CHAPTER 1

INTRODUCTION

The ruminant is dependent upon the predigestion of its food constituents to a utilisable form by the rumen microbial population and it is now recognised that the characteristics of this fermentation can change when protozoa are present (Preston and Leng, 1987). A number of results indicate that the presence of ciliate protozoa in the rumen may reduce the amounts of protein reaching the lower digestive tract (Leng, 1976; Bergen and Yokoyama, 1977).

According to Preston and Leng (1987) and Nolan (1989) this reduction of total protein flow to the duodenum is due to three main causes. Firstly, protozoa engulf bacteria and fungal zoospores, thereby reducing the yield of total microbial protein for host digestion (Leng, 1976; Bergen and Yokoyama, 1977). Secondly, protozoa tend to be preferentially retained in the rumen because they sequester to large feed particles and/or to the rumen wall (Weller and Pilgrim, 1974). Thirdly, it has been reported that digestion of dietary protein in the normal rumen is higher than in the protozoa-free rumen, thereby reducing the total quantity of dietary amino acids available for absorption from the lower digestive tract (Ushida and Jouany, 1985). Therefore on low-protein diets the presence of protozoa in the rumen could be expected to limit production, since a reduction of total microbial protein available for absorption in the lower part of the digestive tract limits growth (if growth limited by protein

availability). Such effects become critical on low-protein diets (Bird *et al.*, 1978, 1979; Bird and Leng, 1978).

Several studies in the past have demonstrated that removal of protozoa from the rumen (defaunation) increased the amount of protein reaching the lower digestive tract (Bird, 1982; Veira *et al.* 1983). Defaunation may also improve the energy status of the rumen by increasing the production of more propionate, (a glucogenic precursor) at the expense of either acetate or butyrate (Chalupa, 1977, Bergen and Yokoyama, 1977). It has been suggested that propionate production is associated with a higher efficiency of microbial protein synthesis in the rumen (Thomas, 1973).

In the absence of protozoa proteolysis of dietary protein in the rumen is reduced and there is an increase in the supply of microbial protein from the rumen, thereby increasing the total quantity of amino acids available for absorption, defaunation should improve production in ruminants fed low-protein diets. Eadie and Gill (1971) have shown that the growth rate of defaunated lambs was improved, compared to controls, at low -protein intakes and Bird *et al.*(1979) found that wool growth rate (the production parameter most closely related to protein absorption from the duodenum) was increased at all levels of protein supplementation in defaunated as compared with faunated lambs.

Chemicals toxic to protozoa have been used to eliminate protozoa from the rumen by delivering the chemical solution directly into the rumen via a length of plastic tubing passed into the mouth and through the oesophagus (Becker and Everett 1930,

Abou Akkada *et al.* 1968). The detergent Teric GN9 has been used to remove protozoa from both cattle (Bird and Leng, 1978) and sheep (Bird *et al.*, 1979), and Alkanate 3SL3 (sodium lauryl ethyl sulphate, also a detergent) has been used to remove protozoa from the rumen of sheep (Burggraaf and Leng, 1980).

However, up to the present time no practical method of satisfactorily controlling protozoa in the rumen of grazing animals has been developed. Among the several modes of defaunation that have been used, chemical drenching is considered to be the most practical one at present (Bird, 1989). But, for the drenching procedure to be successful, animals need to be drenched on three consecutive days (Bird,1989), which is impractical for extensive grazing systems and difficult to ensure amongst unskilled small-holders. Drenching is also often followed by a period of depressed feed intake and animal performance (Burggraaf and Leng, 1980 and Bird, 1982). Solutions to these problems of impracticality in administering the drench need to be found. The molasses block may be a suitable means of administering Teric GN9 and Alkanate 3SL3. Molasses blocks have been used as vehicles for the administration of other drugs, including anthelmintic (Knox, 1995). This possibility is investigated in the present study.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The strategic approach to nutrition of ruminants aims at maximising the conversion of fibrous feed and non-protein nitrogen (NPN) into nutrients which can be efficiently utilised by animal tissues. As the feeds of ruminants in the tropics are dominated by poor quality roughage (low digestibility, low N content), the utilisation of such feeds is inefficient and animal productivity is poor (Preston and Leng, 1987). Dietary supplements aimed at providing nutrients either for the rumen microbes or for the animal (rumen escape nutrients) are likely to improve the efficiency of utilisation of basal ration. However this strategy is often costly.

The elimination of protozoa (defaunation) is one form of rumen manipulation which offers considerable promise as a viable method of increasing ruminant production (Bird, 1991). Manipulation of the rumen aims to increase the rate and extent of digestion of fibre and/or optimise the supply of specific nutrients to the host animal. To understand why the defaunated animal is more efficient in utilising feed intake and thus more productive it is necessary to consider the role of protozoa in the rumen function. Moreover to gain the knowledge of how to control the protozoa population in the rumen, it is necessary to understand the factors that affect the

protozoal population and the methods that have been used to eliminate protozoa from the rumen. Therefore, these aspects are reviewed in this section.

2.2 General description of protozoa

In the rumen and reticulum of ruminants, single - celled microscopic animals called protozoa are present as one of the major populations of anaerobic microbes which are active in the fermentation of ingested food (Hungate, 1966). Protozoal numbers can range from 0 to 20 x 10^5/ml of rumen fluid (Church, 1976). This number is very low relative to the number of bacteria found in the rumen (15 to 80 x 10^9 / ml; Bryant, 1970). However, because most protozoal cells are 20 - 200 μm in length (1000 x bacterial size), protozoa can constitute a significant proportion of the microbial biomass in the rumen (Coleman 1979). They can be seen with the unaided eyes, especially when they are allowed to sediment out of rumen fluid (Yokoyama and Johnson, 1988). The morphology of protozoan cells, such as the ciliary zones, nuclei, skeletal plates, contractile vacuoles, etc. can readily be studied at 100-400 X using light microscopy (Ogimoto and Imai, 1981).

