Exploiting Gastrointestinal Microbes for Livestock and Industrial Development - Review -

Birbal Singh*, Tej K. Bhat and Bhupinder Singh
Indian Veterinary Research Institute, Regional Station Palampur-176 061, H.P., India

ABSTRACT : Gastrointestinal tract of ruminants as well as monogastric animals are colonised by a variety of microorganisms including bacteria, fungi and protozoa. Gastrointestinal ecosystem, especially the rumen is emerging as an important source for enrichment and natural selection of microbes adapted to specific conditions. It represents a virtually untapped source of novel products (e.g. enzymes, antibiotics, bacteriocins, detoxificants and aromatic compounds) for industrial and therapeutic applications. Several gastrointestinal bacteria and fungi implicated in detoxification of anti-nutritional factors (ANFs) can be modified and manipulated into promising system for detoxifying feed stuffs and enhancing fibre fermentation both naturally by adaptation or through genetic engineering techniques. Intestinal lactobacilli, bifidobacteria and butyrivibrios are being thoroughly investigated and widely recommended as probiotics. Restriction endonucleases and native plasmids, as stable vectors and efficient DNA delivery systems of ruminal and intestinal bacteria, are increasingly recognised as promising tools for genetic manipulation and development of industrially useful recombinant microbes. Enzymes can improve the nutrient availability from feed stuffs, lower feed costs and reduce release of wastes into the environment. Characterization of genes encoding a variety of commercially important enzymes such as cellulases, xylanases, β-glucanases, pectinases, amylases and phytases will foster the development of more efficacious and viable enzyme supplements and enzyme expression systems for enhancing livestock production. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 4 : 567-586*)

Key Words : Gastrointestinal Microbes, Anti-Nutritional Factors, Detoxification, Enzymes, Industrial Applications, Biotherapeutics

INTRODUCTION

Gastrointestinal tract of animals and the microbes inhabiting it represents a remarkable symbiosis between the microbes and host animal. The first section of ruminant gastrointestinal tract, the rumen, is a fermentation chamber filled with microorganisms that are largely responsible for feed digestion (Gregg, 1995). These microbes (bacteria, fungi and protozoa) are capable of carrying out an enormous range of metabolic activity and their enzymes are generally specific for individual compounds. However, it has been found that prior exposure of the rumen bacteria to dietary phytotoxins induces and increases the rate of subsequent detoxification with the phenomenon "adaptation" being a key factor. The ruminal microbes provide an ecophysiological advantage to the host animal through supply of nutrients (energy from volatile fatty acids, proteins from microbial cells and B-vitamins synthesized by bacteria) from lignified fibrous feeds which are low in protein and available energy. Prior exposure of the ruminants to the feed containing toxins can promote the proliferation of ruminal microbes capable of tolerating or detoxifying some of these compounds (Odenyo et al.,1997). A major challenge for biotechnology in animal nutrition is to improve feed in developing countries (Cunningham, 1990). The GI ecosystem provides a unique niche for ongoing enrichment and natural selection of microbes adapted to specific conditions. Fibrolytic enzymes, phosphatases, pectinases and tannases from GI microbes are enzymes of choice in pulp and paper, textile, detergent, food and beverage and pharmaceutical industries. Fibrolytic enzymes have applications in livestock industry as feed additives and the potential of other enzymes in the prevention of environmental pollution is well recognised (Cheng et al.,1999). Application of molecular biology techniques to rumen microbiology has made it possible to realise some of its potential like cloning of genes from GI microbes in *E. coli*, ecological analysis with non-culturing methods and transmission, colonization and population of a particular species of organism once it is released into the environment. This review is focused on (i) biotransformation of potential anti-nutritional factors present in forages and grasses, (ii) industrially important enzymes and miscellaneous novel products identified from GI microbes and (iii) the role of the microbial products in livestock nutrition and health promotion.

* Address reprint request to Birbal Singh. E-mail: ivripip @nde.vsnl.net.in.

MICROBIAL DEGRADATION OF ANTI-NUTRITIONAL FACTORS

Nutrition and toxicology are closely intertwined. Many forage grasses and plants produce astringic or toxic secondary metabolites such as tannins (in a wide

range of plants), tryptamine alkaloids (in *Phalaris spp.*), oxalates (in numerous grasses and legumes), cyanogenic glycosides (in sorghum and sudan grasses), photosensitising agents such as saponins (in various grasses and legumes) and fluoroacetates (in *Gastrolobium* and *Oxylobium* species) (McEvan 1964; Aplin, 1967; Cheeke et al., 1988). These metabolites are produced by the plants as defence against predation by herbivores. Exposure of livestock to these compounds arises from weed infestation of pastures, contamination of feeds and inclusion of plants in feed rations (Gregg, 1995). In fact toxin production may be considered a successful evolutionary strategy for adaptation to predation or a form of chemical warfare practiced by a range of species as varied as insects, frogs and plants (Rosenthal and Janzen, 1979). Upon ingestion by animals the toxic compounds can interfere with vital functions of gastrointestinal tract (such as digestion, absorption and excretion) or systemic level resulting in significant losses due to animal mortality, morbidity and decreased growth performance. The toxins may reduce animal productivity by reducing intake of feed, palatability, enzyme activity of gut flora, ruminal fermentation rates or by inducing toxicosis (Kumar and Singh, 1984; Bae et al., 1993). Alternatively the exposure to phytotoxins may induce microbial synthesis of enzymes which detoxify them (Rosenthal and Janzen, 1979; Majak et al., 1982). Pregastric microbial detoxification of plant toxins in ruminants renders them more flexible in diet choice compared to non-ruminant herbivores. The protection of ruminants from plant poisons by altering ruminal microbial population has been demonstrated by Jones and Megarrity (1986). The resistance to the feed toxins was attributed to a phenomenon known as 'Adaptation' which involves different biochemical, growth and molecular mechanisms or processes (Clark, 1984; Van der Meer et al., 1992). The adaptive response of gastrointestinal microbes to a plant toxin may also involve the induction of enzyme(s) involved in detoxification process. In many cases degradative pathways for a toxin involve a consortium or group of microorganisms as the enzymes needed may not be present in one organism. Several distinct strains of a species may be present in the rumen though a single species is capable of degrading a particular toxin (Allison et al, 1992). Tannins, mimosine, saponins, phytoestrogens, oxalates, fluoroacetates, nitrotoxins and pyrrolizidine alkaloides etc. are some of the most widely encountered and studied secondary plant metabolites or phytotoxins with respect to livestock nutrition. Detoxification of these compounds is mainly a characteristic of ruminants.

