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Manipulation of the gut microflora: experimental approach in animals

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The microbiologist and the nutritionist sometimes, with different objectives, attempt to manipulate the animals' digestive flora in order to optimize the beneficial effects of the microflora and to diminish its harmful effects, to study its main functions or to determine the role of a given microbial species in the digestive ecosystem or that of microbial associations of varying degrees of complexity.

These manipulations are delicate and the results do not always live up to expectations, often being difficult to interpret, because throughout evolution the different microbial species have formed complex relationships to create great stability in the digestive ecosystems. Each species has a specific, limited function but the species are so numerous that their functions may overlap and if necessary be substituted one for another. The equilibrium of the microbial populations is the result of many events that we are unable to control. Attempts to improve performance in farm animals by optimizing the functions of the digestive flora have largely relied on an empirical approach consisting of experimenting with diet composition or using feed additives. In these animals, the main aim is to influence microbial metabolism or to modify microbial enzyme activity. Analytical study of the functions of the flora has been performed mostly in laboratory animals (rats, mice) but also in a few instances in small ruminants. In this case the animal serves as a biological tool and different experimental animal models, inoculated with a more or less defined flora have been developed.

There have been many studies dealing with manipulation of the flora and several reviews have already been published (Ducluzeau & Raibaud, 1979; Durand, 1982; Ducluzeau, 1988; Van Nevel & Demeyer, 1988). In the present paper we shall describe and discuss a few examples of manipulation of the digestive flora by the two means currently in use: inoculation of animals with selected microbial species alone or in association; modification of the flora or of its activity by abiotic factors.

MANIPULATION OF THE DIGESTIVE FLORA BY MICROBIAL INOCULATION

Simple-stomached animals

There is an abiding idea that it is sufficient to introduce living bacteria into the digestive tract of a simple-stomached animal for them to automatically become established. Inoculation of a bacterial strain is only one of the conditions of its establishment, a necessary one but not sufficient in itself; thereafter the biotic factors (other micro-organisms, host cells) and abiotic factors (diet components and host secretions and excretions) of the ecosystem must also be favourable. These conditions considerably limit the possibilities of manipulating the gastrointestinal flora in man and simple-

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stomached animals by means of inoculation. Ducluzeau *et al.* (1970) studied the fate of ten bacterial strains introduced orally into the digestive tract of adult conventionally-reared mice. Five of the strains were eliminated from the digestive tract at the same rate as the spores of a strict thermophile *Bacillus* used as a transit marker, three strains were eliminated more quickly than the transit marker and two strains persisted longer than the marker at population levels not exceeding 10^5 per g faeces. Nevertheless, after a single inoculation, all ten strains became established at high and stable levels in axenic mice. Thus, depending on the strain, the resident flora exerted a drastic barrier effect (elimination of the target strain by the interplay of the bacteriostatic and bactericidal factors) or permissive barrier effect (allowing the target strain to be maintained as a sub-dominant flora).

Although little is known about the conditions in which a strain becomes established, several experiments have been performed in an attempt to establish strains selected by the experimenter or to modify empirically the equilibrium of the dominant bacterial flora.

Using gnotoxenic mice, Duval-Iflah *et al.* (1981) showed that a human strain of *Escherichia coli* cured of its plasmids had a permissive and sometimes drastic barrier effect on transconjugant from a conjugation between the cured strain and enterobacteria carrying different conjugative plasmids, in particular plasmids resistant to antibiotics. When used in gnotoxenic piglets, this same strain protected the animals against a non-isogenic enteroxinogenic strain of *E. coli* (Duval-Iflah *et al.* 1983). The study of Dubos *et al.* (1984) is another example of a successful manipulation of the intestinal ecosystem. Holoxenic young hares reared indoors were inoculated at birth with a suspension of faeces from gnotoxenic mice harbouring the caecal flora of a healthy hare. At weaning none of these hares had died whereas in a group of non-inoculated hares 35% died as a result of diarrhoea, due to the development of *Clostridium difficile* in their digestive tract. In axenic mice the inoculation of the caecal flora of the healthy hares had a drastic barrier effect against *C. difficile*. However, the manipulation of the intestinal ecosystem in this case also has its limits, since, when the hares were reared outside an enclosed environment, no significant results were obtained.

