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Dietary effects on the microbiological safety of food

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The high mortality rate associated with human infections caused by *Escherichia coli* strains of the serotype O157:H7 has brought to public attention the importance of ruminants as reservoirs of food-borne pathogens. In addition to established examples such as salmonella, campylobacter and listeria, recent evidence is emerging of the role of food in the transmission of *Helicobacter pylori* and *Mycobacterium paratuberculosis*. Food-borne pathogens harboured by ruminants are spread through shedding in the faeces and subsequent faecal contamination of raw food. Ruminant shedding appears to be affected by diet and, of particular concern, may be increased during fasting regimens imposed during transport to the slaughterhouse. The survival of food-borne pathogens in the ruminant gut is affected by many factors including microbe–microbe interactions, interactions involving plant metabolites and the presence of inhibitory end-product metabolites such as short-chain fatty acids. The potential importance of digesta flow and bacterial detachment in shedding of food-borne pathogens is discussed. Experimental procedures with dangerous pathogens have constraints, particularly in animal experimentation. This situation may be overcome by the use of rumen-simulating fermentors. One such system which, like the natural rumen, has a different turnover rate for solid and liquid digesta, was found to maintain rumen-like variables over an 11 d period. This system may prove useful for the study of dietary effects on food-borne pathogens.

Ruminant diet: *Escherichia coli* O157:H7: Shedding: Food-borne pathogens

Escherichia coli strains of serotype O157:H7 (*E. coli* O157) have evolved from more mildly pathogenic progenitors (Whittam, 1998), and now present the food industry with a severe challenge. This pathogen has a very low infectious dose and it may be carried asymptomatically by farm animals. In November 1996 an outbreak of food poisoning caused by *E. coli* O157 was identified in Central Scotland. By April 1997 eighteen deaths had occurred, at that time the worst single outbreak due to this pathogen (Pennington, 1997). More deaths followed (Ahmed & Donaghy, 1998). In the UK this event, more than any other, focussed attention on the issue of the carriage and spread of human pathogens by farm animals, particularly ruminants.

The spread of food-borne pathogens from ruminants to the human food chain generally occurs through faecal contamination of milk on the farm and meat at the slaughterhouse. Gross microbial contamination of the carcass with gut contents may occur during evisceration, but it is thought that most contamination is of faecal origin and occurs during removal of the hide or cross contamination from hide to carcass via hands and instruments of slaughterhouse workers (Gannon, 1999). Measures to reduce the levels of faeces on hides of slaughter animals have been introduced within the last decade, largely in response to *E. coli* O157. These measures include the withdrawal of feed from animals during transport to slaughter, and the introduction of the 'clean livestock policy' which empowers official veterinary surgeons and meat hygiene inspectors to reject animals with heavily-soiled hides when presented for slaughter. This latter measure was emphasised by Pennington (1997). Developing the ability to manipulate conditions within the rumen to ultimately reduce the levels of pathogens in faeces, and subsequently in the raw products, depends on our understanding of the microbial ecology of the ruminant gut.

Here, some of the different human pathogens which may be shed by ruminants are identified; thereafter, particular emphasis is placed on *E. coli* O157. The effects of the ruminant diet, including fasting and dietary changes, on faecal shedding of food-borne pathogens are discussed. Diet plays a major role in determining the composition of the ruminant gut microbial flora and hence the metabolites produced. The effect of such factors on the survival of food-borne pathogens in the ruminant gut are explored. Finally,

Abbreviations: *E. coli* O157, *Escherichia coli* strains of serotype O157:H7; SCFA, short-chain fatty acids.
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experimental approaches to the study of the carriage and shedding of *E. coli* O157 are considered.