Tannins

Tannins are naturally occurring water soluble polyphenols of varying molecular weight and are the most abundant natural phenolic compounds with their ability to precipitate proteins from solutions (Spencer et al., 1988). Two forms of tannins namely condensed tannins (proanthocyanadins) and hydrolysable tannins (gallotannins, ellagitannins and teragallotannins) are commonly found in a large array of higher plant species of both herbaceous and woody types (pteridophytes and spermatophytes). Tannins can accumulate in large amounts in particular organs or tissues such as bark, root, wood, leaves and fruits (Sanderson et al., 1975; Hoff and Singleton, 1977). Various vegetables, fruits, tea and wines also contain variable quantities of tannins (Deshpande et al., 1984; Salunkhe et al., 1989).

When food tannins are ingested initial contact is with the gastrointestinal tract which is the most active metabolic site in body (Chung, 1996). Adverse effects of tannins include inhibition of growth of many gastrointestinal microbes through various mechanisms. Gupta et al. (1977) have correlated the tannins directly with reduction in body weight and changes in intestine, liver, spleen and kidney of ruminants. Tannins may bind to proteins and may form complexes with digestive enzymes (Kumar and Singh, 1984), may cause reduction in milk yield and production of tainted eggs in poultry (Blair and Richart, 1984; Kozlowska et al., 1990). Condensed tannins (CTs) reduce digestibility of protein and carbohydrates in the rumen and may form tannin-protein complexes that are stable in the pH range of 3.5 to 7.0 (Mangan, 1988). They thus reduce the digestibility of forage by protecting them from ruminal microbial fermentation. CTs may also form indigestible complexes with cell wall carbohydrates, including cellulose and hemicellulose (Reed, 1995). Some tannins are recalcitrant to biodegradation and inhibit the growth of some ruminal bacteria (Field and Lettinga, 1987, 1992). Bae et al. (1993) showed that CTs from birdsfoot trefoil (*Lotus corniculatus*) were inhibitory towards endoglucanase activity of *Fibrobacter succinogenes* S85 in the rumen. CTs of sainfoin (*Onobrychus viciifolia*) bind to the cell coat polymers and inhibit the associated proteolytic activty in ruminal bacteria, *Butyrivibrio fibrisolvens* and *Streptococcus bovis* by 48% and 52% respectively (Jones et al., 1994). Very limited information is available on inhibitory effects of tannins on human intestinal bacteria. Chung et al. (1998) found tannic acid inhibitory towards human gastrointestinal microflora namely *Bacteroides fragilis* ATCC25285, *Clostridium clostridiforme* ATCC25537, *C. perferengenes* ATCC 13124, *C. paraputrificum* ATCC 25780, *Escherichia coli* ATCC25922, *Enterobactor cloacae* ATCC 13047, *Salmonella typhimurium* TA99 and *S. typhimurium* YG 1041. *Lactobacillus*

acidophilus and *Bifidobacterium infestans* were not inhibited by tannic acid.

Despite the antimicrobial properties many fungi, bacteria and yeasts are quite resistant to tannins and are able to grow and flourish on them (Deschamps, 1989). Some molds develop easily on tannin rich woods or on surface of liquids of tannery pits and tannery wastes (Rajakumar and Nandy, 1983). A number of gastrointestinal microorganisms that degrade tannin-protein complex have been reported from adapted domesticated and feral animals. These microorganisms play an important role in deriving dietary proteins from tannin-rich foliage as demonstrated in koalas (Osawa, 1992). *Lonepinella koalarum*. gen. nov., a tannin-protein complex degrading bacterium representing as much as 60% of the fecal flora has been isolated from koalas, which feed on tannin rich *Eucalyptus spp.* (Osawa et al., 1995). The tannin-protein complex degrading GI microbes play important role in deriving dietary proteins from tannin rich foliage as demonstrated in koalas (Osawa, 1992). There are numerous ruminal microorganisms capable of degrading hydrolysable tannins (Nelson et al., 1995; Skene and Brooker, 1995; Bhat et al., 1998). Tannin degrading *Lactobacillus spp.* from human intestine and fermented foods have been reported recently (Osawa et al., 2000). Table 1 shows various gastrointestinal microflora involved in biotransformation of potential ANFs.

Toxic non-protein amino acids

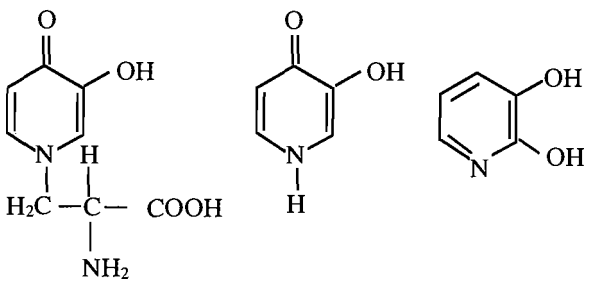
Ruminant as well as non-ruminant livestock suffer toxicity caused by non-protein amino acids present in the seeds and leaves of some agronomically important legumes. *Leucaena leucocephala*, is a tropical, rapidly growing, drought resistant and high yielding forage legume. It is a rich source of proteins, β-carotenes and minerals to animals (Akbar and Gupta, 1985). Its

Table 1. Gastrointestinal microbes involved in detoxification of antinutritional plant secondary metabolites

Organisms	Host/habitat	Substrate	References
Aspergillus niger	Bovine intestine	Tannic acid	Bhat et al., 1996
Butyrivibrio fibrisolvens (Genetically modified)	Bovine rumen	Fluoroacetate	Gregg et al., 1994, 1998
Clostridia	Rumen	nitrotoxins	Angermaier and Simon, 1983
Clostridium sp.	Sheep rumen	mimosine and 3, 4 DHP	Dominguezz-Bello and Stewart, 1991.
Cl. thermoaceticum	Horse manure	Oxalates	Daniel and Drake, 1993
Denitrobacterium detoxificans gen. nov., sp. nov	Bovine rumen	Nitrotoxins	Anderson et al., 2000
Enterobacterium agglomerans	Koala caecum	Tannins	Osawa, 1992
Enterococcus faecalis	Human faeces	Oxalates	Hokama et al., 2000
Eubacterium oxidoreducens sp. nov.	Koala intestine	Tannins	Krumholz and Bryant, 1986
Eubacterium ramulus	Human faeces	Flavonoids	Schneider and Blaut, 2000
Lactobacillus spp.	Human intestine	Tannins	Osawa et al., 2000
Lonepinella koalarum	Koala caecum	Tannins	Osawa et al., 1995
Mitsuokella multiacidus gen. nov., sp. nov.	Bovine rumen	Phytate	Yanke et al., 1998
Oxalobacter formigenes	Rumen and intestine	Oxalates	Allison et al., 1985
Peptococcus heliotrine-reducens sp. nov.	Sheep rumen	Pyrrolizidine alkaloids	Russel and Smith, 1968; Lenigan, 1976
Prevotella ruminicola	Bovine and sheep rumen	Phytate	Yanke et al., 1998
Selenomonas spp.	Antelope, bovine and sheep rumen	Tannins	Odenyo and Osuji, 1998
Selenomonas ruminantium	Bovine rumen	Phytate	Yanke et al., 1998, 1999
Selenomonas ruminantium	Bovine rumen	Tannins	Skene and Brooker, 1995
Sterptococcus caprinus sp. nov.	Feral goat rumen	Tannins	Brooker et al., 1994
Streptococcus gallolyticus	Bovine rumen	Gallic acid	Osawa et al., 1995
Streptococus sp.	Koalas caecum	Tannins	Osawa and Sly, 1992
Synergistes jonesii	Goat rumen	Miosine	Allison et al., 1992
Treponema sp.	Bovine and ovine rumen	Phytate	Yanke et al., 1998

