BACTERIOPHAGES IN THE RUMEN; TYPES PRESENT, POPULATION SIZE AND IMPLICATIONS FOR THE EFFICIENCY OF FEED UTILISATION

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SUMMARY

One cause of a reduction in the efficiency of feed utilisation in the rumen is the non-specific lysis of bacteria within the rumen and subsequent fermentation of the bacterial protoplasm. Bacteriophages are implicated in this lysis, are obligate pathogens of bacteria and occur in dense populations in the rumen. Large numbers are present (up to 10^{11} per millilitre of fluid) in the rumen. These viruses are morphologically diverse with 26 distinct types from three viral families *(Myoviridae, Siphoviridae* and *Podoviridae)* being represented. The use of the DNA-based methodology, Pulsed Field Gel Electrophoresis, has allowed an estimate of phage numbers in the rumen at a point in time. This procedure will enable investigations of changes in the phage population in relation to changing dietary regimes. Preliminary evidence suggests that diet may influence viral activity and therefore dietary manipulation could, in the future, be used to reduce viral activity and improve the flow of microbial protein to the intestines.
Keywords: bacterial viruses, rumen, lysis, phage

INTRODUCTION

The nutrition of ruminants depends on fermentative digestion of plant biomass to supply protein (in the form of micro-organisms) and volatile fatty acids which can then be utilised by the animal. The protein to energy ratio in these end-products is critical to the efficiency of animal growth and it is the most important factor affecting efficiency of feed utilisation. In hot climates, such as occur in Australia, the protein to energy ratio has been shown to be more critical than in temperate countries (Leng 1990). Improving the efficiency of feed utilisation decreases production costs by decreasing feed costs per unit of animal product and reduces waste, including emissions of the greenhouse gas methane. Grazing animals rarely achieve more than 30% of their genetic potential for production, largely due to their inefficient use of feed. One cause of reduced efficiency is the non-specific lysis of bacteria within the rumen and subsequent fermentation of the bacterial protoplasm. This phenomenon has not been explained but at times a large proportion of the bacterial pool can be affected (Nolan and Stachiw 1979; Firkins *et al.* 1992). Bacteriophages (bacterial viruses) are implicated in this lysis (Jarvis 1968). Bacteriophages are obligate pathogens of bacteria and occur in dense populations in the rumen.

Until recently little knowledge of these viruses and their role in the rumen has been available. This paper is an overview of work undertaken over the past 10 years to establish the significance of ruminal phages.

PHAGE TYPES

The phage populations in rumen fluid samples from 2 cattle (fed a diet of chopped rice straw and a commercial mineral and vitamin mix) and 17 sheep (7 fed chopped oaten hay plus a commercial mineral and vitamin mix and 10 grazing improved native pasture) were isolated and concentrated by differential centrifugation and ultrafiltration (Klieve and Bauchop 1988) prior to examination by transmission electron microscopy (TEM). From these studies, 26 morphologically distinct types of phage were identified. These viruses belonged to 3 distinct families of tailed phages; the *Myoviridae, Siphoviridae,* and *Podoviridae.* It was found that the diversity in this and other TEM studies was far greater than that found amongst the phages cultured on ruminal and non-ruminal bacteria (Klieve and Bauchop 1988). These authors felt that 3 explanations were possible for this discrepancy: (1) the phages of the rumen may exist in harmony with their hosts in a state of lysogeny or pseudolysogeny; (2) the phages have very narrow host ranges and susceptible hosts are yet to be found; and (3) very few bacteria that are regarded as true ruminal inhabitants had been used as prospective hosts in previous studies.

LYTIC VERSUS TEMPERATE PHAGES

Phages that infect and lyse their host are lytic. However, some phages have the ability to lyse the host and also to integrate their DNA into the bacterial chromosome, and then to exist from one generation to

another as an integral part of the bacterium. These phages are known as temperate phages and the hosts are lysogenised.

Lytic phages were isolated from rumen fluid using the soft-agar-overlay technique of Gratia (1936), as described by Adams (1959). Rumen fluid samples from 13 cattle (1 fed a diet of chopped rice straw; the rest slaughtered at an abattoir, diet unknown) and 30 sheep (3 fed chopped oaten hay; 10 from the abattoir, diet unknown; the remainder had been grazing improved native pasture and 5 of these were defaunated) were screened for lytic phage to a variety of rumen bacteria *(Eubacterium ruminantium, Streptococcus bovis, Ruminococcus albus, Ruminococcus flavifaciens, Prevotella (Bacteroides) ruminicola* ss *brevis, Butyvivibrio fibrisolvens,* and *Fibrobacter succinogenes).*

A single lytic phage was isolated on *Streptococcus bovis* 2B from a pooled rumen sample from cattle slaughtered at the abattoir. No other lytic phages were isolated from rumen contents, although many phages of *Prevotella ruminicola* were isolated from sewage (Klieve *et al.* 199 1; Klieve unpublished data).