Types of human food-borne pathogens carried by ruminants

Farm ruminants are important reservoirs of food-borne pathogens such as *Listeria* spp., *Campylobacter* spp., *Yersinia enterocolitica, Cryptosporidium* spp., *E. coli* O157 and *Salmonella* spp. Many such human pathogens may be carried by apparently-healthy adult animals without clinical signs of disease, and they are not detected by ante- or post-mortem inspection at the slaughterhouse. However, cryptosporidium, some species or serovars of *Salmonella* (e.g. *S. dublin*) and *E. coli* O157 may cause diarrhoea in calves. The significance of the carriage of pathogens by farm animals relates not only to the potential for contamination of milk at the farm and the carcass at slaughter, but also to contamination of water and the environment during the disposal of abattoir effluents and slurries (Wallace, 1999). The major site of amplification of enteric organisms such as *E. coli*, salmonella, campylobacter and listeria is the intestines. Very low numbers of campylobacter may be found in the rumen, where their presence may indicate recent ingestion. However, the numbers of campylobacter in the lumen of the large intestine of cattle and sheep at slaughter may reach 10^7 cells/g fresh material (Stanley *et al*. 1998*a*,*b*). In contrast, Harmon *et al*. (1999*b*) found that following its introduction by oral inoculation *E coli* O157 was present in higher numbers in the rumen than in other sites of the intestinal tract.

Ruminants are also an important reservoir of emerging food-borne pathogens such as *Arcobacter* spp. and of pathogens such as *Mycobacterium paratuberculosis* (Collins, 1997) and *Helicobacter pylori*, for which there is recent evidence of food-borne transmission. *M. paratuberculosis* causes Johne's disease in cattle and is implicated in the aetiology of Crohn's disease, a chronic inflammatory bowel disease in human subjects (Collins, 1997). This organism is excreted in the faeces and sometimes the milk of chronically-infected cattle, thus potentially contaminating raw products. As detection of *H. pylori* is sometimes difficult, indirect evidence for the transmission of *H. pylori* from meat animals to human subjects has been provided by serum surveys or blood tests of veterinarians and slaughterhouse workers (Wesley, 1997). A number of observations suggest a role for sheep in the transmission of *H. pylori* (Dore *et al*. 1999). These observations include a significantly higher prevalence of *H. pylori* in Sardinian shepherds (98 %) compared with other members of their household, the isolation of *H. pylori* from sheep's milk and the detection by polymerase chain reaction of *Helicobacter* spp. in mucosal samples from sheeps' stomachs.

Dietary factors affecting shedding

Seasonal variation

The patterns of faecal shedding of pathogens by ruminants show seasonal variation. In dairy cattle, the prevalence of *Listeria monocytogenes* in the faeces is higher in winter than summer (Husu, 1990), whereas shedding of campylobacter (Stanley *et al*. 1998*b*) and *E. coli* O157 (Wallace, 1999) peak in late spring and autumn. The number of campylobacter in lambs at slaughter (Stanley *et al*. 1998*a*) and shed by grazing sheep (Jones *et al*. 1999) peaks in March. This peak coincides with lambing, weaning and movement onto new pasture. Factors subject to seasonal change which might affect the survival of *E. coli* O157 in the environment include incident radiation, temperature and transmission by possible vectors such as wild birds (Wallace, 1999). There is also evidence to suggest that shedding by ruminants may reflect seasonal changes in the diet. Feedstuffs used during winter, such as silage, hay and concentrates, are the major sources of both pathogenic and non-pathogenic species of *Listeria*, whereas the low prevalence of *L. monocytogenes* in summer may be due to grazing on pasture (Husu, 1990). Peaks of campylobacter shedding roughly correlate with the move from winter housing to summer pasture and back again, and may reflect changes in diet.

Effects of dietary composition

As diet is one of the most important factors affecting microbial numbers and composition in the ruminant gut (Dehority & Orpin, 1988), it is not surprising that dietary composition may affect the faecal shedding of pathogens. Some examples are summarised in Table 1. Although within individual studies diet appears to affect shedding of specific pathogens, there is no overall discernible pattern between the type of diet fed and the rate of shedding. This observation may be due to experimental differences. In particular, the length of time for adaptation to a new diet may confound results, since sudden dietary changes can

Table 1. Some effects of the ruminant diet on carriage or shedding of selected pathogens

Pathogen	Carrier	Dietary effect	Reference
Listeria spp.	Sheep	Shedding lowest on pasture	Husu (1990)
Campylobacter spp.	Sheep	Shedding lower on hay and silage than on pasture	Jones *et al*. (1999)
C. jejuni	Cattle	Feeding cottonseed hulls a risk factor	Wesley *et al*. (2000)
Arcobacter spp.	Cattle	Prevalence lowered by brewer's by-products, whole cottonseed or lucerne	Wesley *et al*. (2000)
Salmonella dublin	Calves	Infections lowered by supplementing grass with maize or silage	Vaessen *et al*. (1998)
E. coli O157	Cattle*	Hay-fed animals shed for longer than grain-fed animals	Hovde *et al*. (1999)
E. coli O157	Cattle*	Similar titres shed by hay- and grain-fed animals	Hovde *et al*. (1999)
E. coli O157	Sheep*	Shedding lower on high-protein diet than high-roughage diet	Kudva *et al*. (1997)

C. jejuni, *Campylobacter jejuni*; *E. coli* ^{O157}, *Escherichia coli* strains of serotype O157:H7.
* Experimentally inoculated.