use in human diet (Van Veen, 1973) and as forage (NRC,1984) is restricted due to the presence of a free amino acid, the mimosine (β-N-3-hydroxy-4-oxo-pyridyl-α-amino-propionic acid) which undergoes autolysis by leaf enzymes during ingestion and mastication (Hegarty et al., 1976; Lowry et al., 1983) or is hydrolyzed by rumen bacteria to a goitrogenic toxin pyridinediol, 3-hydroxy-4-(1H)-pyridone or 3,4-DHP (figure 1). Biochemical mode of action of mimosine is not very clear. Hegarty (1978) suggested that it may act as amino acid antagonist and may complex with metals like zinc and copper. Under physiological conditions mimosine binds to zinc and copper more strongly compared to most amino acids (Stunzi et al., 1979). Mimosine acts as cell-specific antagonist of folate metabolism (Oppenheim et al., 2000). Treatment of mammalian cells with mimosine have been found generating DNA breaks (Mikhailov et al., 2000). Level of leucaena meal at about 5-10% of diet for swine, poultry and rabbits generally results in poor animal performance. Animals exhibit symptoms of alopecia, eye cataracts and reproductive problems. Ruminants grazing on leucaena may show various symptoms such as poor growth, alopecia, swollen and raw coronets above the hooves, lameness, mouth and oesophageal lesions, depressed serum thyroxine levels and goiter.

Some ruminants such as Hawaii and Indonesian cattle and goats can consume leucaena with impunity and the tolerance was correlated to the presence or absence of ruminal microbes capable of degrading 3,4-DHP (Jones, 1981). It was demonstrated that transfer of ruminal fluid from animals in Hawaii and Indonesia to mimosine sensitive Australian cattle resulted in complete elimination of mimosine toxicity (Jones and Lowry, 1984; Meggarity and Jones, 1986). *Synergistes jonesii* 78-1, a ruminal bacterial species capable of degrading the toxic pyridinediols has been isolated from the adapted Hawaiian goats (Allison et al., 1992). This unique anaerobic ruminal bacterium is excreted in faeces and is spread among animals via dust in cattle yards and in areas of animal



Mimosine 3, 4-DHP 2, 3-DHP

Figure 1. Ruminal metabolism of mimosine

concentrations (Jones, 1994). Ruminal *Clostridium spp.* have also been found capable of degrading mimosine and 3,4-DHP (Dominguezz-Bello and Stewart, 1991). Presumably these organisms have evolved in the areas where leucaena is native.

Many other forage trees and shrubs contain toxic amino acids which induce toxicity in susceptible animals. Canavanine found in jack bean (*Canavaliaeusi formis*) seeds are toxic to non-ruminants whereas ruminants are not affected as canavanine is metabolized by sheep rumen bacteria (Dominguez-Bellow and Stewart, 1990). *Indigofera spictata* is a potential tropical leguminous forage crop having soil improving properties. It contains an unusual amino acid, indospicine which inhibits arginine incorporation in tissue proteins causing hepatic necrosis in cattle and sheep consuming this forage. Horses consuming *Indigofera* suffer severely from a neurological disorder called 'Birdville horse disease' (Morton, 1989). The flat pea (*Lathyrus sylvestris*), a perennial legume forage contains toxic amino acids including diaminobutyric acid (DABA) and other lathyrogens such as oxalyl diaminopropionic acid (ODAP). While flatpea hay is toxic to sheep (Rowe et al., 1993), other animals also exhibit variable susceptibility to flat pea. Rasmussen et al. (1993) have found evidence for detoxification of toxic amino acid by ruminal microbes and ruminal adaptation to flatpea in the diet.

Saponins

Saponins are glycosidic compounds composed of steroids (C_{27}) (Mahato et al., 1982) or triterpenoid (C_{30}) (Kulshreshtha et al., 1992) sapogenin nucleus with one or more carbohydrate branch. Various plants of economic importance like leguminous forage and a variety of human feed stuffs contain various saponins (Oakenfull and Sidhu, 1989; Price et al., 1987). Saponins are often implicated in rumen bloat (Mathison et al., 1999) and may affect rumen fermentation. Lu and Jorgenson (1987) and Klita et al. (1996) reported reduction in ruminal protozoal population and reduced amount of microbial protein flowing to small intestine and decreased apparent digestibility of diet when alfalfa saponins were administered to sheep. They attributed antiprotozoal effect to the reaction of saponins with cholesterol in the protozoal cell membrane, causing the lysis of protozoa. Steroidal saponins from *Yucca schidigera* inhibited cellulolytic ruminal bacteria and fungi, but their effects towards amylolytic bacteria were species dependent and similar to the effects of ionophores (Wang et al., 2000). However, bacteria were tolerant to saponins as their cell wall does not contain cholesterol. Saponins may influence nutrient digestion and absorption by interacting with cell membranes, causing permeability changes or the loss of activity of

membrane bound enzymes (Oleszek et al., 1994). Southon et al. (1988) found inhibition of iron absorption by dietary saponins. Saponins act as inhibitors of smooth muscles activity and have profound effects on animals. Alfalfa (*Medicago sativa*) saponins can completely inhibit rumen and reticular contractions (Klita et al., 1996). Studies with ruminants suggest that some ruminal microflora are capable of metabolizing saponins (Gutierrez et al., 1958; Gutierrez and Davis, 1962; Lu and Jorgenson, 1987). Mathison et al. (1999) confirmed extensive metabolism of saponins in sheep. However, no information on actual digestibility or whole animal digestibility of saponins is available (Mathison et al., 1999). Moreover, information is lacking on the involvement of a particular ruminal or intestinal microorganism in metabolism of saponins and the end products formed.