The healthy carriage of *Salmonella typhimurium* which is the result of a permissive barrier effect could be suppressed by inoculating newborn chicks with a collection of forty-eight bacterial strains (Impey *et al.* 1982). Large-scale trials were carried out in Sweden in which more than two million chicks were treated with non-defined cultures of chicken caecum contents devoid of salmonellae. Healthy carriage was significantly decreased but not entirely eliminated. Berchieri & Barrow (1990) obtained profound protection lasting several weeks by inoculating an avirulent mutant of *S. typhimurium* at birth. Hudault *et al.* (1985) tried unsuccessfully to determine the bacterial strains capable of eradicating *S. typhimurium* in gnotoxenic chicks.

The day of birth is probably the only time at which inoculation of selected strains can be attempted. Beyond this date trying to establish selected bacteria falls foul of the difficulties due to our incomplete understanding of the mechanisms of the establishment of the gastrointestinal flora. Nevertheless, one may question whether the ingestion of live bacterial strains, such as probiotics, by animals whose gastrointestinal flora is already in place is a means of manipulating the flora. There is no published evidence of a probiotic becoming established in the intestinal ecosystem. However, the passage of probiotics through the small intestine has been shown to affect the organ's digestive capacities and

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the immune system of the host. The first demonstration of such an effect was in human subjects with a deficient intestinal lactose system who, when given lactose in conjunction with yoghurt, showed an improvement in lactose digestion (Savaiano *et al.* 1984). Lactic bacteria have been shown to be capable of activating the cells involved in specific and non-specific immunity (Perdignon & Alvarez, 1992). A decrease in faecal β-glucuronidase activity has been observed in subjects given a strain of *Lactobacillus acidophilus* (Goldin & Gorbach, 1984). A similar decrease in this enzyme activity was also observed in the faeces of gnotoxenic rats, harbouring human faecal flora, when given the same strain of *Lactobacillus* (Cole *et al.* 1989). In neither case was any modification of the equilibrium of the populations shown. Probiotics could also be used to modify the genome of certain resident bacteria by transfer *in vivo* of plasmids. Such a transfer has been observed for plasmids encoding resistance to antibiotics. Thus, resistance to fourteen antibiotics of a strain of *Serratia liquefaciens* was transferred to a strain of *E. coli* in the digestive tract of gnotoxenic mice harbouring a population of forty-one bacterial strains of human origin (Duval-Iflah *et al.* 1980). This study also showed the limits of such a manipulation since the transconjugants, i.e. the *E. coli* strains carrying the plasmid from *S. liquefaciens*, were eliminated from the digestive tract in the same way as the donor. The transconjugants were enabled to establish in the digestive tract by adding ampicillin to the drinking water of the mice. Hence, the transconjugants simply replaced the resident *E. coli*, which were sensitive to ampicillin, even after the antibiotic treatment had been discontinued. The use of probiotics as a mechanism for delivering exogenous DNA to resident gastrointestinal bacteria, although potentially useful, is limited by bacterial antagonism. However, it cannot be ruled out that plasmids encoding functions useful to the host may be transmitted from a probiotic to a resident strain without incurring ecological disadvantages for the transconjugant.

Preruminants and ruminants

(a) *Effect of isolation and inoculation of conventionally reared ruminants on rumen flora.* Numerous experiments have been carried out in which newborn preruminants were separated from their dams a few hours after birth or inoculated with rumen contents from adult animals to determine whether their rumen flora would be qualitatively or quantitatively modified. In these experimental conditions the cellulolytic species became established only in small numbers in the rumen flora of calves (Bryant & Small, 1960) isolated immediately after birth in comparison with levels observed in controls not separated from their dams. At no time during the experimental periods did these bacterial species become established in the rumen of lambs isolated after birth (Fonty *et al.* 1987). Inoculating isolated calves with rumen contents of adult cattle (Bryant & Small, 1960) had no significant effect on the kinetics of establishment of the common rumen bacterial species but did accelerate that of ciliate protozoa. The inoculation of a mixture of bacterial species in newborn ruminants isolated from adult animals strengthened their resistance to infection and increased weight gain immediately after birth (Cheng & Costerton, 1986). The inoculation of the rumen fluid of adult animals adapted to a cereal diet can also prevent acidosis at weaning (Allison *et al.* 1964) probably by accelerating the establishment of bacterial species that use lactate. Likewise, the introduction of rumen contents from goats reared in Hawaii into the rumen of goats reared in Australia has been shown to prevent 3-hydroxy-4(1H)-pyridone (3,4-DHP)

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toxicity resulting from the transformation of mimosine, an aromatic amino acid present in the tropical legume *Leucaena leucocephala*, presumably by establishment of the 3,4-DHP-degrading bacterium in the rumen population (Allison *et al.* 1983).