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affect the composition of the rumen flora dramatically (Grubb & Dehority, 1975). The effect of seasonal dietary change on shedding of campylobacters has been discussed earlier. For sheep shedding *E. coli* O157, a dietary change from hay to maize and lucerne (*Medicago sativa*) or vice versa resulted in an increase in the number of culture-positive animals compared with animals remaining on the same diet throughout the study (Kudva *et al*. 1997).

Fasting

Feed is commonly withheld from animals during transport to slaughterhouses and between farms to reduce faecal excretion. However, feed deprivation is thought to predispose cattle to *E. coli* and salmonella carriage (Brownlie & Grau, 1967). More recent investigation of shedding of *E. coli* O157 detected no increase in numbers of this organism in adult sheep (Kudva *et al*. 1997) or weaned calves (Harmon *et al*. 1999*a*; Brown *et al*. 1997) that were experimentally inoculated then fasted for 24 and 48 h respectively. A similar finding was apparent in a study of weaned calves (Cray *et al*. 1998). However, when the calves were fasted for 2 d before inoculation, significantly greater numbers of *E. coli* O157 were shed compared with non-fasted calves. Gradual adaptation to certain diets may help to limit shedding induced by fasting. Midgley *et al*. (1999) fasted cattle during transportation, then fed diets in which the grain was gradually increased. The coliform counts remained stable throughout, and there was no difference in the percentage of samples containing DNA of verocyto-toxin-producing *E. coli* such as *E. coli* O157 over the 117 d period of the study. Similarly, Kudva *et al*. (1995) found that feeding native sagebrush (*Artemisia* spp., mainly *A. tridentata*)–bunch grass (mainly *Poa* spp. and *Festuca* spp.) increased the incidence of shedding of *E. coli* O157 in lambs and ewes initially. However, on cessation of shedding, samples from the animals remained negative for *E. coli* O157 despite subsequent periods of fasting and dietary change.

Other dietary factors

The shedding of *E. coli* O157 in the faeces of naturally-infected calves was found to be associated with the regular use of antibiotics but not with the use of ionophores (Shere *et al*. 1998). Other factors not associated with shedding in this study included feeding clover (*Trifolium* spp.) hay as the first forage, feeding whole cottonseed to heifers before first calving, or feeding milk substitute. Age-related dietary effects may also be important. *E. coli* O157 shedding was associated with grain feeding in calves less than 5 d old, but not in calves greater than 5 d old (Garber *et al*. 1995). Other problems arise from the survival of pathogens in stored feed and other products on farms. The persistence of salmonella in recycled litter, contaminated commercial protein feeds and hay contaminated on the farm causes many problems in the management of the carriage and shedding of this organism by cattle (Vaessen *et al*. 1998).

Factors influencing the survival of food-borne pathogens in the rumen

Microbe–microbe interactions involving Escherichia coli *O157:H7*

The rumen is a pre-peptic compartment of the ruminant stomach. The digesta is held there for a period sufficient to allow the predominantly-anaerobic mixed microbial population present to hydrolyse dietary polymers such as cellulose, arabinoxylans and starch, releasing sugars which are fermented to form short-chain fatty acids (SCFA). These products are used by the host animal as energy sources and as C skeletons for biosynthetic reactions. Microbial cells provide the bulk of the host's protein requirement (Hungate, 1966; van Soest, 1994). Secondary metabolites present in the dietary plant material may also be released and trans-formed by the rumen micro-organisms (Chesson *et al*. 1982; Fig. 1). Coliform bacteria are usually present in rumen contents at about 10^4/ml (Diez-Gonzalez *et al*. 1998), whereas the total number of anaerobes in the rumen is comparatively very high (10^8–10^{11} viable cells/ml; Hungate, 1966). Competitive and amensalistic interactions with anaerobes and their products probably limit the population size of *E. coli* (Stewart, 2000).

