	A	P.P. 125g	21.66	2.40
		P.L. 125g	22.36	3.10
		PEG 200g	17.33	3.90
	B	P.P. 50g	23.11	4.66
		P.L. 125g	20.69	3.78
		PEG 200g	18.99	3.27
	C	P.P. 125g	19.86	3.27
		P.L. 50g	22.30	5.06
		PEG 200g	21.33	3.80

TABLE III. 5

The total and endogenous water inflow rates and the feed dry matter intakes of each steer in all PEG treatments in runs X and Y and in the Poloxalene treatments in run Y.

Run	Steer	Treatment	Total water inflow (L. h^{-1})	Endogenous water inflow (L. h^{-1})	D.M. intake Kg. h^{-1}
X	A	PEG 200g	3.19	1.92	0.119
		PEG 200g	3.04	1.67	0.234
	B	PEG 200g	3.86	2.88	0.105
		PEG 200g	4.90	3.20	0.179
	C	PEG 200g	4.04	2.19	0.184
		PEG 200g	5.06	3.17	0.176
Y	A	P.L. 125g	7.19	5.02	0.365
		PEG 200g	4.80	2.44	0.341
	B	P.L. 125g	8.00	5.71	0.357
		PEG 200g	6.21	3.94	0.374
	C	P.L. 50g	6.28	3.49	0.411
		PEG 200g	5.72	3.33	0.359

CHAPTER 4

Field trials to investigate the efficacy of anti-bloat pastes.

INTRODUCTION

During the course of this study a number of field trials were carried out. Two farms in the Manawatu with a history of severe bloat were used (the farm trials), and a further trial was carried out on the No. 4 Dairy Unit at the Ruakura Animal Research Centre in Hamilton (the Ruakura trial).

The objectives of the field trials were:

1) To determine whether the bloat controlling efficacy of paste formulations of detergent was better than liquid formulations of the same active materials.
2) To determine whether the effectiveness of the pastes in the field was of similar degree to that predicted from the laboratory investigations and
3) To test and develop techniques for conducting field trials on bloat preventives.

EXPERIMENTAL DESIGN

In each farm trial, the experimental herd was randomly divided into three groups which received either paste or liquid detergent, or remained untreated as a control group.

In the Ruakura trial, the experimental herd was divided into four groups. One was an untreated control group, one received ethoxylate liquid detergent, one received ethoxylate paste detergent and the fourth group received Poloxalene paste detergent.

In each trial, the groups were identified either by numbers alone or by numbers plus coloured neck bands.

The normal farming routine continued as usual.

The animals were appropriately treated night and morning (farm trials) or at night only (Ruakura trial). After treatment, the herd was turned out to graze

as usual, but each animal was regularly checked and scored for signs of bloat during the daylight hours.

Animals which became seriously bloated were immediately treated with paraffin oil.

MATERIALS AND METHODS

Materials

In the farm trials, the ethoxylate paste $KB_3/34A$ and an ethoxylate liquid formulation of the paste were tested.

In the Ruakura trial, the ethoxylate paste KB3/32B, ethoxylate liquid and Poloxalene paste $KB_6/16$, were tested.

The rumen model experiments had shown that both ethoxylate pastes markedly slowed the washout of detergent from the model but that Poloxalene did not (see Chapter I., p 25). The in vivo detergent decay curves for the Poloxalene preparations had supported the rumen model findings (see Chapter III., p78).

Approximately 5.0 to 5.5g of active material was the dose administered at each treatment.

Animals

In the farm trials 120 milking cows of mixed ages and breeds (Jersey, Friesian) made up the three experimental groups.

In the Ruakura trial, 12 sets of identical twin cows (24 cows) of mixed ages and breeds were randomly divided in a four way split into the four experimental groups. The bloating behaviour of each of these animals was well known, having been defined in earlier research.

Drenching

A paste dosing gun (see p 57) was used to administer the paste dose.

Liquid detergent was given by drenching gun, as was paraffin oil (28ml) when necessary.

Bloat Scoring System

The classification used was as follows:

Stage 0 - The cow was normal

Stage 1 - Mild bloat with the cow's left flank distended

Stage 2 - Moderate bloat with the left and right flanks distended

Stage 3 - Flanks tightly distended, the animal not eating but only mildly distressed

Stage 4 - Severe bloat with the animal in marked distress

Stage 5 - The animal was dead

Bloat Score Recording

Bloat scores of individual animals were recorded on specially prepared score sheets. The person observing the cows at pasture either recorded the bloat scores himself directly or relayed them to a recorder at base, using a "walkie-talkie" radio telephone system. The latter had the advantage of being able to summon help quickly if it became necessary.

Pasture

The pastures encountered varied from lush clover ryegrass swards with an average of 50-70% clover in the farm trials, to pastures at Ruakura containing 85-95% clover.

RESULTS - DISCUSSION

The Ruakura trial was the only trial in which substantial bloat occurred. However, the challenge was relatively light and occurred on only three successive days so that insufficient data for statistical analysis was accumulated.