Phytoestrogens (Isoflavones and coumestans)

Pasture species such as subterranean clover (*Trifolium subterran*) widely sown as sheep pasture, red clover (*Trifolium pretense*) and alfalfa (*Medicago sativa*) contain various estrogenic compounds in the form of water soluble glycosides. Most of the leguminous plants have been reported to contain phytoestrogens and their content varies depending upon a number of factors Alfalfa contains little phytoestrogens unless it is suffering from foliar disease. Plants with genetic resistance to attack by aphids and fungal pathogens suffer less damage and therefore are less estrogenic (Loper et al., 1967). *Medicago spp.* can produce coumestrol or 4-methoxy coumestrol if infected with fungal pathogens. Subterranean clover may contain as much as 5% dry weight of estrogenic isoflavones including genistein, formononetin and biochanin A (figure 2). Soybean (*Glycine sp.*) products may contain up to 0.25% isoflavones mainly genistein, daidzein, glycetin and coumestrol. Various leguminous and non-leguminous forages are also found to have various amounts of these compounds (Kaur et al., 1999). Kaur and co-workers found high content of daidzein, genistein, coumestrol and biochanin A in sprouting gram.

Phytoestrogens have structure similar to that of endogenous estrogen and they have a similar interaction with the nuclear estrogen receptors in different tissues (Rosenblum et al., 1993; Whitten et al., 1995). The most important estrogenic compounds in legumes are isoflavones and coumestans. Estrogenic compounds of clovers are usually isoflavones while alfalfa contain coumestans which apparently resemble estradiol. Phytoestrogens mimic the action of estradiol though their actions are not identical. Phytoestrogens are also found to have antiestrogenic activities. Folman and Pope (1966) indicated that phytoestrogens compete with endogenous steroids so that the balance between estrogen and estrogenic activity is determined by the ratio of phytoestrogens to estrogens. This may explain why estrogenic effects may predominate in sheep, but antiestrogenic effects are mainly reported in humans, in which circulatory concentrations of estradiol estrogens are relatively high (Adlercreutz et al., 1991). Laboratory animals fed soy based diets exhibited estrogenic effects (Sharma et al., 1992). Phytoestrogens have little effect on male ruminants. The coumestans are more estrogenic compared to isoflavones as the later are readily hydrolyzed by plant enzymes or ruminal microbes (Beck, 1964). The metabolism of isoflavones in sheep has been extensively reported (Cox and Davies, 1988; Price and Fenwick, 1985). However, ruminal metabolism of isoflavones in other species has been suggested to be qualitatively similar to that in sheep (Adams, 1989). Ruminal metabolism of isoflavones may result in both the activation of compounds that are intrinsically estrogenic and the bioactivation of others. Biochanin A is demethylated to genistein and non-estrogenic p-ethyl phenol and organic acid (Batterham et al., 1965; Braden et al., 1967). There is conclusive evidence that the gut flora play a crucial role in the metabolism of phytoestrogens with the parent compounds and bacterial metabolites having different biological activities (Rowland et al., 1999).

Oxalates

Plants of *Oxalidaceae* and *Chenopodiaceae* familes and various tropical grasses such as bufflegrass (*Cenchrus ciliaris*), pangolagrass (*Digitaria decumbens*), setaria (*Setaria sphacelata*) and kikuyu grass (*Pennisetum clandestenum*) contain soluble oxalates (oxalic acid, potassium oxalate, calcium oxalate and sodium oxalate) in sufficient concentration to induce calcium deficiency in grazing animals (Cheeke, 1998). Oxalates react with calcium to produce insoluble calcium oxalate, reducing its absorption (figure 3). This leads to disturbance in the absorbed Ca:P ratio, resulting in mobilization of bone minerals to alleviate the hypocalcaemia. Prolonged mobilization of bone minerals in horses may induce or result in nutritional secondary hyperparathyroidism (NSHP) or osteodystrophy fibrosa (Blaney et al., 1982; McKenzie et al., 1981). Interstitial concentrations of oxalate ions damage capillaries in lungs and tubular epithelial cells of kidneys. Characteristic crystals of calcium oxalate precipitate in lamina of nephrons (Allison and Reddy, 1984). Oxalate-induced death of renal epithelial cells exhibits several features of characteristic of apoptotic cell death, including increased production of ceramide, abundance of apoptotic bodies, and marked sensitivity to the level of expression of apoptotic gene bcl-2 (Miller et al., 2000).