There are two groups of protozoa in the rumen, flagellates and ciliates. 'The flagellates are in the Orders Polymastigida and Trichomonadida of Class Mastigophora'(Clarke, 1977). Flagellate protozoa have been observed in young pre-ruminant animals and in adult animals from which the ciliate population has been completely eliminated (Eadie, 1962a). The number of Flagellates in the rumen is

generally low (Eadie, 1962b.) and their mass small (Clarke, 1977) and they are therefore not discussed further.

In adult ruminants most of the protozoa are normally ciliates (most of them are 20-200 μm in length; they are classified into three Orders : Prostomatida, Trichostomatida and Entodiniomorphida (Ogimoto and Imai, 1981). Rumen Prostomatida contains a few species belonging to the family Buetschliidae, but the frequency of their appearance in the rumen is usually very low (Ogimoto and Imai, 1981). Trichostomatida (family *Isotrichidae, butschi*), commonly called the holotrichs, have hair-like cilia over their entire bodies. The number of species in this family is relatively few, but their frequency of appearance is high. In contrast the Entodiniomorphida (family *Ophryoscolecidae, Stein*), or oligotrichs, which are commonly referred to as entodiniomorphs, have only tufts of cilia on the anterior parts of the body and compose most of the rumen ciliates, and both their number and frequency of appearance in the rumen are high (Clarke, 1977; Ogimoto and Imai, 1981). Taxonomic relationships between the major classes of rumen protozoa are shown in Table 2.1.

The ciliate protozoa in the rumen are important in the digestion of major dietary carbohydrate, protein components; they are also responsible for the engulfment and degradation of large numbers of rumen bacteria For example, *Entodinium spp.* actively ingest and digest bacteria (Coleman, 1975;).

Their predatory activity substantially reduces the amount of protein which is available for intestinal digestion. In many ruminant production systems, this behaviour of the rumen ciliates results in a critical loss of protein to the host animal. Therefore, removing ciliates protozoa from the rumen (defaunation) may be beneficial.

The role of individual species of protozoa in the rumen needs clarification because not all ciliate protozoa may be detrimental to the host animal; for example, inoculation of the protozoan *Polyplastron multivesiculatum* into a defaunated sheep's rumen increased cellulose digestion by 3-10% (Jouany and Senaud, 1979). At present it is not possible to selectively kill individual species, hence defaunation procedures are designed to remove all ciliate protozoa in the rumen.

Table 2.1 The taxa of rumen ciliate protozoa, including families and their genera and the sources of their classification

Phylum Ciliophora DOFLEIN, 1901
Class I. Kinetofragminophora DE PUYTORAC *et al.*, 1974
Subclass (1) Gymnostomata BÜTSCHLI, 1889
Order 1. Prostomatida SCHEWIAKOFF, 1896
Suborder (1) Archistomatina DE PUYTORAC *et al.*, 1974
Family BUETSCHLIIDAE POCHE, 1913
Genus: *Buetschlia* SCHUBERG, 1888

Subclass (2) Vestibulifera DE PUYTORAC *et al.*, 1974
Order 1. Trichostomatida BÜTSCHLI, 1889
Suborder (1) Trichostomatina BÜTSCHLI, 1889
Family ISOTRICHIDAE BÜTSCHLI, 1889
Genus: *Isotricha* STEIN, 1859
Genus: *Oligoisotricha* IMAI, 1981
Genus: *Dasytricha* SCHUBERG, 1888
Suborder (2) Blepharocorythina WOLSKA, 1971
Family BLEPHAROCORYTHIDAE HSIUNG, 1929
Genus: *Charonina* STRAND, 1928
Order 2. Entodiniomorphida REICHENOW in DOFLEIN et REICHENOW, 1929
family OPHRYOSCOLECIDAE STEIN, 1859
Subfamily Entodiniinae LUBINSKY, 1957
Genus: *Entodinium* STEIN, 1859
Genus: *Campylodinium* JANKOWSKI, 1975
Subfamily Diplodiniinae LUBINSKY, 1957
Genus: *Diplodinium* SCHUBERG, 1888
Genus: *Eodinium* KOFOID et MACLENNAN, 1932
Genus: *Eremoplastron* KOFOID et MACLENNAN, 1932
Genus: *Eudiplodinium* DOGIEL, 1927
Genus: *Diploplastron* KOFOID et MACLENNAN, 1932
Genus: *Polyplastron* DOGIEL, 1927
Genus: *Elytroplastron* KOFOID et MACLENNAN, 1932
Genus: *Metadinium* AWERINZEW et MUTAFOWA, 1914
Genus: *Ostracodinium* DOGIEL, 1927
Genus: *Enoploplastron* KOFOID et MACLENNAN, 1932
Subfamily: Ophryoscolecinae LUBINSKY, 1957
Genus: *Opryoscolex* STEIN, 1859
Genus: *Epidinium* CRAWLEY, 1923
Genus: *Epiplastron* KOFOID et MACLENNAN, 1933
Genus: *Opisthotrichum* BUISSON, 1923
Genus: *Caloscolex* DOGIEL, 1926

Adapted from Ogimoto and Imai (1981). All references in this table can be obtained from Ogimoto and Imai (1981).

2.3 Factors affecting the population of rumen protozoa

The population of ciliates in rumen fluid varies both diurnally within any one animal and also between animals kept under identical feed management. Variations in protozoan numbers in rumen fluid of a particular host are likely to determined by a complex set of factors which are the subject of this section.

2.3.1 The quality and type of diet

The quality of forage and type of diet determine both the nutrients available to rumen microorganisms and the physico-chemical conditions of their environment. Therefore, the type and population densities of protozoa present in the rumen are strongly affected by the animal's diet. Purser and Moir (1966) found that diets of high rumen digestibility supported high numbers of both holotrichs and entodiniomorphs. Holotrichs often predominate in the rumen of animals fed diets rich in soluble carbohydrates (Hungate, 1960), such as sugar cane (Valdez, *et. al.*, 1977) and diets based on molasses (Preston and Willis, 1970). On the other hand entodiniomorphs, especially *entodinia* and *epidinium spp.*, become numerically predominant in animals fed large quantities of starch-containing rations (Hungate,1966). The explanation for the large population of protozoa in ruminants fed on higher quality forage is thought to be the availability of suitable substrates for bacterial growth and thus a higher biomass of bacteria that can be preferentially used by protozoa (Jouany, 1989).