Exploitation of antagonistic microbial interactions has led to the development of probiotics with inhibitory activity against *E. coli* O157. Zhao *et al*. (1998) isolated seventeen strains of *E. coli* and one of *Proteus mirabilis* from cattle faeces which were able to inhibit the growth of *E. coli* O157. When introduced experimentally, *E. coli* O157 did not survive in six calves dosed with a cocktail of these strains, but was detected in most of the control animals. Duncan *et al*. (1999*a*) screened aerobic ovine rumen isolates for inhibitory activity against *E. coli* O157. In 50 % of samples studied the predominant inhibitory isolates were strains of *Pseudomonas aeruginosa*. These studies suggest that an appropriate probiotic strategy may reduce the carriage of *E. coli* O157 by ruminants (Harmon *et al*. 1999*b*).

The particular strains of *E. coli* that persist in the ruminant gut may be influenced by the production of colicins, plasmid-encoded proteins produced by

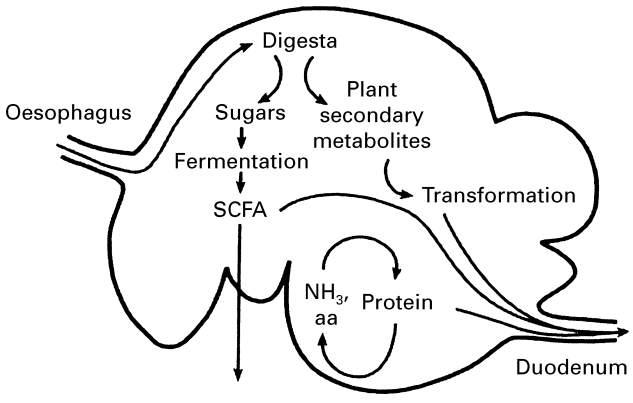

Fig. 1. Schematic representation of key events in the rumen fermentation. SCFA, short-chain fatty acids; aa, amino acids.

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enterobacteriaceae which inhibit the growth of closely-related strains (Pugsley, 1985; Riley & Gordon, 1996). The fact that many gut strains of *E. coli* are colicinogenic suggests that this property may confer an ecological advantage (Tan & Riley, 1996). Bradley & Howard (1991) found colicins G and H inhibited all *E. coli* O157 strains that were tested, while colicins E2 and V inhibited 60 and 90 % of the strains respectively. Colicins A, B, D and Ia had no inhibitory effect. Similarly, Murinda *et al.* (1996) found that a range of *E. coli* O157 strains were sensitive to colicins G and H, and microcin B17, a low-molecular-weight oligopeptide bacteriocin. Bradley & Howard (1991) reported that many *E. coli* O157 strains were themselves colicinogenic, and predominantly produced colicin D-like colicins, D157. A non-verocytotoxic *E. coli* O157 strain 12900 was also found to be sensitive to fewer colicins than some rumen *E. coli* isolates (Duncan *et al.* 1997). In this investigation, colicinogenic *E. coli* strains isolated from a range of animal intestinal samples were not inhibitory to *E. coli* O157 strain 12900.

The outer-membrane colicin receptors of colicin-sensitive cells may also serve as the attachment sites for bacteriophages. There are large numbers of bacteriophages in the rumen, and they are commonly present at between 3×10^9 and $1{\cdot}6\times10^{10}$ virions/ml rumen fluid following differential centrifugation and ultrafiltration (Klieve & Swain, 1993). They are largely maintained in high numbers by lysis of rumen bacteria, but their role in the ecology of *E. coli* and other bacteria in this ecosystem remains unclear. The genes encoding the verocytotoxins of *E. coli* O157 are carried by bacteriophages (O'Brien *et al.* 1984).

Microbe–microbe interactions may determine the fate of *E. coli* following its shedding into the environment. For example, *E. coli* O157 was found to survive and replicate in a protozoan, *Acanthamoeba polyphaga, in vitro* (Barker *et al.* 1999). This protozoan, a common inhabitant of effluents, may have a significant role in pathogen transmission.