Cattle and sheep are less affected because of

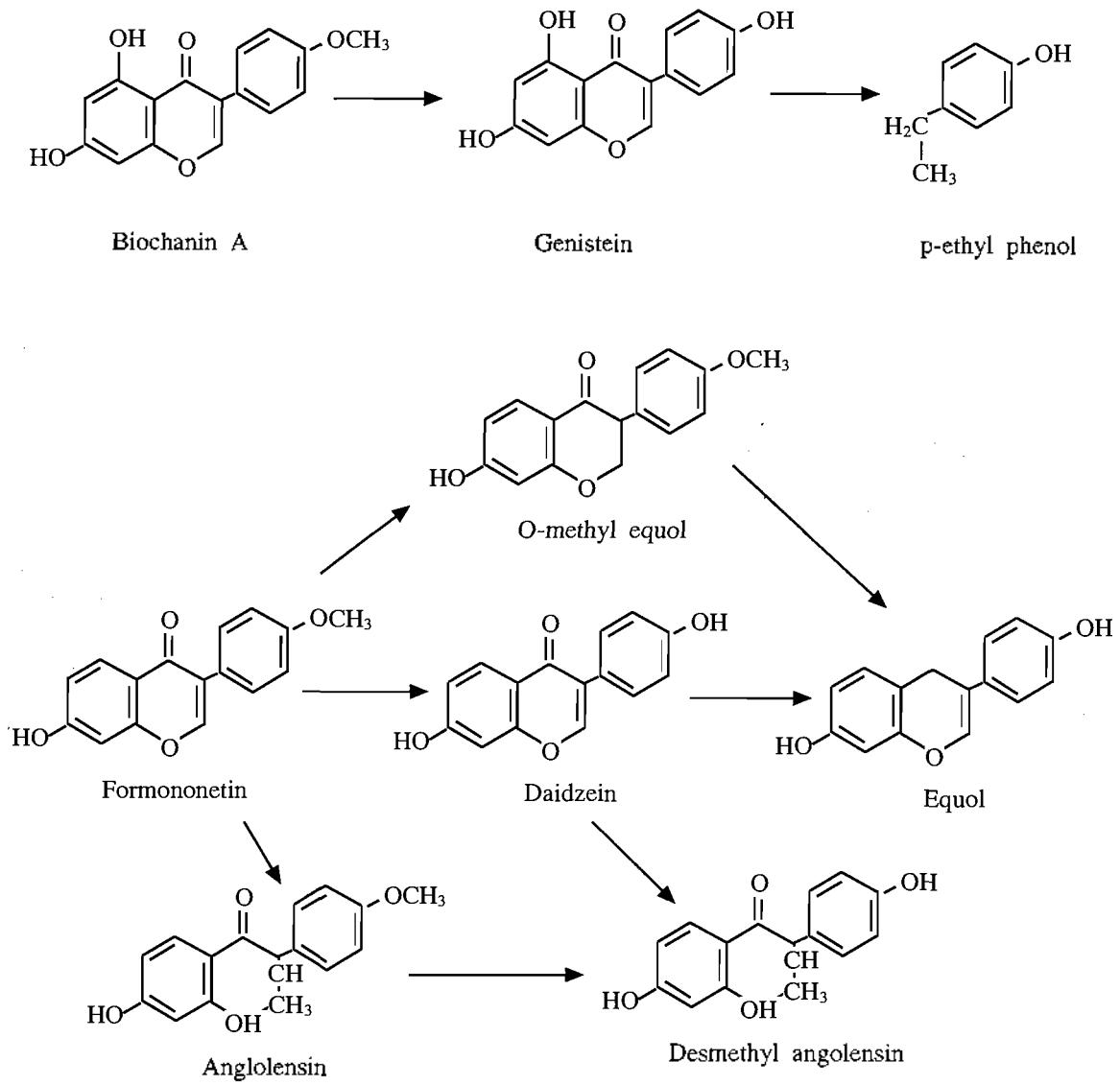


Figure 2. Ruminal metaolism of isoflavones (Biochanin A, genistein, and daidzein). The major metabolic pathway of formononetin is via daidzein to equol, rather than via o-methyl equol and to desmethyl anglonensin.

degradation of dietary oxalates in rumen. Ruminants adapted to diet containing oxalates are resistant to high levels of oxalates whereas the non-adapted animals suffer acute oxalate toxicity (Allison and Reddy, 1984). Acquired tolerance to oxalate toxicity is attributed to the increased population of organisms that metabolise oxalate in the rumen (Dawson et al., 1980). Bacteria that use oxalate as a carbon and energy source have been isolated from many environments including the gastrointestinal tract of animals. The only organism capable of degrading dietary oxalate has been isolated from the rumen of sheep is *Oxalobacter formigenes* (Allison et al., 1985; Dawson et al., 1980). This bacterium is of great economic importance because its population in the rumen of sheep has been found to increase dramatically when sheep are slowly fed increasing levels of oxalate (Allison et al., 1977; Dawson et al., 1980). *O. formigenes* requires oxalate only as source of energy for growth and catabolises it to CO_2 and formate in approximately 1:1 ratio (Baetz and Allison, 1989). This organism also appears to be present in caecal and rectal contents of simple-stomached animals (Allison et al., 1981). *Clostridium thermoaceticum* originally isolated from horse manure (Daniel and Drake, 1993), has also been implicated in metabolism of oxalate and glyoxylate to form acetate.

Fluoroaceate

A somewhat enigmatic example of the role of selection in microbial detoxification is provided by fluoroacetate. Fluoroacetate occurs in leguminous shrubby browse plants including various species of *Acacia*, *Gastrolobium* and *Oxylobium* and renders them

poisonous by accumulating within leaves, stems and seeds (McEwan, 1964; Aplin, 1967). Fluoroacetate is a very potent toxin inhibiting tricarboxylic acid (TCA) cycle. Fluoroacetate can substitute for acetate and be converted into fluorocitrate in the first step of TCA cycle. Fluorocitrate acts as competitive inhibitor of aconitate hydratase and blocks TCA at the citrate stage. This results in the accumulation of citrate in the tissues, energy deprivation of the cells and their death (Twigg and King, 1991). Fluoroacetate may also impair citrate transport through mitochondrial membrane. The domestic animals exhibits variable sensitivity to fluoroacetate poisoning. While some of the native animals in Western Australia are able to consume plant materials of this type without ill effects (Oliver et al., 1979; McIlory, 1984; Twig et al., 1988), the others suffer fatal fluoroacetate poisoning (Seawright, 1982; McCosker, 1989). Canines are highly sensitive, while birds are more tolerant than mammals. The emu is especially resistant to fluoroacetate poisoning (Twigg et al., 1988). Because of its economic importance in livestock production and lack of naturally occuring gastrointestinal microflora that could degrade fluoroacetate, ruminal bacteria were genetically engineered for this purpose (Gregg et al., 1994). The genes encoding fluoroacetate-dehalogenase (H1), were isolated from *Moraxella sp.* strain B and the plasmid containing a gene for hydrolytic dehalogenation of fluoroacetate introduced into *Butyrivibrio fibrisolvens* OB156. The recombinant plasmid was found stably maintained in this strain *in vitro*, and the bacterium was maintained in measurable numbers following the inoculation into the rumen of sheep. Markedly reduced toxicological symptoms were exhibited by sheep inoculated with these recombinant strains (Gregg et al., 1998).

Aliphatic nitro compounds

Various aliphatic compounds containing nitrate moiety (nitrotoxins), e.g. 3-nitro-1-propionic acid (NPA) and 3-nitro-1-propanol (NPOH) found in *Astragalus* spp. (Fabaceae) are responsible for poisoning of thousands of sheep and cattle every year (Williams and Barneby, 1977a,b; Williams et al., 1979). More than 450 plant species are found to contain either NPA or NPOH (Williams and Barneby, 1977a,b). Several plants of agricultural importance including the genera *Coronilla*, *Indigofera* and *Lotus* (Majak and Pass,1989), contain varying amounts of NPA. The nitro componds in *Astragalus spp.* are of highest significance to livestock. Nitro-containing glucosides may cause 'cocoism' or may accumulate toxic levels of selenium. Both acute and chronic toxicity (often refered to as Craker heels) occur in livestock that consume *Astragalus spp.* containing glycosides. When absorbed into the blood stream, NPA inhibits succinate dehydrogenase and while not toxic per se, NPOH is converted to NPA by hepatic alcohol dehydrogenase (Majak and Pass, 1989). The nitro compound toxicity is associated with development of methaemoglobinemia which occurs when haemoglobin is oxidized by nitrite released from the absorbed nitrotoxins. Methaemoglobin is incapable of carrying oxygen. NPA and NPOH also appear to be inhibitory to some of ruminal microbes at concentrations 4.2 mM occurring in the rumen of animals grazing nitrotoxin enriched pasture plants (Anderson et al., 1993).