In contrast to high quality diet, the number of protozoa on high roughage diets are generally lower (10^4 - 10^5/ml) than that on concentrate diet (Jouany and Senaud, 1982), presumably, due to lack of nutrient availability to support protozoa growth. When protein content of the food is low, reduction of microbial protein flow from the rumen may limit the productivity of animals; therefore, defaunation is essential in animals on high roughage diet. Defaunation increased the production of cattle fed molasses-based diet (Bird and Leng, 1978) and increased the growth rate and wool production of lambs fed a roughage-based diet (Bird *et al*, 1994) even though these diets only supported small population of protozoa in the rumen.

2.3.2 Rumen environment

a. Rumen dilution rates

The type of diet is not the only factor controlling the protozoal population in the rumen. The physical form of the ration influences the rate of passage of digesta which, in turn, influences the population density of protozoa in the rumen. For instance, the feeding of pelleted or high concentrate diets, especially at high intakes, reduces the selective retention of fibre and may cause elimination of protozoa through "wash out" (Bauchop 1977). A high rate of passage of solids from the rumen has also been associated with low numbers of protozoa in the rumen (Forster, 1989). In *in vitro* studies Hungate (1966) found that many rumen protozoa do not survive in continuous cultures if the turnover is more rapid than once every 24-48 h.

The frequency and level of feeding have been suggested as other factors influencing the protozoal population in the rumen. Jouany (1989) found that feeding prairie hay on a daily basis reduced the protozoan population as opposed to feeding the same total amount in 12 equal amounts throughout the day.

b. Rumen pH

The ciliate protozoal population is profoundly influenced by pH conditions within the rumen. Purser and Moir (1959) have demonstrated the relationship between minimum rumen pH and ciliate numbers in mature sheep, and have shown that protozoal motility is inhibited completely at pH levels below 5 and protozoa were all dead at pH 4.5. Phillipson (1955) quotes the range of pH values within the rumen for animals on roughage rations as 5.9-7.4 for cattle and 5.3-7.3 for sheep. Slyter *et al.*, (1970) showed that protozoa were practically eliminated from rumen of ruminants fed high-concentrate diets due to the acidic conditions in the rumen.

2.3.3 Microbial antagonisms

Bacteria are both a competitor for nutrients and space with protozoa and a food source. The possibility of inter-relationships with other microbes is thus another factor that may influence the protozoal population. It was known that the inter-relationship between protozoa and bacteria is an inverse one : elimination of protozoa

from the rumen increases the bacterial population (Bryant and Small, 1960; Eadie and Hobson, 1962; Eadie and Gill, 1971; Kurihara *et al.*, 1968).

In contrast, Satapathy and Purser (1967) demonstrated that protozoal numbers doubled when the numbers of bacteria were reduced by feeding of tylosin to sheep. The inverse relationship between bacteria and protozoa is partially explained by competition for nutrients, the evidence for which was demonstrated by Walker and Hope (1964) when a potato starch diet was given to faunated and defaunated sheep. In that work it was found that in defaunated animals the starch-degrading enzyme was found in the bacteria while in faunated animals it was found in the protozoal fraction.

The intense competition for substrate, however, is not the only explanation for the inverse relationship between protozoa and bacteria. Coleman (1964) and Yokoyama and Johnson (1988) both reported that engulfment of bacteria by protozoa occurs in the rumen. Protozoa ingest both rumen bacterial species and non-rumen species derived from the feed as a source of protein for their cell synthesis. Due to engulfment of bacteria by protozoa, the presence of ciliate protozoa always reduces (by 50 to 90%) the number of fluid phase bacteria present in the rumen (Coleman, 1989).

Protozoa are not only predators of bacteria; various workers have reported that predation of protozoa by other protozoa also occurs in the rumen. Several types of protozoal population have been observed in the rumen of sheep and cattle. These populations consist of a mixture of *entodinium sp.*, *holotrich sp.* and either *Polyplastron*, *Diploplastron* and *Ophryoscolecids* (type A) or *Eudiplodinium*,

Epidinium, *Eremoplastron* and *Ostracodinium* (type B). Thus, *Polyplastron multivesiculatum* (type A) will eliminate *Eudiplodinium*, *Epidinium*, *Eremoplastron* and *Ostracodinium* (type B) from a population (Eadie, 1962a, 1967).

2.3.5 Determination of rumen protozoal populations

Obtaining accurate estimates of protozoal numbers in the rumen is difficult because of the heterogenous nature of rumen contents and a number of potential sources of error associated with this estimate (Bird, 1982). Firstly, there are difficulties in obtaining a representative sample from the rumen. Protozoa may not be uniformly distributed through the rumen for some species or for some diets, therefore taking a small sample of rumen liquid may not be representative. For while Boyne *et al.* (1957) and Purser and Moir (1959) found no difference in the population densities between the top and the bottom of the rumer of sheep fed high roughage diets, it is unlikely that protozoa will be uniformly distributed in the rumen of animal fed sugar cane diets. These diets support a large population of holotrichs which are able to rapidly assimilate storage amylopectin and sink to the bottom of the rumen (Preston and Leng, 1987).

The second problem identified by Bird (1982) was that sampling time in relation to feeding would influence the estimate of protozoan population density. It has been observed that that protozoal population density varies with the time and frequency of feeding (Jouany, 1989) necessarily affects the accuracy of population estimates. Potter and Dehority (1973) and Jouany (1989) reported that during the first 4 hours after the initiation of feeding, the concentration of protozoa decreased rapidly

and that it then increased during the next four-hour period. Recently, Senaud *et al.* (1995) observed that entodiniomorphid ciliates decreased during the first 3 hours following a meal and then increased during the next 3 hours. In contrast, isotricha increased slightly in the rumen after feeding. The decrease in protozoal numbers after feeding can be explained by dilution of protozoa associated with saliva flow, drinking and the passage of ingesta from the rumen during feeding and possibly attatchment to feed particles. Therefore when comparing protozoal population between animals, consideration of sampling time in relation to feeding time is essential.