In one farm trial, one day of serious bloat occurred otherwise the bloat challenge was insignificant. In one other farm trial, one cow under paste treatment died of bloat during the night. However, because of the circumstances in this trial, the farmer himself had to drench the cows. The reason was that there were no drenching facilities separate from the actual milking area and unless the farmer himself did the drenching,

the cows became very upset by strangers in the shed. It is possible therefore that this particular cow did not receive her treatment with the paste formulation $KB_3/34A$.

The total of the bloat scores recorded at two hours post milking per group per day in the Ruakura trial is shown in the Table IV. 1. Light to moderate bloat occurred on three successive days only. Treatments were administered in the evening (3.30pm) and the only group to record bloat within 2 hours of returning to pasture was the control group. Hence, it appeared that ethoxylate paste ($KB_3/32B$), ethoxylate liquid, and Poloxalene paste ($KB_6/16$), prevented bloat for at least two hours post treatment. On the three following mornings when moderate bloat again occurred in the control group (approx. 18 hours post treatment), the group under $KB_3/32B$ treatment consistently recorded the least amount of bloat. Ethoxylate liquid and Poloxalene paste were always similar to each other and recorded more bloat than $KB_3/32B$.

This may suggest that the ethoxylate paste formulation was still active in spite of the very low dose rate (5.0 to 5.5g active). The usual d ose rate of detergents is around 7.5g of active ingredient.

Unfortunately the bloat was not severe enough and it did not continue long enough to provide any conclusive evidence as to the efficacy of $KB_3/32B$.

The reason the bloat challenge at Ruakura was relatively light was that the field trial had to be undertaken in conjunction with other bloat work. Consequently, the trial could not get under way until late October by which time conditions conducive to bloat had passed.

The almost complete absence of bloat in the farm trials may be explained by extraordinary climatic conditions experienced in the autumn and spring of 1976 in the Manawatu. The weather was unusually wet and cold and the lush pasture growth normally seen at this time of year did not appear.

Criticism of the paste dosing gun was evident from comments of the farm owners and the experienced livestock staff at Ruakura. Problems of the gun included:

1) Oozing of paste from the nozzle which was estimated to cause about 25-30% of the paste in the cartridge to be wasted
2) The straight nozzle of the gun resulted in difficulty in delivering the paste dose to the back of the cow's throat to reduce chewing. It meant the animals had to be dosed from the front of the mouth rather than from the "corner" as is possible with a curved nozzle.

It was generally agreed that farmers would be reluctant to accept the present paste dosing gun as a means of administering paste to cattle.

The lack of sufficient bloat amongst control animals prevented definitive data being obtained regarding:

1) The usefulness of the rumen model as a screening system for anti-bloat pastes
2) The bloat controlling efficacy of anti-bloat pastes.

The rumen model had shown that the two ethoxylate paste formulations (KB_3/34A and KB_3/32B) slowed the washout of detergent quite markedly (Chapter I., p 34). Had it been possible to prove conclusively that KB_3/32B needed to be administered to cattle only once a day to reliably prevent bloat, then this would have given support to the validity of the rumen model as a screening system for new paste formulations.

In all, much valuable experience was gained in designing and implementing bloat field trials. The more important requirements for satisfactory field trials were found to include:

1) Selection of a farm with a history of serious bloat, and a farmer who is genuinly interested in the trial.
2) A strong reliable drenching race at the cowshed but separate from the actual milking area.

3) A workable transportable drenching race for treatment of seriously bloated animals in the paddock.
4) A "walkie-talkie" radio telephone system for use in wet weather and to summon help in emergency.
5) Sufficient personnel to cope with any emergency.
6) An easily recognisable system of identifying animals and treatment groups.

A good working relationship with the farmer is essential to the smooth running of the trial. The necessity for a separate drenching race at the cowshed is to allow the normal milking routine to continue without interference from strangers which could lead to a reduction in the quantity of milk obtained. A transportable drenching race may prevent the necessity of removing the whole herd from the pasture, to the cowshed, if an emergency arises. A good animal identification system is essential to make bloat scoring of individual animals easier.

It is concluded that further field trials are necessary to test the paste's bloat controlling efficacy. However, for greatest value, the approach attempted here should be repeated and improved upon. Thus the materials selected for field testing should be selected on the basis of an *in vitro* test (rumen model) and established, accurate, *in vivo* dilution curves. The results from such a combined approach will enable decisions to be made as to the most efficient means of identifying promising formulations and establishing the reason why they are effective.

Should the bloat controlling efficacy of anti-bloat pastes be proved greater than liquids, then it is likely that an improved method of paste administration will need to be developed before being accepted by farmers.

TABLE IV. 1

Total of the bloat scores per group per day. (Only the bloat scores recorded at 2 hours post milking are included).

DAY	1	2	3	4
GROUP				
Control	14.0	23.0	23.0	23.0
Ethoxylate liquid	11.0	17.0	8.0	9.0
Ethoxylate paste	7.0	11.0	5.5	7.0
Poloxalene paste	12.0	11.5	7.5	10.5

GENERAL DISCUSSION AND CONCLUSIONS

The intention of this study was to investigate some factors relating to the bloat-controlling efficacy of some anti-bloat paste formulations.