Some ruminants can tolerate higher concentrations of nitrotoxins whereas monogastrics are susceptible to nitrotoxin poisoning. Nitrotoxin degrading ruminal microbes rapidly adapt to nitro compounds in the diet with enhanced detoxification rates (Majak et al., 1982; Anderson et al., 1993). However, the degree of poisoning depends on the level of intake of toxins and their rate of metabolism in the rumen. Observations clearly demonstrate that rumen environment can be manipulated to enhance microbial detoxification of NPOH (Majak and Cheng, 1983; Majak et al., 1982). Since NPA is degraded more rapidly than NPOH in the rumen (Gustine et al., 1977; Anderson et al., 1993), the former is less toxic to the animal. The primary pathway of metabolism of NPA and NPOH by ruminal microorganism from cattle and sheep was the reduction of nitro group in the toxins and the conversion to β-alanine and 3-amino-1-propanol, respectively (Anderson et al., 1993). In view of the economic importance of forage containing nitrotoxins, practical strategies are being developed to enhance their detoxification by ruminal microorganisms. By supplementing the microbial suspensions with ferrous and sulfide ions, active microbial preparations against these toxins were obtained (Anderson et al., 1993). Their investigations on the microbial metabolism of NPOH and NPA have shown that ferrous and sulfide ions stimulate, while CO reduces the rate of reduction

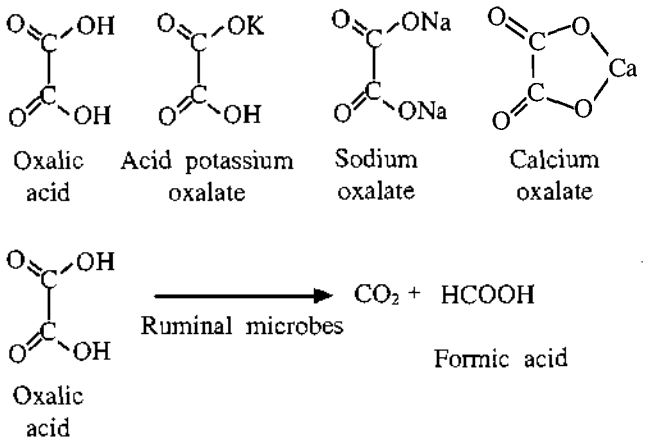


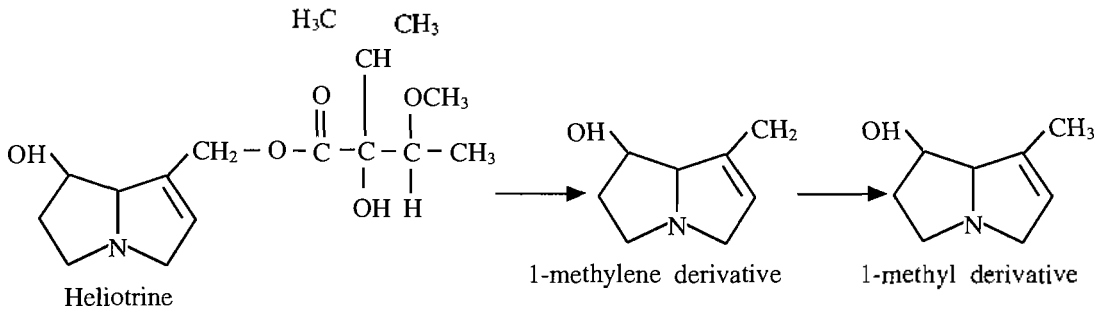
Figure 3. Plant oxalates and their ruminal metabolism

of NPOH but not NPA. This effect of ferrous and sulfide ions on NPOH metabolism was further enhanced when the incubations were done under H_2, suggesting that NPOH-degrading bacteria like Clostridia, may use a non specific hydrogenase-ferredoxin system to reduce NPOH (Angermaier and Simon, 1983). A bacterium capable of metabolizing the naturally occurring nitrotoxins NPOH and NPA was isolated from a ruminal population enriched for enhanced nitrotoxin metabolism (Anderson et al., 1996). Rates of ruminal metabolism of both the nitrotoxins are enhanced when animals are fed sublethal amounts of milkvetch forage (*A. miser* var. *serotinus*) or a non-toxic analogue (Majak, 1992).

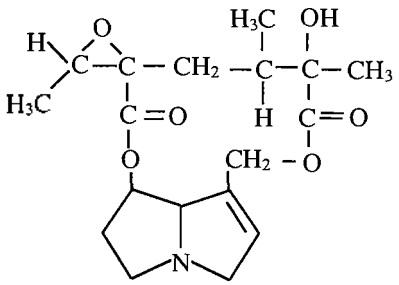
Pyrrolizidine alkaloids

The plants belonging to Families *Boraginaceae*, *Compositae* and *Leguminaceae* contain hepatotoxic pyrrolizidine alkaloids (PAs). *Senecio*, *Crotolaria*, *Heliotropium*, *Amsinckia* and *Echium* are the ubiquitously distributed genera which contain PAs as principal toxins. *S. brasliensis* is most often implicated in PAs poisoning (Lombardo de Barros et al., 1992). Most of the PAs are esters of bases retronecine and heliotridine and are hepatotoxic in nature (figure 4). Hundreds of the PAs have been identified and their structures determined (Mattocks, 1986). The toxic effects of PAs are due to their bioactivation in liver to chemically reactive metabolites called pyrroles or dihydropyrrolizidine (DHP) derivatives (Bull et al., 1968) or trans-4-hydroxy-2-hexenal by the liver (Segall et al., 1985). Pyrroles are highly reactive alkylating agents that bind with vital cellular components including DNA.

The domestic livestock species exhibit variable susceptibilities to PA toxicity. Grazing animals like cattle and horses are equally susceptible to PA poisoning whereas browsing livestock like sheep and goat appear quite resistant (Sharrow and Mosher, 1982; Cheeke, 1988). Although it is not entirely clear what specific host factors confer resistance and/or susceptibility, evidence suggests that preliminary protective mechanism *in vivo* is localized in the anaerobic ruminal microflora (Craig et al., 1985). Rabbits and guinea pigs are resistant to effects of dietary PAs (Cheeke, 1988). A ruminal bacterium *Peptococcus heliotrinereducens*, isolated from sheep rumen metabolizes PA, heliotrine to non-toxic α -hydroxy-1-α-methylene 8-α-pyrrolizidine and 1-methyl pyrrolizidine derivatives (Russle and Smith, 1968; Lanigan, 1976). Metabolism of *Senecio* PAs in sheep, goat and bovine rumen has been reported (Wachenheim et al., 1992) with the suggestion that the tolerance to PAs toxicity could be due to ruminal detoxification of these compounds (Craig et al., 1992). Inhibition of the methanogens in the rumen increases the rate of metabolism of heliotrine to non-toxic metabolites (Lenigan, 1972). The ruminal bacteria



(a)



(b)

Figure 4. (a) Ruminal metabolism of pyrorlizidine alkaloid heliotrine, and (b) structure of alkaloid jacobine

responsible for detoxification of PAs have not been identified. However, biotransformation of tansy ragwort (*Senecio jacobaea*) PAs, in the sheep provides evidence for the ability of sheep to detoxify PAs through ruminal metabolim.