(b) *Effect of the probiotics.* Probiotics seem in certain conditions to stimulate some functions of the rumen flora. At present, yeasts of the species *Saccharomyces cerevisiae* and the fungi *Aspergillus oryzae* have proved to be the most effective. The probiotics seem to stabilize the pH, increase acetate production to the detriment of that of propionate and decrease the production of methane and ammonia (Williams, 1989; Wallace & Newbold, 1992). The total number of cellulolytic bacteria and those using lactate would then increase in the presence of these probiotics. The use of lactate by *Selenomonas ruminantium*, one of the dominant rumen species, seemed to be stimulated by a culture of *S. cerevisiae* because of its high production of L-malic acid (Nisbet & Martin, 1991). The mechanisms of action of probiotics are still incompletely understood and results obtained so far will have to be confirmed since they seem to depend on both diet and the microbial strains used (Jouany *et al.* 1991; Wallace & Newbold, 1992).

(c) *Defaunation and specific refaunation of the rumen.* Rumen microflora are less abundant in faunated animals than in their counterparts devoid of ciliates (Ushida *et al.* 1991; Williams & Coleman, 1992). With diets rich in concentrates, defaunation results in an increase in the number of amylolytic bacteria and of those using simple sugars. Hemicellulolytic and cellulolytic flora, which attach mainly to fibres, are less vulnerable to predation (Prins, 1991). The absence of ciliates also increases bacterial growth rate (g bacterial N/d flowing to duodenum) and bacterial N yield (g bacterial N/kg fermented organic matter). The ciliates ingest zoospores of fungi and mycelium fixed on plant particles (Williams & Coleman, 1992).

The specific reintroduction of ciliates into the rumen of previously defaunated sheep modified the equilibrium of the microbial populations and consequently the fermentation pattern (Jouany *et al.* 1988; Jouany & Ushida, 1990). The introduction of *Entodinium spp.* decreased the total bacterial count and that of the amylolytic and cellulolytic species, whereas species using sugars increased. In contrast, a drop in cellulolytic numbers was no longer observed when *Polyplastron spp.* was inoculated together with *Entodinium spp.*, probably because *Polyplastron* is a predator of *Entodinium*.

(d) *Introduction of genetically engineered bacteria into the digestive tract.* The advances in recombinant DNA techniques and molecular biology have prompted many workers to envisage the introduction into digestive ecosystems of bacterial strains or species genetically engineered for a specific function. Considerable progress has been made recently in the engineering of rumen bacteria, and numerous genes encoding hydrolytic activities (cellulase (*EC* 3.2.1.4), xylanase (*EC* 3.2.1.32), endoglucanase (*EC* 3.2.1.6) etc.) have been cloned in *E. coli* (Hazlewood & Theather, 1988; Gregg & Sharpe, 1991; Teather & Ohmiya, 1991). Most of these genes come from three major cellulolytic species, *Fibrobacter succinogenes, Ruminococcus flavefaciens* and *Ruminococcus albus* but also from the hemicellulolytic species *Butyrivibrio fibrisolvens*. The main objective is to increase the degradation of plant fibres by selecting the most efficient bacterial hydrolytic enzymes and introducing them into one or several dominant species of the rumen. However, numerous problems such as the stability of the gene within the host bacterium, the ease of detection and control of the engineered bacterium, will have to be solved before engineered bacteria can be introduced into digestive ecosystems. It has not yet been proven that they can become firmly established and remain so in sufficiently

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high numbers to compete with the indigenous species. The different (gnotobiotic) animal models could be excellent tools for following the development and fate of new strains produced by genetic engineering.

(e) *Establishment of defined microbial populations in gnotobiotic ruminants*. Germ-free or gnotoxenic lambs fed on milk from birth to a few weeks old have been used to study the development of pathogenic bacteria potentially capable of producing intestinal disturbance and to test the possible role of commensal bacteria (lactobacilli) in controlling the proliferation of pathogens in the digestive tract (Cushnie *et al.* 1979; Mann *et al.* 1980).