Interactions involving plant metabolites

The breakdown of plant material in the rumen may yield anti-microbial substances. Coumarins, found mainly in leguminous plants including clover, are glycosides which are hydrolysed in the gut to produce aglycone and sugar residues. Many of the predominant bacterial species from the gut possess 1,4-β-glucosidase which hydrolyses the coumarin esculin, releasing the aglycone esculetin (Stewart *et al.* 1997). The growth and survival of the non-verocytotoxic *E. coli* O157 strain 12900 was found to be reduced in the presence of the aglycones esculetin, umbelliferone, coumarin and scopoletin under both aerobic and anaerobic conditions *in vitro*, but was unaffected by the presence of the glycoside esculin. The effects of the simultaneous presence of esculetin and SCFA at the concentrations likely to be encountered in the gut (50–100mM) were additive (Duncan *et al.* 1998). The addition of esculin to batch cultures of ovine rumen contents inoculated with *E. coli* O157 resulted in a greater than 2000-fold decrease in the number of cells surviving over a 24 h incubation period relative to controls without this compound. In contrast, the total number of anaerobes was little affected by the presence of esculin.

Other plant compounds may have similar effects. Nagy & Tengerdy (1968) reported that the essential oils from sage-brush had anti-microbial activity, but whether this activity could selectively affect *E. coli* has not been tested. The growth of *E. coli* was found to be inhibited by several essential oils from plants by Hammer *et al.* (1999). Feeding cottonseed to ruminants has been reported to reduce shedding of *E. coli* O157 (Rasmussen *et al.* 1999). This effect may be related to the presence in this plant of anti-microbial factors, although gossypol, one of the major anti-microbial factors present in this plant, had little effect on the growth of *E. coli* (Rasmussen *et al.* 1999).

Effect of short-chain fatty acids

The SCFA produced in the rumen, such as acetate, propionate and butyrate, are weak acids with bactericidal properties at low pH. These acids are mainly undissociated at low pH and are consequently lipophilic. This characteristic allows them to freely traverse the cytoplasmic membrane where they dissociate at the slightly alkaline pH which generally exists in bacterial cells. Protons and acid anions are liberated and the toxicity of weak acids has been ascribed to cytoplasmic acid anion accumulation (Russell, 1992). Cherrington *et al.* (1990) showed that propionic acid anions inhibited the synthesis of DNA, RNA, protein, lipid and cell walls of *E. coli* K12. Some rumen bacteria and *E. coli* strains, including *E. coli* O157, are relatively resistant to weak acids (Diez-Gonzalez & Russell, 1997). In such strains the maintenance of low intracellular pH decreases the proportion of dissociated acid anions in the cytoplasm (Russell, 1992).

Although the pH of the healthy rumen is only slightly acidic (van Soest, 1994), some acid-producing organisms, such as the lactic acid bacteria, may cause a localised reduction in the pH of their microenvironment. The low pH values required to overcome resistance to weak acids in *E. coli* O157 strains may be achieved in these microhabitats. Lactic acid bacteria may be important in the control of *E. coli* O157 in the rumen, since lactic acid is more inhibitory than other SCFA at low pH (Jordan *et al.* 1999; Leitch & Stewart, 2000). In addition, numbers of lactic acid bacteria tend to increase when the pH of rumen contents is allowed to fall (Stewart, 1977); the numbers of such bacteria might be expected to decrease concomitantly as the rumen pH rises during fasting. Duncan *et al.* (1999b) showed that propionate was more inhibitory than acetate or butyrate for *E. coli* O157 under anaerobic conditions. At pH 7, acetate, propionate and butyrate at concentrations found in the rumen inhibited the growth of *E. coli* O157 (Duncan *et al.* 1998). Similarly, the growth rate of *E. coli* O157 was reduced when the SCFA concentration increased at normal rumen pH (Rasmussen *et al.* 1993).

In addition to the rumen microenvironments created by acid producers, bacteria may also encounter toxic concentrations of SCFA at low pH in the abomasum. They must survive this environment in order to reach the colon and be shed in the faeces. The gene *rpoS* is induced in *E. coli* during adverse conditions such as stationary phase, and

plays a role in acid resistance. Price *et al*. (2000) showed that an *E. coli* O157 mutant lacking this gene was shed in lower numbers by weaned calves than the wild type. This finding suggests that acid resistance may be an important factor in the survival of *E. coli* O157 in the ruminant gut. However, the acid-resistant mutant was shed for a similar length of time to that of the wild type, suggesting that survivors of abomasum acidity that reach the colon where the pH is close to neutral no longer require acid resistance for survival.