Another factor that affects the accuracy of counting protozoa is the error associated with actual counting. It was suggested by Clarke (1977) that in view of the size of protozoa, a counting chamber with a depth of not less than 200 μm must be used, and only for the smaller flagellates is a standard haemocytometer suitable. However, using a deep chamber may lead to counting difficulties because some protozoa can not be seen in the same field of focus. Boyne *et al.* (1957) emphasised the need to fix protozoa before counting begins, and recommended dilution with equal volumes of either 10 % (v/v) formalin in water or of 0.85% (w/v) sodium chloride. Clarke (1977) recommended the staining of the test suspension with 0.5 % (w/v) methyl green for 10 minutes to stain the nuclei of the protozoa and improve their stability, and hence the accuracy of counting.

2.4 Summary

It appears that the protozoal population is strongly influenced by diet. In addition considerable variation may occur between different animals fed the same diet in the absence of any apparent external stimuli. Diurnal variations within individual animals are presumably due to variations in: eating and drinking habits, the composition and flow of saliva, and in changes in rumen fluid volume. Therefore, it is important to standardise procedures for sample collection and cell counting. An important conclusion to be reached from these findings is that experimental treatment effects on protozoal population will need to be both sustained and large if the results are to be considered significant.

2.5 The role of protozoa in host nutrition

The role of rumen protozoa in the nutrition of the host animals is still, in part, debatable. From a knowledge of their ability to digest the major feed components (Coleman, 1985) and of their large population in the rumen (Harrison and McAllan, 1980), it is presumed that protozoa play an important role in ruminant fermentation. In direct support of this, Veira *et al.* (1983) reported that the larger the protozoal population in the rumen, the higher the rate and extent of organic matter and starch digestion. On the other hand, Van Soest (1994) argued that the importance of protozoa relative to rumen metabolism and digestion is much less clear. The problem

of assessing the exact role of protozoa arises from the difficulty of growing protozoa away from rumen bacteria; rumen protozoa are also strictly anaerobic and the end-products of their fermentation are often similar to those of bacteria (Czerkawski,1986).

Just how important protozoa are to host nutrition can be determined by assessing their role in the degradation of ingested feed in the rumen, by their effects on the rumen environment, and the effect of protozoa on the end-products of rumen fermentation. Therefore, this part of the review focuses on those aspects.

2.5.1 Degradation of ingested feed in the rumen

a. The role of protozoa in the digestion of dietary protein

All foodstuffs consumed by ruminants are initially exposed to microbial enzyme activity in the rumen. The rumen microbes digest feed proteins to amino acids and both assimilate and dissimilate them (Weller *et al.*, 1958). Pilgrim *et al.* (1970) estimated that, depending on the diet and feeding level, approximately 58 to 75 % of dietary protein is degraded in the rumen. The process of nitrogen metabolism in the rumen can be seen in Figure 2.3, from which it is clear that the flow of protein to the intestines does not depend merely on the protein content of the diet, but also on the synthesis of microbial cells in the rumen.

The active proteolytic enzymes found in ciliates and their ability to engulf feed particles are factors that contribute to increased dietary breakdown of dietary protein in the rumen of faunated animals (Coleman,1983; Wallace *et al.*, 1987). The

degradability of several protein meals was 13-20% higher in faunated than in fauna-free sheep (Ushida and Jouany, 1985).