GASTROINTESTINAL MICROBES AS SOURCE OF ENZYMES, NOVEL PRODUCTS AND AS PROBIOTICS

The history of industrial enzyme application is believed to begin in the late 19th century with the first patenting of an industrial enzyme 'Taka-Diastase' (derived from *Aspergillus oryzae*) by Dr. Jokiche Takamine, a Japanese immigrant. The global enzyme market was estimated in 1994 to be worth US$ 400 million annually (Hodgson, 1994). This market was more recently valued at US$ 1.4 billion with an increase of 4 to 5 percent annually as suggested by Cowan (1996). The rumen microbial ecosystem represents a wealth of microbial resource- cellular, enzymatic and genetic- with tremendous potential of exploitation in industrial applications (Cheng, 1999). It also includes a wide array of potentially useful organisms probably awaiting discovery for a particular purpose. Cloning of the genes encoding polysaccharidases in *E. coli* have accelerated characterization of their structure and origin by sequencing and of their products by purification (Kobayashi and Onodera, 1999). Some of commercially important enzymes obtained from gastrointestinal microbes and their applications to livestock nutrition and prevention of environmental pollution are emphasised here.

Fibrolytic enzymes (cellulases, hemicellulases, endoglucanases and pectinases)

Plant structural carbohydrates are the major contributors to the energy requirements of the ruminants and other herbivores. The plant cell wall is composed primarily of fibrils of cellulose, the most abundant polymer on the earth. Cellulose is a hydrogen bonded β-1-4-linked D-glucan, which accounts for 20-30% dry weight of most plant primary cell wall (Mc Neil et al., 1984). Enzyme system digesting crystalline cellulose comprises mainly of endoglucanase (β-1-4 D-glucan glucanohydrolase; EC 3.2.1.4), exoglucanase (exocellobiohydrolase, β-1-4-D-glucan cellobiohydrolase; EC 3.2.1.91), exoglucano-hydrolase (β-1-4-D-glucan glucohydrolase; EC 3.2.1.74) and β-glucosidase, (β-D-glucoside gluco-hydrolase; EC 3.2.1.21). The most commonly found exoglucanase is a cellobiohydrolase that cleaves cellobiose units from non-reducing ends of cellulose chains. Endoglucanases cleave 1-4-linkages of cellulose at random. Glucosidase mainly hydrolyses cellobiose produced by exocellobiohydrolase and some β-glucosidases remove glucose residues from short oligosaccharides (Shewale, 1982; Wood, 1991).

Among the ruminal microbes fungi are the major sources of fibrolytic enzymes described to date (Trinci et al., 1994). Ruminal fungi have been shown to exhibit as much as 5 times higher cellulolysis than those of predominant bacteria (Lee et al., 1996), and their enzymes have therefore sparked interest for a number of biotechnological applications. Hemicellulose, the second most important polysaccharide in nature is a heteropolymer composed primarily of β-1-4-linked β-D-xylose backbone with various amounts of arabinose, glucose, uronic acid, methylated glucouronic acid and other sugars as side groups depending upon the plant source (Whistler and Richards, 1970). Hemicellulose represents a potential energy source for the host animal especially to the herbivores. Since the herbivores do not produce hemicellulase, they entirely depend upon their gastrointestinal microflora for the degradation of hemicellulose. Rumen bacteria exhibiting a wide array of endoglucanases, cellobiases and β-glucosidases have been reviewed in detail (Chesson and Forsberg, 1997; Selinger et al., 1996; Lee et al., 1999). Hind gut of other vertebrate herbivores is also inhabited by various fibrolytic microflora. The dominant xylanolytic bacterium in the rabbit was identified as *Bacteroids ruminicola* whilst *Butyrivibrio fibrisolvens* and *B. ruminicola* were predominant in mice. Cellulolytic *Eubacterium*, *Bacteróides* and *Ruminococcus spp.* have been isolated from large intestine of herbivores (Davies, 1965; Boulahrouf et al., 1986). The hemicellulolytic potential of these bacteria is indicated by their analogy with ruminal isolates. Julliand et al. (1999) have identified *Ruminococcus flavefaciens* as predominant cellulolytic bacterial species colonizing the equine caecum. The number of cellulolytic and hemicellulolytic bacteria were found to increase within 3 days in swine receiving fibrous diets (Varel, 1987). *B. fibrisolvens, Bacteroides ruminicola, B. succinogenes* and *R. flavefaciens* were the predominant bacterial species. It has been established that microbial population from the hind gut of ruminants (Bailey and MacRae, 1970), horse (Bonhomme-Florentin, 1988), rat (Rowland et al., 1986) and human (Englyst et al., 1987) degrade hemicellulose. A number of ruminal bacteria (Clarke et al., 1969; Pettifer and Latham, 1979) and protozoa (Prins, 1977) have been described as producer of hemicellulases. Cloning and characterization of a xylanase encoding gene in ruminal anaerobic protozoan *Polyplastron multivasiculatum* reveal that protozoa play significant role in dietary fibre digestion (Devillard et al., 1999). Anaerobic fungi colonize specific regions of intestinal tract of mammalian herbivores and are most abundant in larger herbivores consuming fibrous diets.

Rumen of domestic and feral ruminants, fore gut of camel and kangaroo, the caecum of ruminants and hind gut fermenters are inhabited by anaerobic Chytridiomycetes (Orpin, 1988).

The fibrolytic enzymes have several applications especially to the animal feed processing (table 2). Amending livestock ration with fibrolytic enzymes such as cellulases, xylanases and endoglucanases have been found to enhance efficiency of non-ruminant livestock. Extensive reviews are available on use of microbial enzymes to improve feed quality (Bedford, 1996; Wallace and Chesson, 1995; Campbell and Bedford, 1992). The use of fibrolytic enzymes in improving the digestibility of fibrous feeds for ruminants is also of considerable interest. Commercially produced cellulases and hemicellulases are used during ensilage to release soluble sugars from degradable fibre components of ensiled grasses (Spoelstra, 1990). Cellulase is sold and used as commercial silage additive and to improve malting and filtration in brewing.

Glucanase is aimed at improving digestibility of non-starch carbohydrate in viscous cereals such as barley and oats. Barley contains anti-nutritional factors called β-glucans which after solubilization yield extremely viscous mixture that inhibits nutrient digestion and assimilation. This inhibition has numerous adverse effects on poultry performance. Adverse effects of β-glucans may be counteracted by the incorporation of β-glucanase preparation in the diet. It reduces the viscosity in gut lumen of broiler chicks and piglets. Xylanases are directed at viscous polymers in wheat, rye and triticales. Pack et al. (1998) have suggested that a mixture of xylanases, proteases and amylases improve digestion in low viscous cereals such as corn and sorghum. The merits of β-glucanases in barley based poultry and swine diet is well documented. β-glucanase is normally added in a dry form to the feed and acts after hydration within digestive tract (Edney et al., 1986). This application has led to wide spread incorporation of barley into poultry feed, an area where barley had previously been used to a very limited extent. Feeding of these enzymes has become common practice in some parts of the world (Cheng et al., 1999).