The importance of digesta turnover in shedding of Escherichia coli *O157:H7*

In the gut, bacteria preferentially attach to surfaces such as the rumen epithelium or feed particles, forming biofilms containing multiple species which may provide metabolically-beneficial associations (van Soest, 1994). The organisms produce a glycocalyx which stabilises adherence and prevents cell washout (van Soest, 1994). Pathogenic *E. coli* strains can adhere to rumen epithelial cells (Galfi *et al*. 1998). However, in weaned calves experimentally inoculated with *E. coli* O157, the rumen contents rather than the mucosal surface appeared to be the main site of colonisation (Brown *et al*. 1997). It is not known whether *E coli* O157 adheres to food particles in the solid phase or remains in the liquid phase of the digesta. Partitioning may be important in determining the significance of digesta flow to the shedding of *E. coli* O157, in that the turnover rates of the solid and liquid phases in the rumen differ.

Dietary particles are subjected to competition between passage and digestion, the tendency of particles to flow with the digesta stream being counteracted by their microbial degradation (for review, see Sauvant, 1997). Autochthonous microbial communities maintain their populations by proliferating at a rate that compensates for loss through passage and predation by certain rumen ciliate protozoa (Hungate, 1966). The fate of *E. coli* O157, which appear to be only transient members of ruminant gut microflora (Hancock *et al*. 1998), provides a special case in that any localised proliferation which may occur does not compensate for their ultimate displacement by digesta flow, and shedding will be influenced strongly by the rate of digesta passage.

In the rumen, particles are sorted by the floating rumen mat, which separates the liquid and gas phases, and by leaf-like structures attached to the distal wall of the omasum (van Soest, 1994). Solids and liquids flow through the gut at different rates. Estimations of rumen turnover times of DM and the liquid phase cited by Hungate (1966) varied considerably, but averaged about 1·9 and 0·6 d respectively. The composition of the diet affects the flow of digesta. Feeding with concentrate leads to increased intake, decreasing the amount of saliva per g food (van Soest, 1994). As saliva provides about 70 % of the water entering the rumen, this decrease reduces the net flow (van Soest, 1994). The particle size of the diet also affects turnover times; larger particles are retained in the rumen longer than small particles (Hungate, 1966; van Soest, 1994). Concentrates, which usually have a smaller particle size, and ground forage are associated with faster passage compared with pasture (van Soest, 1994). Finely-ground whole diets cause cessation of rumination and the diminution of the rumen mat, allowing passage of particles which would normally be entrapped (van Soest, 1994). For bacteria adherent to feed particles, reducing the retention time in the rumen is likely to increase shedding. These considerations, which currently have not been explored in relation to shedding of food-borne pathogens, potentially complicate the view that effects of SCFA and pH are the major factor affecting whether forage or grain-fed animals shed greater numbers of *E. coli* O157 (Diez-Gonzalez *et al*. 1998).

Cell detachment

Whether *E. coli* O157 associates mainly with the liquid or particulate phases of the digesta is not known. If *E. coli* attaches to particles, then biofilm detachment mechanisms may have an important role in shedding due to the greater turnover rate of the liquid phase compared with the solid phase. Boyd & Chakrabarty (1994) suggested that some bacterial species actively detach from surfaces to escape adverse environmental states or to disseminate and colonise new surfaces. Detachment can be mediated through enzymic cleavage of matrix polymers, including the alginate lyase of *P. aeruginosa* (Boyd & Chakrabarty, 1994), or by changes in the physiology of the attached cells such as the active release of surface proteins of *Streptococcus mutans* (Lee *et al*. 1996; Baehler & Moxley, 2000). For some Gram-negative bacteria, including *E. coli*, detachment occurs at a particular point during the division cycle (Allison *et al*. 1990). Cell adhesive structures and cell surface hydrophobicity are minimised during and immediately after the division period, leading to daughter cell separation and dispersal (Allison *et al*. 1990). Surface hydrophobicity of *E. coli* biofilm and planktonic cells also decreases with increasing growth rate (Allison *et al*. 1990). Cell detachment has not yet been examined as a potential contributor to the shedding of food-borne pathogens from ruminants. However, it can be speculated that conditions which favour increased numbers of *E. coli* in the rumen, such as dietary change and fasting, may also favour increased detachment of cells from biofilms in order to colonise new surfaces.