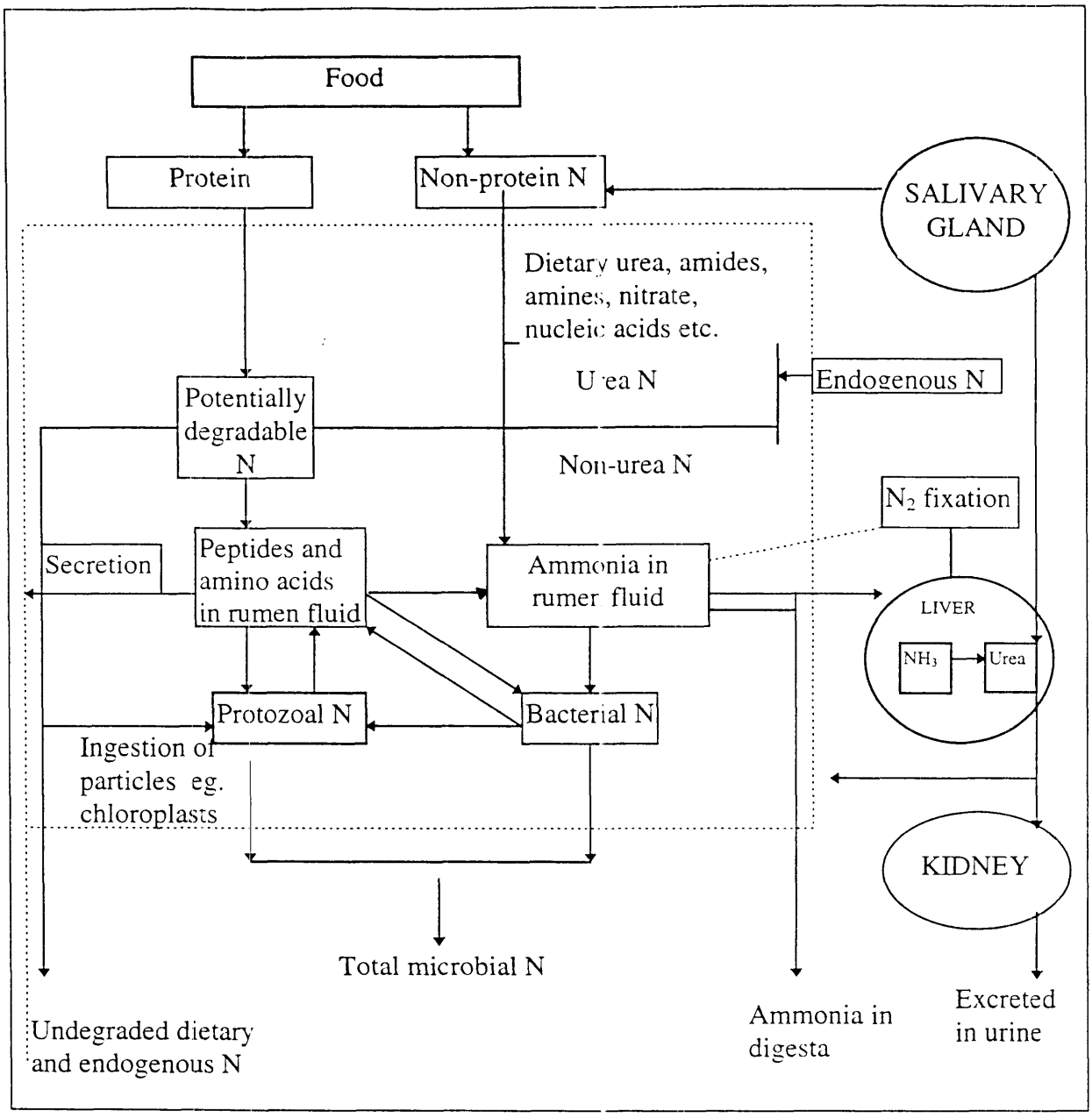

Figure 2.1 A model of the metabolism of nitrogen in the rumen (Sources: Leng and Nolan 1984 and McDonald, *et al.*,1988)

b. The role of protozoa in carbohydrate digestion

The principal carbohydrate substrates available for microbial fermentation are the plant-storage and structural polysaccharides (Williams, 1989). The role of protozoa in the degradation of cell-wall material is still under debate (Veira, 1986; Punia *et al.*, 1987; Ushida *et al.*, 1987; Ushida *et al.*, 1991). Ushida *et al.* (1991) found that the addition of large quantities of starch to a roughage diet improved the digestibility by protozoa of cell-wall carbohydrates, presumably due to an increased number of protozoa as a result of more substrate being available to them from the starch. However, this does not mean that maximum fibre digestion necessarily occurs when protozoa are present. The effects of defaunation on fibre digestion in the rumen will be influenced by the enzymic activity of the protozoa relative to the bacteria and fungal populations.

The many species of protozoa found in the rumen vary in their ability to utilise carbohydrates as energy sources. Some protozoa have cellulolytic activity (Coleman, 1978) but more commonly they ingest starch and sugar which exist free in solution and use these to generate ATP for maintenance and growth. The holotrichs are attracted to soluble carbohydrates by chemotaxis (Orpin, 1977a) and rapidly attach to the damaged ends (eg. as a result of mastication) of plant material in the rumen (Bauchop, 1989). The holotrichs assimilate soluble sugars and convert them to storage polysaccharides within their cell (William and Harefoot, 1976). However, there are differences in the types of sugars metabolised by the two dominant genera of

holotrichs in the rumen, the *Isotricha* and *Dasytricha*. *Isotricha* do not ferment maltose but can utilise starch of a suitably small grain size, whereas *Dasytricha* can utilise maltose but not starch (Clarke,1977). The oligotrichs, on the other hand, use mainly particulate materials including starch (Yokoyama and Johnson, 1988).

Some species of protozoa can metabolise lactic acid thereby preventing the potentially harmful accumulation of lactate in the rumen (Newbold *et al.*, 1986). The engulfment of starch and sugar by protozoa may have a major effect in preventing rumen acidosis by reducing the rate and extent of starch digestion (Mendoza *et al.*, 1993). It has also been shown that some species of protozoa have specific effects on rumen fermentation. Thus *Polyplastron multivesiculatum* significantly increased both dry matter and organic matter digestibility when inoculated into the rumen, while *Isotricha prostoma* significantly decreased these parameters (Jouany, *et al.*, 1981).

The entodiniomorphs, with the possible exception of the smallest Entodinium spp., utilise both starch and more complex carbohydrates (Hungate,1966). Some genera, including *Entodinium* and *Epidinium* (Coleman,1969) and *Polyplastron* (Abou Akkada *et al.*, 1963) can metabolise some soluble sugars for energy and polysaccharide storage. Several entodiniomorphs are active in the degradation of hemicelluloses (Clarke,1977), but their contribution to total cellulose metabolism in the rumen is probably small (Hungate,1975). Van Soest (1994) argued that in the digestion of particulate carbohydrate substances, protozoa may not be independent from bacterial inclusions. However, Williams and Strachan (1984) found that the capacity of protozoa to degrade starch on some diets was greater than that of bacteria.

In ciliate-free animals given concentrate and roughage diets digestibilities of plant cell wall polysaccharides (Jouany, 1989) and starch (Veira *et al.* 1983) are lower than in faunated animals, presumably due to the removal of cellulolytic protozoa (Coleman, 1985) and/or the cellulolytic bacteria directly or indirectly associated with them (Demeyer, 1981). However this situation may not always be true; the digestibility of untreated wheat straw increased from 26 % in faunated sheep to 33 % in defaunated sheep (Bird *et al.*, 1994).

The digestion of fibre in ciliate-free animals need not necessarily be depressed because of a compensatory increase in the size of the bacterial (Bryant and Small, 1960; Eadie and Hobson, 1962) and fungal populations (Soetanto, 1986; Romulo *et al.*, 1989) which in digestion potential could equal or surpass that of protozoa in the faunated rumen. For example, defaunation had no effect on the digestibility of Timothy grass (*Phleum pratense*) in sheep given high roughage diets (Orpin and Letcher, 1983/1984) and the digestibility of cereal straw was increased by defaunation (Bird *et al.*, 1994).

2.5.2 The modifying influences of protozoa on other aspects of rumen function

As well as their general effects on the digestion of dietary protein and carbohydrate, protozoa are now known to modify specific aspects of rumen function including digesta outflow, pH and NH_3 metabolism.

a. Digesta outflow rate

Although there is no proven explanation for the existence of a relationship between digesta kinetics and the presence of ciliate protozoa, the absence of ciliates in the rumen (defaunation) is often associated with changes in rumen volume and digesta outflow (Orpin and Letcher, 1983/1984 and Kayouli *et al.*, 1983/1984).