Experiments in which gnotobiotic lambs were fed on milk and then on a solid diet and in which the rumen was inoculated with a limited and defined flora designed to carry out normal rumen digestion, showed that most of the predominant rumen bacterial species typical of the adult ruminant were able to colonize the rumen of the gnotoxenic lambs with the exception of the cellulolytic species. Ureolysis, amylolysis and methanogenesis appeared to be relatively easy to establish in the rumen (Cook, 1976; Lysons *et al.* 1976, 1977; Barr *et al.* 1980; Hobson *et al.* 1981). In contrast, cellulolysis is much more difficult to establish. In all experiments performed with gnotoxenic lambs inoculated with limited floras made up entirely of the main species present in the dominant flora of adult ruminants, the cellulolytic species (*Ruminococcus spp.* or *F. succinogenes*) either did not establish or reached only a very low population level (Mann & Stewart, 1974; Lysons *et al.* 1976, 1977; Hobson *et al.* 1981; Stewart *et al.* 1988). To become established in the rumen *F. succinogenes* needs the presence of a complex non-cellulolytic bacterial microflora to satisfy its nutritional requirements for branched-chain volatile fatty acids and vitamins (Fonty *et al.* 1983, 1988*a*). The rumen microflora of lambs aged 24 h, although different from that of the adult (Fonty *et al.* 1987), not only allows the subsequent establishment of cellulolytic bacteria by creating a biotope favourable to their development (Fonty *et al.* 1988*b*, 1989) but also fulfils the essential digestive functions of the ruminant (Fonty *et al.* 1991).

Lambs placed in isolators at 24 h of age, before the natural establishment of the cellulolytic micro-organisms (bacteria, fungi, protozoa) and methanogenic bacteria which occur during the first week of life (Fonty *et al.* 1987), and then inoculated with one or several cellulolytic species have been used to study the effectiveness of *F. succinogenes*, *R. flavefaciens* and *R. albus*, alone or in association, in degrading cellulosic substrates (Fonty *et al.* 1988*b*). *R. flavefaciens* was the most effective in degrading the cellulose of ryegrass. However, its activity was weaker than that of the cellulolytic flora of conventionally reared control lambs. In contrast, *F. succinogenes* was better at degrading the crystalline cellulose of straw. The introduction of a second cellulolytic species into the rumen of these animals resulted in a better degradation of the ryegrass but had less effect on that of straw. The association of the three species was not more effective than *F. succinogenes* alone. Anaerobic fungi can also become established as the only cellulolytic micro-organisms in the rumen of lambs placed in sterile isolators at the age of 24 h and fed on a dehydrated lucerne (*Medicago sativa*)-based diet. The fungal population, as estimated by the number of zoospores, established at a level comparable with that observed in the adult animal. This animal model was used to assess the cellulolytic activity of *Neocallimastix frontalis*, *Piromyces communis* or of an association of several fungal species *in vivo* (Fonty & Joblin, 1991).

The different animal models described previously are also ideal biological tools for the

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study of microbial interactions *in vivo*. In meroxenic lambs, G. Fonty, A. Williams, F. Bonnemoy & Ph. Gouet (unpublished results) observed that the establishment of a fungal flora slightly increased the *in sacco* degradation of the plant cell walls and the activities of most of the microbial glycosidases and polysaccharidases. The increase in these activities was particularly marked in the microbial population attached to plant materials. The interactions between H_2-producing cellulolytic micro-organisms and hydrogenotrophic bacteria can be assessed in lambs isolated at 24 h of life before the natural establishment of methanogen-producing bacteria. The stimulation of bacterial or fungal cellulolysis observed *in vitro* (Bauchop & Mountfort, 1981) has been confirmed, albeit to a lesser extent, *in vivo* (G. Fonty, A. Williams, F. Bonnemoy, B. Morvan, J. Dore & Ph. Gouet, unpublished results).

MANIPULATION OF THE DIGESTIVE FLORA BY ORAL ADMINISTRATION OF ABIOTIC FACTORS

Simple-stomached animals

Another means of manipulating the gastrointestinal flora is to administer various inert substances capable of resisting digestion in the small intestine so that they reach the caecum, or which are able to modify the secretions of the host after their absorption in the small intestine.

(a) *Antibiotics*. Of the different substances used, antibiotics added in small doses to feed have been among the most common, although their administration is now strictly regulated. The mechanisms of action of these 'growth factors' in simple-stomached animals and birds have not been clearly demonstrated. Very few studies of axenic and holoxenic animals have been made comparing those receiving antibiotics with those receiving none in order to determine whether these substances act via the gastrointestinal flora or directly on the digestive capacities of the intestine.