Experimental approaches to investigating dietary effects on *Escherichia coli* O157:H7

Constraints on investigating Escherichia coli *O157:H7*

Experimental procedures with pathogenic bacteria are regulated by the (UK) Health and Safety Executive. Under the relevant guidelines (Advisory Committee on Dangerous Pathogens, 1995) verocytotoxin-positive *E. coli* O157 are now listed in hazard group 3. A key feature of the required working practices relates to the safe containment of the pathogen in specialised containment facilities. The requirements of these regulations, and those governing experimentation with animals described in the Home Office (1986) guide to the Animals (Scientific Procedures) Act (1986), have to be met by investigators, and they impose

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limits and costs on the experimental procedures that can be applied. Moxley & Francis (1998) have reviewed the use of animal models for the study of *E. coli* O157.

In vivo *experiments*

A further disadvantage of animal experimentation is the large variation between animals in the numbers of *E. coli* O157 shed in the faeces following experimental inoculation. Cray *et al*. (1998) found the range of the numbers of *E. coli* O157 shed in faeces to be greater than 10^6 colony-forming units/g between calves. Similarly, the range of the numbers of *E. coli* O157 shed from individual sheep during dietary change was as much as 10^4 colony-forming units/g (Kudva *et al*. 1997). With such variation, it is likely that large numbers of animals would be required to supply meaningful results.

In vitro *experiments*

Batch cultures. Batch cultures were employed to investigate amensalistic interactions between strains of *E. coli* by Chao & Levin (1981) and Tan & Riley (1996). The cultures were transferred at intervals to enable long-term changes in populations to be followed. Such simple experiments could readily be carried out under anaerobic conditions, using media containing particles of different animal feeds in suspension and inoculated with mixed rumen bacteria. A similar approach has been used for studies on effects of plant metabolites (Theodorou *et al*. 1987). As rumen protozoa do not survive well in batch cultures, more complex simulations are needed for the study of their effects.

Fermentor simulations. A number of different devices have been used to simulate the rumen fermentation *in vitro*. The best known is the Rusitec, originally devised by Czerkawski & Breckenridge (1977). In this system the feed is contained in nylon-mesh bags incubated in a liquid phase of artificial saliva inoculated with mixed rumen micro-organisms. The bags are replaced at intervals to allow studies on the digestive process. Other rumen-simulating fermentor systems have been developed (for review, see Cheng & McAllister, 1997). The system of Teather & Sauer (1988) has several advantages for studies on pathogens. In this system the fermentor contents pass out into a container that can be sealed, minimising the spread of bacteria in aerosols, and the equipment can be contained within appropriately-equipped laboratories. Several fermentor vessels containing the same rumen inoculum can be run in series, which probably provides more reproducible conditions than those found within the gut of different animals.