Defaunation causes a decrease in the rumen fluid flow rate, with an average decrease of 95 ml/h observed in sheep (Orpin and Letcher, 1983/1984), whereas rumen fluid volume (6.5 - 7.8 l) is larger than in normal animals (2.8 - 6.0 l), an increase of from 17 to 43.5%. This could explain why defaunated animals possessed a larger girth and a pot-bellied appearance compared with normal sheep (Eadie, 1962a). Such changes to the dynamics of the ruminal ecosystem could affect both microbes and the retention time of feed stuffs, both of which can alter overall fermentation.

b. pH of rumen fluid

Protozoa may play an important role in stabilising ruminal pH particularly if the diet is rich in starch (Veira *et al.*, 1983). The pH of rumen fluid in defaunated animals is often lower than in normal animals (Whitelaw, *et al.*, 1972). This effect may be due to an increase in the rate of starch fermentation in the high-concentrate diets (Mendoza, *et al.*, 1993), as a result of an increase in amylolytic activity of bacteria in the absence of ciliates protozoa (Kurihara, *et al.*, 1978). A decrease in pH may reduce the digestion of fibre in the rumen..

Some protozoal species also metabolise lactic acid (Newbold, *et al.*, 1986), therefore when grains are given as a major food , the stabilising effect of protozoa plays an important role in preventing acidosis (Mackie *et al.*, 1978).

c. Metabolism of ammonia (NH_3)

Ammonia is very important substrate for the efficient synthesis of bacterial protein (Preston and Leng, 1987). A large protozoa population was associated with increased ruminal ammonia concentration and urea concentration in blood plasma (Veira *et. al.*, 1983). Increased ruminal ammonia is probably due to fewer bacteria able to utilise ammonia as a result from engulfment of bacteria by protozoa (Coleman, 1975) and a high level of dietary protein degradation in the rumen liquor (McDonald *et al.*, 1988) as a result of high proteolytic activity of protozoa (Bergen *et al.*, 1968).

Unlike bacteria, which utilise between 40 and 60% of ruminal NH_3-N for protein synthesis (Nolan *et al.*, 1976), protozoa do not utilise ammonia directly. Protozoa use amino acids and peptide as a major source of N (Purser and Moir, 1966). Therefore, in ciliate-free animals the level of ammonia may be reduced (Abou Akkada and El-shazly, 1964; Males and Purser, 1970) due to a compensatory increase in the number of bacteria, which can be expected to more extensively use the rumen ammonia than would have the protozoa they replaced. In contrast, ammonia concentrations in the rumen of faunated animals are higher than ciliate-free animals due to greater recycling of microbial protein within the rumen,

fewer bacteria to utilise the ammonia, and increased dietary protein breakdown (Leng and Nolan, 1984).

The presence of active proteolytic enzymes in the ciliates results in a higher rate of protein degradation to ammonia in the rumen when protozoa are present compared with that in ciliate-free animals (Coleman, 1983; Wallace *et al.* 1987). This effect has been attributed to the significant contribution of protozoa to dietary or bacterial protein degradation and deamination (Abou Akkada and El-shazly, 1965).

Low ammonia concentration in the defaunated animals may limit carbohydrate digestion (Eadie and Hobson, 1962), but it can be solved by varying levels of urea supplementation (see Bird and Leng, 1984).

5.3 The modifying influences of protozoa on the end-products of rumen fermentation

Volatile fatty acids (acetate, propionate, butyrate), along with carbon dioxide, methane and microbial cells, are the major end-products of fermentation of all diets in the rumen.

a. VFA

The proportion of the main energy-yielding end-products, the volatile fatty acids (VFA), are often changed by the presence or absence of protozoa (Jouany *et al.*, 1981). 0However the change in VFA proportions is in the absence of protozoa is not

consistant (Bird,1991). Often the absence of protozoa is associated with an increase in propionate and a decrease in butyrate (Males and Purser, 1970). The effect of protozoa upon VFA ratios also has been demonstrated by Eadie and Mann (1970), who showed that an increase in propionate relative to acetate is associated with the absence of ciliates from the rumen, particularly on high-grain diets, a condition which is beneficial in retaining more substrate energy in the products of fermentation (Leng *et al.*, 1980). In one experiment where defaunation resulted in a doubling of the proportion of propionate, the metabolisability of the gross energy increased by 5% (Whitelaw, *et al.*, 1984).

b. Methane

The efficiency of energy utilisation of most feeds in ruminants is negatively correlated to the methane concentration in the rumen (Miltimore and McArthur, 1962), due to the eructation of 95% of that methane to the atmosphere. Much research has been carried out into rumen manipulation in an attempt to inhibit methane production and thus improve overall energetic efficiency (Blaxter and Czerkawski; 1966; Czerkawski, 1972; Chalupa, 1977).

The major agents of methane production in the rumen are bacteria and fermentative protozoa (Bryant, 1979). A symbiotic relationship normally exists between ciliate protozoa and methanogens that attach to the protozoal surface. Finlay *et al.* (1994) reported that on average there were 96 and 520 endosymbiotic methanogens attached to individual ciliates of *Entodinium spp.* and *Dasytricha*

ruminantium respectively. Defaunation of the rumen is thus of practical importance not only because it increases ruminant productivity (Leng, 1991; Williams and Coleman, 1991), but also because it reduces ruminal methanogenesis (Demeyer and Nevel, 1979; Williams and Coleman, 1991) and thus reduces methane emissions to the atmosphere. Defaunation could thus have a significant role to play in reducing the environmental (greenhouse) effect of farmed ruminants.

c. Microbial protein

The total microbial biomass in the rumen is made up largely of bacteria, protozoa and fungi. The ability of the rumen ciliates to assimilate and transform both dietary and microbial proteins, and the fact that ciliates comprise a large proportion of the ruminal microbial mass in the rumen (McNaught *et al.*, 1954; Blackburn and Hobson, 1960; Bergen *et al.*, 1968;) led to the assumption that protozoa made a significant contribution to protein availability to the host ruminant.