Rumen bacterial isolates have also been screened for the presence of temperate phages by challenging them with mitomycin C (Iverson and Millis 1976; Klieve *et al.* 1989). Thirty eight ruminal bacteria (14 species) were screened (Klieve *et al.* 1989). Twenty four per cent of the rumen bacterial isolates examined were found to be lysogenic and to harbour inducible temperate phages (Klieve *et al.* 1989). This suggested that the origin of the phages observed by TEM in ruminal contents was probably from the induction of temperate phages. A major discrepancy, however, was that all except one of the phages identified by mitomycin C challenge were of a small and common group of morphotypes belonging to the family *Siphoviridae.* This did not correspond with the diversity of morphotypes directly observed by TEM.

The presence of viral genetic material in a significant percentage of ruminal bacteria indicates that viral genetic material is a normal constituent of the genome of ruminal bacteria.

PHAGE POPULATION ANALYSIS

In order to better study the phage population of the rumen *in vivo,* and to study factors that impact on the extent of phage mediated bacterial lysis, a method of estimating the size and composition of the phage population of the rumen was required. To achieve this Klieve and Swain (1993) developed a method using pulsed field gel electrophoresis (PFGE) and laser densitometry to estimate the phage population from phage DNA that can be isolated directly from rumen fluid samples.

Using the above techniques Klieve and Swain (1993) demonstrated that the rumen phage population was commonly between 10^9 and 10^{11} particles per ml of rumen fluid. They also demonstrated that this population, as gauged by phage DNA banding profiles on PFGE gels, appears to have 2 distinct components. A broad region of DNA between 30 and 200 kilobase pairs in size was always present and it appeared that this region comprised DNA from many different phages including temperate phages. In addition, discrete DNA bands were observed frequently. It has been proven that one of these bands corresponds to a single phage and it appears that these discrete bands are due to epidemics and 'blooms' of phage activity, probably on one or a few bacterial species (Klieve and Swain 1993).

The variety of DNA banding patterns is in agreement with TEM observations (Klieve and Bauchop 1988) and indicates that the diversity of the bacteriophage population of the rumen is considerably greater than the few morphotypes isolated from culturable rumen bacteria (Klieve and Bauchop 1988; Klieve *et al.* 1989). In turn this could indicate that the bacteria currently cultured from the rumen are not necessarily representative of the true diversity of bacteria present.

SCREENING SECONDARY PLANT COMPOUNDS

Recently it has been shown that sheep grazing pasture had significantly elevated total phage populations in comparison to counterparts fed a diet of chopped oaten and luceme hays (70:30) (Swain *et al.* 1996a). It is possible therefore that diet and dietary components affect phage activity. One way that this could occur would be where components are acting as inducing agents and inducing bacterial lysis by temperate phages.

Due to the suspected prevalence of temperate phage in the rumen (Klieve *et al.* 1989) the possible impact of secondary plant compounds on ruminal phage activity is being investigated. Many secondary plant compounds are produced to combat infection by microorganisms, or herbivory. It could therefore be expected that some of the compounds may be antagonistic to rumen microorganisms.

To determine the possible effects of different plant components on phage activity in the rumen a variety of model secondary plant compounds, including tannic acid, saponin, sparteine, catechin, rutin, quercetin, and p-coumaric acid, have been examined (Swain *et al.* 1996b). However, to date none of these compounds has been found to induce bacterial lysis. Interestingly, tannic acid has been shown to have an impact on

phage. At a low concentration (0.1% w/v) tannic acid precipitates phage particles and therefore reduces the numbers and infectivity of the phage in aqueous media (Swain *et al.* 1995). The concentration of tannic acid that is effective is below the concentration that significantly reduces the growth rate of bacteria.

The impact of secondary plant compounds on phage activity, and the difference between animals on different dietary regimes, is still under investigation.

It is envisaged that further knowledge of the impact of feed components on phage activity in the rumen will lead to a better understanding of the factors that control phage activity and allow for the reduction of phage activity in the rumen by dietary manipulation.

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