A rumen model was successful in demonstrating clearly, differences in the detergent release rate between the formulations. This finding was important for it showed that there were differences in the dissolution rates of the various formulations and therefore raised the possibility that some formulations might maintain an effective level of detergent in the rumen liquor longer than others.

The differences in detergent release rate could be related to the method of preparation of the paste and to its physical properties of melting point and consistency.

In spite of providing useful data on the physical effects of differences in formulation, the value of the rumen model was limited because:

1) It was not possible to simulate rumen motility and the effects of that and rumen ingesta movements on the paste.

2) Water had to be used as the exchange fluid because a suitable assay method which would operate with rumen liquor as the medium was not available. Had such an assay method been available, it might have been used to determine the degree of adsorption of detergent onto particulate matter.

3) The rumen model findings could neither be supported by <u>in vivo</u> detergent decay curves because a suitable assay method was unavailable, nor by field trial results because the prevalence of bloat was low (Chapter IV.)

The dosing of fistulated steers with each paste formulation and the recovery of the swallowed paste bolus at the cardia showed that the degree of physical

disruption inflicted on the paste could be severe and could range widely. This was found to depend largely on the reaction of the animal to the dosing procedure and this in turn was shown to vary both within and between animals.

The importance of this work was that it demonstrated that any advantage one paste formulation might have over another in terms of a slower *in vitro* detergent release rate, might be lost because of the extent of physical break-up it might suffer during administration to the live animal. In this respect, mild agitation of broken-up paste boli was shown to result in a more rapid dissolution than the same agitation of intact boli. The practical significance of this finding could be interpreted if it was shown that the persistence of detergent in the rumen, (and thereby protection against bloat), was determined by the surface area of the paste dose exposed to rumen liquor on entering the rumen. Unfortunately, lack of an appropriate analytical method prevented this investigation being carried out.

The intactness of the paste bolus entering the rumen might be increased if the "stiffness" of the formulation were to be increased. However, this could then lead to alterations in the rate and degree of detergent release, to problems of administration, and possibly to regurgitation of the paste.

Because animal reaction to dosing was found to largely determine the degree of paste bolus intactness, the culling of fractious animals from a herd may be necessary to realise the full potential of anti-bloat pastes.

The use of three fistulated steers to establish the *in vivo* detergent decay curves of Poloxalene demonstrated that the paste formulation ($KB_6/16$) did not prolong the persistence of detergent in the rumen by more than 2 hours in comparison with a liquid preparation of the same materials. The same experiment showed that

abnormally high doses of Poloxalene caused a marked increase in the endogenous water inflow to the rumen and thereby increased the dilution rate of Poloxalene in the rumen. Perhaps the most important findings in Chapter 3 were the following:

1) The *in vivo* detergent decay curves for Poloxalene were very similar to those predicted from the rumen model.

2) It was realised that a detergent analytical method operable in rumen liquor would markedly advance developments in the field of anti-bloat detergent pastes.

3) The experimental design in Chapter 3 was workable and with minor improvements would be an excellent model to work from in the future.

The several field trials undertaken were disappointing in terms of the results obtained but they provided valuable experience and confirmed that the general experimental design and day to day operational methods were appropriate. There was some suggestion that one of the ethoxylate paste formulations, $KB_3/32B$, did provide extended protection against bloat, even when administered at a very low dose rate.

If this could be proven conclusively, then it would give further support to the *in vitro* findings which showed $KB_3/32B$ to be superior in terms of the persistence of detergent in the rumen model.

It is concluded that:

1) Anti-bloat pastes can be formulated with differing release rates.

2) The physical break up that can occur as a result of animal reaction at the time of administration,

may nullify any formulatory advantage of one product over another which otherwise would produce a superior detergent release rate.

3) The development of a suitable detergent analytical method for use on rumen liquor, would allow rapid progress to be made in the development of anti-bloat pastes. Without such an assay method, the investigation of anti-bloat pastes is restricted to field trials which have been shown to be expensive and unpredictable in terms of the bloat challenge.

4) Field trials will not provide answers to the fundamental question of why certain formulations of anti-bloat pastes will or will not influence the persistence of detergent in the rumen.

5) Further progress in developing anti-bloat pastes might be approached on either a systematic product selection or an ad hoc basis. Difficulties associated with field work suggest that promising paste formulations should first be screened in some way and the best tested further.

Provided an assay system for detecting detergent in rumen liquor can be satisfactorily developed, in vivo detergent persistence after oral dosing of paste formulations will give an indication of those formulations which are likely to offer practical advantages over liquid preparations. Such an assay system in operation would allow selection of paste formulations that provided persistent levels of detergent in rumen liquor. The assay method would also allow each new paste formulation to be tested in the rumen model, using rumen liquor as the exchange fluid.

On the basis of the performance of each formulation in the rumen model and its in vivo detergent decay curves established in rumen fistulated cattle, only

the most promising products would be worth taking forward to testing in the field. Hopefully, a product could be found which necessitated no more than once daily administration to cattle. Such a product if found to be safe and effective in other respects, would be a great labour saving invention for dairy farmers and would help lift the production of an industry vital to New Zealand.

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