However, since protozoa are also responsible for the engulfment and degradation of large numbers of rumen bacteria (Coleman, 1989), and it was calculated by Coleman (1975) that protozoa remove 30% of the total bacterial biomass, the presence of protozoa in the rumen may decrease rumen microbial protein output due to the resultant recycling of bacterial Nitrogen through protozoa (Kurihara *et al.*, 1968; Van Soest, 1994). This situation leads to a reduction in the total amount of microbial N available in the intestines and thus to reduced amino acid absorption, and production. Nolan (1989) stated that the presence of protozoa in the rumen

significantly decreases the amount of bacterial protein leaving that organ and thus becoming available for digestion in the intestines. In terms of the ratio of microbial protein to energy from volatile fatty acids (VFA) in the nutrients absorbed, this may change that ratio from 25 g microbial protein/ MJ VFA in fauna-free ruminants to below 12g microbial protein/MJ VFA in faunated ruminants (Preston and Leng, 1987). This reduction of the P/E ratio may limit the efficiency of use of absorbed energy-supplying nutrients, whereas elimination of protozoa from rumen enhanced the efficiency of microbial growth and more microbial and dietary protein flows from the rumen (Weller and Pilgrim, 1974).

Protozoal protein is not a large component of ruminal outflow (Weller and Pilgrim, 1974; Bergen *et al.*, 1968) due to sequestration of protozoa onto feed particles and to the walls of the reticulum and the dorsal sac of the rumen. Protozoa have a slow turnover rate (Leng, 1982) and this results in a reduction in the net synthesis of microbial protein. Their extended residence time in the rumen and their engulfment of bacteria and fungi (Orpin and Letcher, 1978) result in a reduction in the total outflow of microbial cells from the rumen. Leng *et al.* (1981) and Leng (1982) stated that from 65 % to 85% of protozoa actually die and are degraded in the rumen, and do not flow to the abomasum.

2.6 Summary

The ability of protozoa to digest the major feed components (Coleman, 1985) and the large proportion of protozoa in the microbial biomass in the rumen (Coleman, 1979) suggest that protozoa play an important role in ruminant fermentation.

However, The presence of protozoa also has a pronounced effect on the removal of 30 % of the total bacterial biomass due to the recycling of bacteria protein through protozoa (Kurihara et al., 1968; Van Soest, 1994). A large number of rumen bacteria are engulfed and degraded by protozoa (Coleman, 1989; Nolan, 1989; Kurihara *et al.*, 1968; Van Soest, 1994). This situation leads to a reduction in the total amount of microbial N available in the intestines and thus reduced amino acid absorption (Nolan, 1989).

In addition, protozoa sequester onto feed particles and the rumen wall (Weller and Pilgrim, 1974; Orpin and Letcher, 1978; Bird and Leng, 1985) and they are degraded in the rumen rather than flowing down to the abomasum (Leng *et al.*, 1981; Leng, 1982). Protozoa also engulf feed particles (Pilgrim *et al.*, 1970) and the presence of active proteolytic enzymes in the bodies of ciliate results in a higher rate of dietary protein breakdown in the rumen (Coleman, 1983; Wallace *et al.*, 1987), with a consequent reduction in intestinal availability of dietary protein.

Although Jouany (1989) stated that in ciliate-free animals the rumen digestibility of plant cell wall polysaccharides are lower than in faunated animals, this situation may not always be true in animals given high roughage diets (see Orpin and Letcher, 1983/1984 and Bird *et al.*, 1994). It can be presumed that there is compensatory increase in the size of the bacteria and fungal population which in digestion potential in defaunated rumen could surpass that of protozoa in the faunated rumen (Soetanto, 1986 and Romulo *et al.*, 1989).

While the preceding literature review has clearly demonstrated the importance of protozoa to rumen function, it is also clear that with the exception of high starch diets their presence is apparently not essential to the host animal and often represents a constraint to production. As a consequence, the protozoa-free condition may improve ruminant production whenever the availability of amino acids to the host animal is the primary limitation to production. There are advantages which are potentially important in ruminants fed roughage and low protein diets, diets which are commonly found in the tropics. The next section of this review will deal the methods available for defaunation, their effectiveness and possible practical applications.

2.7 Methods for defaunation, their effectiveness and possible practical applications

It was concluded in the previous section that the removal of protozoa (defaunation) from the rumen is likely to result in an improvement in the nutrient supply to the host, and since defaunation increases the total quantity of amino acids available for absorption, defaunation should improve production in ruminants fed low-protein diets (Soetanto, 1986; Romulo *et al.*, 1989; Bird and Leng, 1984). There are a number of methods that have been investigated in order to obtain ruminants free of ciliate protozoa; these are reviewed in the following sections.

2.7.1 Methods of obtaining ruminants free of protozoa

a. Isolation of new-born animals

New-born animals are free of protozoa and rumen protozoa are not acquired in the first week after birth (Eadie and Gill, 1971). New-born animals then become faunated either by direct transfer from an adult, or by saliva containing the active organisms (Bird, 1989). Common routes of faunation are when the mother licks or grooms her offspring and when the young consume feed freshly contaminated with protozoa. Since protozoa are strictly anaerobic organisms, and resistant cysts have never been found, protozoa-free ruminants isolated from all other ruminants will remain free of protozoa.