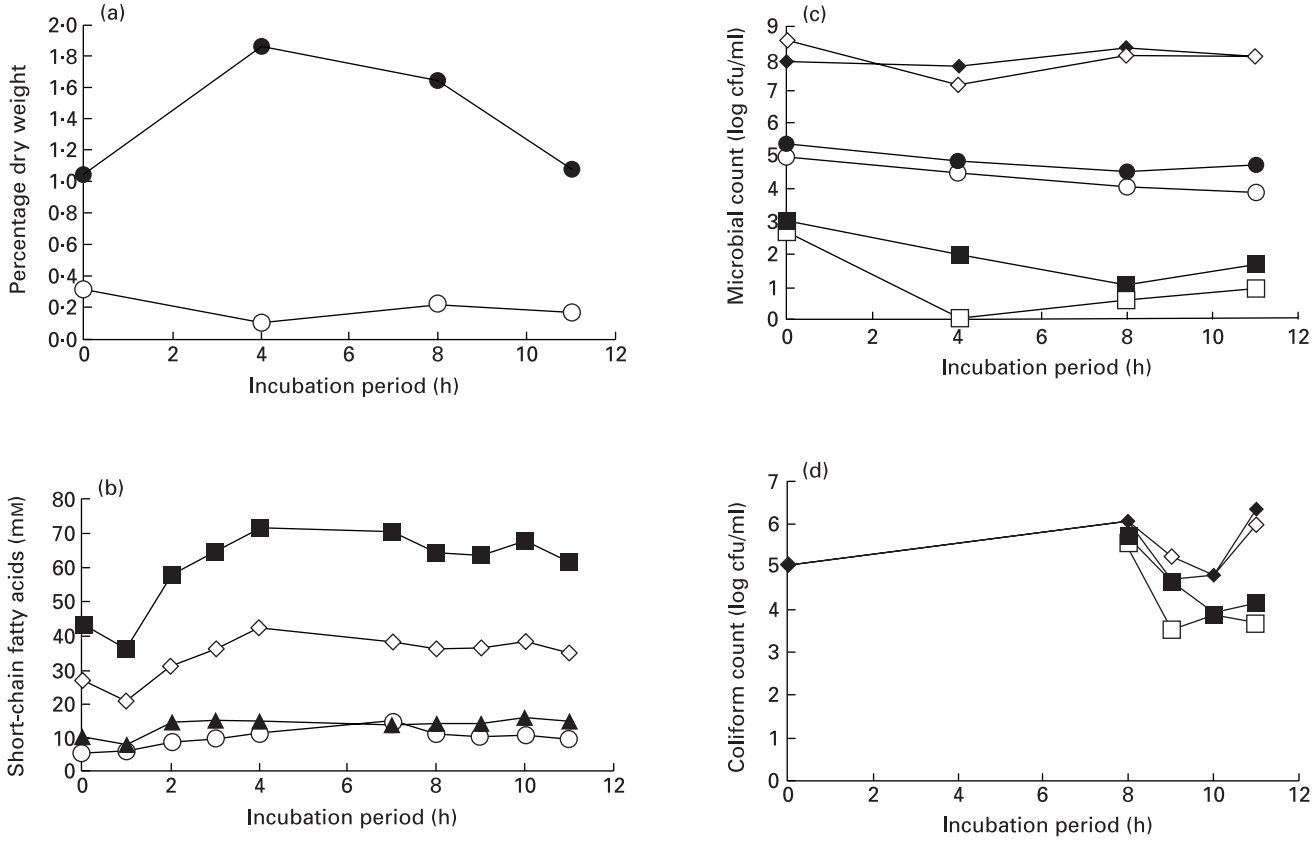

Fig. 2. (a) The percentage dry weight of the rumen-simulating fermentor contents from the vessel (●) and the overflow (○). (b) The concentration of total short-chain fatty acids (■), acetate (◇), propionate (▲) and *n*-butyrate (○) from the rumen-simulating fermentor vessel contents. (c) The number of colony-forming units (cfu) of anaerobic bacteria (vessel, ◆; overflow, ◇) and anaerobic fungi (vessel, ■; overflow, □) and the number of protozoa (vessel, ●; overflow, ○) in the rumen-simulating fermentor. (d) The number of cfu of coliforms (vessel, ◆; overflow, ◇) and a rumen *E. coli* isolate (vessel, ■; overflow, □) in the rumen-simulating fermentor.

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The system of Teather & Sauer (1988) also has a major advantage over some other rumen-simulating fermentors for analysis of dietary effects in which washout may be an important factor; as with the natural rumen, the liquid turnover rate is greater than the solid turnover rate. This characteristic is achieved by the positioning of the overflow in the liquid phase of the fermentation, allowing feed particles to stratify according to density. Using this system, we were able to verify the stratification of the feed both visually and by the greater dry weight content found in the mixed vessel contents compared with that of the overflow (Fig. 2(a)). The SCFA concentrations reached steady-state after 4 d, and thereafter were maintained at levels comparable with those *in vivo* (Fig. 2(b)). Microbial counts were performed on samples from both the vessel and the overflow and were similar. Throughout the experiment the microbial population was maintained in similar numbers (Fig. 2 (c and d), with the exception of the anaerobic fungi, whose numbers initially decreased but subsequently recovered. A rumen *E. coli* isolate introduced into the system reached steady-state within 2 d, and caused a temporary decrease in coliform numbers (Fig. 2 (d)), perhaps due to antagonistic interactions, as the strain introduced was colicinogenic (Duncan *et al*. 1997). The results of this preliminary study suggest this system may be useful for studying the effects of fasting and diet on the survival and washout rate of *E. coli* O157 in the rumen.

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