(b) *Dietary fibres*. The role of dietary fibres in the production of different bacterial metabolites in the large intestine (short-chain fatty acids, H_2, CH_4) or in the activity of bacterial enzymes has been well documented, particularly in man and gnotoxenic rats harbouring human faecal flora (Ducluzeau, 1988). The activities of biotransformation enzymes of bacterial origin were compared in the caeca of holoxenic rats and gnotoxenic rats harbouring a complex human flora receiving or not receiving pectin (Mallett *et al.* 1987). In the same host animal, the rat, pectin had different effects: it increased the activity of β-glucosidases, β-glucuronidases and nitrate reductases in holoxenic rats but not in those with human flora. Nitroreductase was not modified in either group. Non-sterilized pectin decreased the absorption coefficient of cholesterol, the plasma cholesterol concentration and the faecal excretion of cholesterol in holoxenic rats but not in axenic rats, which suggest that there were functional modifications of the gastrointestinal flora (Sacquet *et al.* 1985). The effect of dietary carbohydrates that are indigestible or difficult to digest on certain metabolic activities of bacterial origin, such as the epimerization of β-muricholic acid into ω-muricholic acid, in holoxenic rats kept in isolators could be reversed (Andrieux *et al.* 1980): the metabolic activity disappeared once the rats ingested the resistant carbohydrate and reappeared when they received a diet containing digestible starch. In contrast, other metabolic activities, for example those resulting in the formation of hyodeoxycholic acid, were not observed again in the faeces after the change of diet. In birds which have no intestinal lactase (*EC* 3.2.1.108),

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lactose can fulfil the role of a dietary fibre. It increased the depressive effect on the healthy carriage of *Salmonella enteritidis* of an inoculation given at birth consisting of an anaerobic culture prepared from the caecum of unaffected chicken (Corrier *et al.* 1991). The level of the plant-cell-wall-degrading bacterial population depends also on the fibre content of the diet (Boulharouf *et al.* 1990, 1991*a*,*b*).

Studies with gnotoxenic animals have shown that it is possible to modify just the microbial balance or metabolic activity alone. For example, the simple addition of 200 g lactose/l to the drinking water of gnotoxenic rats harbouring a strain of *Bacteroides spp.* and a strain of *Lactobacillus murinus* resulted in the *Lactobacillus* strain eliminating the other. However, in gnotoxenic mice harbouring a strain of *E. coli* and the same *Lactobacillus* strain the lactose had no effect (Ducluzeau *et al.* 1971). In gnotoxenic rats harbouring fourteen microbial strains the only effect of the lactose was to increase the population of only two of the fourteen strains (Raibaud *et al.* 1972).

It has also been shown that bacterial metabolic activity can be modified without causing changes in population levels. When inoculated with a strain of *C. difficile*, axenic mice receiving a semi-synthetic diet rich in carbohydrate stopped producing enterotoxin and produced less cytotoxin (Mahé *et al.* 1987; Dubos-Ramaré & Corthier, 1990). A more complex feed but low in protein had the same effect, but the addition of fish meal to the diet resulted in the death of all the animals by an increased production of the two toxins. In neither of the two experiments was the population level of *C. difficile* significantly modified (Dubos-Ramaré & Corthier, 1990).

(c) *Other factors*. The barrier effect exerted by the digestive microflora can be modified by various other abiotic factors. For example, *Clostridium perfringens* was eliminated by three anaerobic species from the digestive tract of gnotoxenic mice receiving a commercial feed sterilized by irradiation but was maintained when the mice were given the same feed but sterilized in an autoclave (Yurdusev *et al.* 1987).

Ruminants

(a) *Effect of diet*. In ruminants the nature of the feed ingested has a considerable effect on the qualitative composition of the rumen microflora and microfauna and determines population balance. Studies on the effect of diet are far too numerous to be listed here and several reviews deal with the subject (for example, Dehority & Orpin, 1988). Modification of rumen metabolism by the addition of lipids or long-chain fatty acids to feed rations has been widely investigated. Lipid supplementation usually decreases the protozoal population (Henderson *et al.* 1977). Long-chain fatty acids, particularly unsaturated acids, are toxic for many bacterial species, especially cellulolytic (Maczulak *et al.* 1981) and methanogenic (Prins *et al.* 1972) bacteria. The effects vary according to species, strain and acid concentration. Likewise the fermentation variables of the rumen and consequently the balance of the populations, their activity and their interactions are affected by the addition of artificial saliva or buffer ($NaHCO_3$, Na_2CO_3) to feed (Van Nevel & Demeyer, 1988).