The poor survival of ciliates outside the rumen has greatly facilitated studies aimed at controlling the ciliate fauna. The isolation technique has been used frequently in the past (Becker and Hsuing, 1929; Abou Akkada and El Shazly, 1964; Eadie, 1962a; Eadie and Gill, 1971; Wallace *et al.*, 1987). However, rearing young animals away from their mothers would not only be time-consuming, but also labour-intensive (Becker and Hsuing, 1929; Wallace *et al.*, 1987).

b. Breeding from ciliate-free dams

Work at the University of New England (Bird, 1989) indicates that ewes which are completely defaunated in the early stages of pregnancy and kept in complete isolation from faunated individuals will rear protozoa-free offspring. After the initial defaunation of the ewes, the remaining microbial population (bacteria and fungi) adjusts to the modified rumen conditions and approaches a new stability before parturition. Despite the technical success of this method, it is impractical to apply in

the field because problems and excessive costs arise from keeping the dams isolated from faunated animals.

c. Chemical Drenching

An oral drench with a suitable anti-protozoal solution delivered directly into the rumen via a length of plastic tubing is the simplest method to eliminate protozoa from the rumen. This method of defaunation has been used for many years (Becker and Everett 1930, Abou Akkada *et al.*., 1968). The effects of a variety of chemical agents have been examined for anti-protozoal activity. Willard and Kodras (1967) reported that heavy metals such as copper and nickel, anthelmintic such as piperazine and those with phenol and imidazole groups, inorganic and organic arsenicals and anion surface detergents showed promise as anti-protozoal agents.

Defaunation was achieved by five days starvation and copper sulfate administration for two consecutive days (Becker and Everett, 1930), but this method has adverse effect on ruminal processes and the animal's general health (Abou Akkada *et al.*, 1968). Surface active agents which are now recognised for their ability to control bloat in cattle fed legumes (Clarke and Reid, 1974) have also been shown to have anti-protozoal activity. Dioctyl sodium sulfosuccinate (trade names 'Manoxol OT', British Drug Houses Ltd. and 'Aerosol OT', American Cyanamid Co.) was the first surface active agent (surfactant) to be used successfully *in vivo.* This compound in the form of 30 g doses of Aerosol OT (*Ca* 1g/10 kg live wt) was given to Jersey cows (350 kg) and it was administered using gelatine capsules via a balling gun, repeated on two consecutive days. This compound produced a range of toxic effects upon all types

of rumen ciliate protozoa, varying from inhibition of motility to cell disintegration, without harming either rumen fermentation rate or the health of the host, and through its use it has been possible to maintain animals in a ciliate- free condition for at least 2 weeks (Abou Akkada *et al.*, 1968). However, Manoxol OT showed toxic effects to host animal (anorexia and scouring) when it was used at higher doses (*Ca* 4g/10 kg live wt) to defaunate wethers (30 kg) (Males and Purser, 1970). To avoid the toxic effect from surfactant, workers found it necessary to maintain an estimated 2 g concentration of surfactant /l rumen fluids continuously for 72 hours (Lindsay and Hogan, 1972).

Teric GN9 (9 moles of ethylene oxide condensed with one mole of nonyl-phenol or nonyl phenol ethoxylate) has subsequently been used to remove protozoa from cattle (Bird and Leng, 1978) and sheep (Bird *et al.*, 1979). Defaunation resulted in an increased growth and improved feed efficiency in cattle and sheep fed high-energy, low-protein diets supplemented with small amounts of protein which would escape ruminal degradation. Alkanate 3SL_3 (sodium lauryl ethyl sulphate) has also been used to completely remove protozoa from the rumen of sheep (Burggraaf and Leng, 1980). However, both Teric GN9 and Alkanate 3 SL_3 are unlikely to be specifically toxic to protozoa and probably also kill other microorganisms and host cells in the rumen (Bird, 1989) and their administration is often followed by a period of depressed feed intake and animal performance.

d. Dietary manipulation

Since it is known that the population of ciliate protozoa in the rumen is strongly influenced by the nature of the diet, withholding feeding or dietary manipulation are two options which may be used to accomplish defaunation (Bird, 1989; Jouany *et al.*, 1988).

Both the size of the protozoal population and other rumen parameters are influenced by rumen acidity (Purser and Moir, 1959) and protozoa were found to be killed at a pH of 4.5 (Hungate, 1966). Feeding cereals (barley) to animals *ad libitum* results in an excess production of organic acids and a high degree of rumen acidity (Kobayashi and Itabashi, 1986). Complete elimination of protozoa has been achieved by feeding cereals (barley) to animals *ad libitum* (Rung *et al.* 1986). Concentrate feeds also may give rise to low protozoal counts (Nour *et al.*, 1979), presumably due to interrelated effects on rumen pH, rumination and salivation.

Prolonged under-nutrition for a few days has been associated with low protozoal counts (Warner, 1965; Potter and Dehority, 1973; Orpin, 1977c) due to lower level of nutrients being available. Nour *et al.* (1979) found that feeding ruminants with low-quality roughage results in low protozoal counts. It has also been reported that restricted feeding results in low rumen volume (Orpin, 1977c), which would normally be associated with a faster turnover of rumen digesta, therefore protozoa are washed out of the rumen at a faster rate.

Dietary manipulation technique is probably safer than chemical drenching, but due to impracticality and to difficulty in achieving complete elimination of protozoa, this method has not been used more widely (Bird, 1989).

2.8 Summary and a proposed method for defaunation

Among the several methods that have so far been investigated, chemical drenching is considered to be the most practical. However, for this procedure to be successful, animals need to be drenched on three consecutive days (Bird, 1989), which is impractical in an extensive grazing system and is often followed by a period of depressed feed intake and animal performance. Continuous treatment with smaller doses of surfactant detergent was shown to be effective in penned animals (Lindsay and Hogan, 1972) but has not been investigated under field conditions.

Molasses blocks have been used widely as a vehicle to administer multinutrients to ruminants since 1920 and have also been used as a carrier of anthelmintic agents (Knox, 1995).

The experimental work presented in Chapter 3 was designed to determine if the molasses block could be used as a suitable means of administering an anti-protozoal agent to reduce or completely remove protozoa from the rumen of sheep. The relevant questions which need to be answered were as follows.

a)Will a molasses block containing anti-protozoal detergent set sufficiently hard to ensure that sheep can achieve a suitable intake of detergent over a prolonged period ?

b)Will sheep consume a molasses block containing anti-protozoal detergent ?

c)Will the consumption of anti-protozoal detergent contained in molasses block

defaunate the rumen ?

d) Will the consumption of a molasses block containing anti-protozoal detergent have any adverse effects on the animal?