(b) *Ionophore antibiotics*. The ionophores, which are seemingly the most active in the rumen, belong to the polyether type and include monensin, lasalocid, salinomycin, abierexin and nigericin. Their activity against bacterial cells depends on the the nature of the cell wall. In general, Gram positive species are sensitive. The rumen bacterial species that are inhibited are those that produce acetate, butyrate, H_2 and formate as

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endproducts of fermentation. Among the resistant species are those that produce succinate and use lactate. The metabolic consequences of changes in the rumen microbial population in presence of ionophores are an increase in propionate production and a decrease in that of CH_4 (Van Nevel & Demeyer, 1977; Chen & Wolin, 1979; Durand, 1982). Lasalocid and, to a lesser extent, monensin, to which lactic bacteria are sensitive, can be used to limit the risks of lactic acidosis. Ionophores also afford protection for dietary amino acids and decrease the production of NH_3. However, among the sensitive species, there are cellulolytic species such as *R. flavefaciens* and *R. albus*, so that with certain diets rumen cellulolytic activity is decreased (Durand, 1982). Microbial protein synthesis is also reduced in the presence of ionophore. However, these two drawbacks can disappear in animals that have been adapted.

(c) *Antibiotics other than ionophores*. Numerous antibiotics have been used in attempts to favourably modify fermentation in the rumen. The positive effects sometimes observed *in vitro* are short-lived *in vivo* because the flora rapidly becomes adapted (Chalupa, 1980). However, three of these antibiotics, avoparcin, thiopeptin and flavomycin, seem to be effective. Avoparcin has effects similar to those observed with ionophore antibiotics. Thiopeptin, by inhibiting the growth of *Streptococcus bovis*, decreases the formation of lactic acid *in vivo*. It could also inhibit the lactic acidosis that is commonly encountered in the rapid transition from a forage diet to a cereal diet. Flavomycin, inhibits methane production and stimulates cellulolysis *in vitro* (Van Nevel & Demeyer, 1988).

(d) *Inhibitors of methane production*. CH_4 production is a process that can be inhibited by numerous chemical compounds such as the halogen analogues of CH_4 (amichloral), sulphites, nitrates, unsaturated long-chain fatty acids and trichloroethylpiralate (Van Nevel & Demeyer, 1988). The inhibition of CH_4 formation is generally accompanied by an increase in the concentration of propionate and butyrate and sometimes in that of lactate.

(e) *Inhibitor of proteolysis or deamination*. The degradation of dietary proteins in the rumen into peptides and acids and then into NH_3 is a process that is unfavourable to the host (Hobson & Wallace, 1982); therefore, the nutritionist tries by technological means to protect the proteins in the rumen but also to control proteolysis and deamination by neutralizing the activities of the micro-organisms. Various chemical compounds known to inhibit proteolysis *in vitro* have also been used *in vivo*. In most cases their mode of action is unknown.

(f) *Other substances as growth factors*. Growth factors (branched-chain volatile fatty acids, phenylpropanoic acid, vitamins) are known to stimulate microbial synthesis and fermentation *in vitro* in certain conditions but their role *in vivo* is not fully understood. Orotic acid, alkylamines and sarsapanin can also modify rumen fermentation but their mechanism of action has yet to be determined (Van Nevel & Demeyer, 1988).

CONCLUSION

The diversity of the rumen microflora, the complexity of the interactions between the different microbial populations and of those between the host and its flora, and our limited understanding of the phenomena make it very difficult to manipulate the digestive flora.

Manipulation of the flora by inoculation of adult animals with bacteria or by

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administration of abiotic factors is still for the moment an empirical process based on hypotheses that for the most part have yet to be borne out by experimental research. The only confirmed method is the inoculation of bacterial strains at birth, the limitations of which have been explained in this survey. Certain feed additives can be used to modify the balance of the microbial populations or to regulate microbial metabolism, or both, but they are difficult to put into widespread use because their effects on several interrelated factors which cannot always be governed (composition of the feed, age of the animal, digestive secretions). In addition the aim of manipulation varies according to the physiological stage reached by the animal. The use of these additives must also comply with the requirements of legislation.

Given the technical difficulties in following the fluctuations of microbial populations it is hard to determine accurately the relative effects of the biotic and abiotic factors on the microflora. It will not be possible to manipulate the intestinal flora in a rigorous way until there has been an exhaustive ecological study of the digestive ecosystem. Work should be continued, therefore, on the description of new species, their physiology and their metabolism and on their metabolic interactions *in vitro* and *in vivo*. This research will involve the development of techniques such as oligonucleotidic probes to study fluctuations of the microbial populations, species and even the strains *in vivo* and nuclear magnetic resonance spectroscopy to follow their metabolic activity.

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