Middle lamella of primary cell wall of higher plants is composed of pectin polymers, the chains of β-1-4-D-galactouronic acid and methoxylated derivatives (Collmer et al., 1988). It is present in vegetables and fruits as a component of the plant cell wall. Lignified tissues contain only small quantity of pectic materials compared to young, actively growing plant tissues (Fogarty and Kelly, 1983). Like other types of dietary fiber, pectin also is not depolymerized by endogenous gastrointestinal enzymes during passage through the stomach or intestine. It is fermented more or less completely by the host's indigenous microflora (Jensen and Canale-Parola, 1986; Gibson et al., 1990; Titgemeyer et al., 1991). A few strains of ruminal fungi exihibit pectinolytic activity (Kopency and Hodrova, 1995). Ruminal bacteria (*Streptococcus bovis*) and protozoa (*Treponema saccharophilum*) are the major pectinolytic microbes (Wojciechowicz and Ziolecki, 1984; Paster and Canale-Parola, 1985). Exo-polygalactournase, endo- and exo-pectate lyase and pectin esterase produced by these microbes act on pectin molecules (Wojciechowicz and Ziolecki, 1984; Paster and Canale-Parola, 1985). Fungi (*Erwinia, Aspergillus, Penicilium, Fusarium* and *Trichoderma*) are primarily used for production of pectinase on commercial scale. Pectinolytic enzymes have several industrial uses. The age old process of retting by which important textile fibres such as flax, hemp and jute are prepared also involve pectinolytic enzymes which degrade pectin in middle lamella of the plant fibres (Fogarty and Kelly, 1983). Pectin esterases, endopectin lyases and endopolygalactouronases are commercially used in fruit processing, paper, textile, detergent and pharmaceutical industries.

Tannase

Tannase (Tannin acyl hydrolase, E.C. 3.1.1.20), primarily catalyzes the hydrolysis of ester and depside bonds in hydrolyzable tannins such as tannic acid releasing glucose and gallic acid. Tannase is now known to be an ubiquitous enzyme of microbes namely yeast, fungi and bacteria (Deschamps, 1989; Field and Lettinga, 1992; Lekha and Lonsane, 1997). Tannase was originally used in Japan to produce commercially quantities of gallic acid from tannic acid. It is extremely useful in food, feed, beverages, brewing and pharmaceutical industries (Lekha and Lonsane, 1997). Preparation of instant tea is one of the most promising applications of tannase. It is used as an inhibitor of creamdown in tea and as a clarifier in the production of beer and fruit juices (Cantarelli et al., 1989). Lactobacilli with tannase activity have been recently reported from human feces and fermented foods (Osawa et al., 2000). A number of gastrointestinal microflora that produce tannases have been documented (table 1).

Phytase

For an environment friendly livestock production phytase is a valuable enzyme. Much of the phosphorus in seeds, cereals and oil cakes occurs as phytate (Graf, 1986), and is found in rather substantial levels in virtually all feed substances originating from plants (figure 5). It comprises from 1-3% of the content of all cereals, nuts, legumes and spores. The corn grain and wheat processing by-products (e.g. wheat bran and wheat middings) are high in phytate content. Because

Table 2. Gastrointestinal microbes as sources of enzymes, and biotherapeutics.

Products	Organisms and references
Enzymes	
Cellulase	*Caecomyces communis* (Bata and Gerbi, 1997); *Cytophaga* (Volokita et al., 2000); *Fibrobacter succinogenes* (Martin and Martin, 1998; Julliand et al., 1999; Bera-Maillet et. al., 2000); *Neocallimastix frontalis* (Fugino and Ushida, 1999); *Orpinomyces joyonii* (Qiu et al., 2000); *Polyplastron multivasicultum* (Bonhomme-Florentin, 1988); *Ruminococcus albus, R. flavefaciens* (Julliand et al., 1999); *Streptococcus bovis* (Ekinsi et al., 1997).
Hemicellulases	*Bacteroides ruminicola, B. succinogenes* (Bailey and MacRae, 1970); *B. ovatus* (Reddy et al., 1984); *Clostridium aerotolerans* (van Gylswick and van der Toorn, 1987).
Xylanases and glucanases	*Bacteroides ruminicola, Butyrivibrio fibrisolvens* (Boulahrouf et al., 1986); *Clostridium aerotolerans* sp. nov., (van Gylswick and van der Toorn, 1987); *Neocallimastix frontalis* (Fugino and Ushida, 1999); *N. patriciarum* (Liu et al., 1999); *Orpinomyces* (Li et al., 1996): *Polyplastron multivasiculatum* (Devillard et al., 1999); *Ruminococcus albus* (Attwood et al., 1996).
Pectinases	*Bacteroides pectinophilus* sp. nov., *B. galacturonicus* sp. nov. (Jensen and Canale-Parola, 1986); *Bacteroides thetaiotaomicron* (Tierny et al., 1994; Dongowski et al., 2000); *Butyrivibrio fibrisolvens* and *Prevotella ruminocola* (Maraunek and Duskova, 1999); *Streptococcus bovis* (Wojciechowicz and Ziolecki, 1984); *Treponema saccharophilum* sp. nov. (Paster and Canale-Parola, 1985)
Phytase	*Mitsuokella multiacidus, Prevotella ruminicola, Selenomonas ruminantium* and *Streptococcus bovis* and *Treponema spp* (Yanke et al., 1998, 1999)
Tannase	*Aspergillus niger* (Bhat et al., 1996); *Eubacterium oxidoreducens* (Krumholz and Bryant, 1986); *Lactobacillus spp.* (Osawa et al., 2000); *Lonepinella koalarum* (Osawa et al., 1995); *Selenomonas ruminantium* (Skene and Brooker, 1995); *Streptococcus caprinus* (Brooker et al., 1994).
Restriction endonucleases	
*Fsu*I	*Fibrobacter succinogenes* (Lee et al., 1992)
Ral 8I, *Rfl* F1	*Ruminococcus albus, R. flavefaciens* FD I (Morrison et al., 1992a,b)
Sbv I	*Streptococcus bovis* (Vanat et al., 1993a)
Sru I, *Sru* 4D1	*Selenomonas ruminantium* (Vanat et al., 1993b)
Sru 30D1	*Selenomonas ruminantium* (Pristas et al., 1995)
*Pst*I isoschizomers	Ruminal selnomonades (Molnarova et al., 1999)
Srl I, *Srl* II	*Selenomons ruminantium* sub sp. lactilytica (Pristas et al., 1998)
Bfi 571, *Bfi* 891	*Butyrivibrio fibrisolvens* (Mohan and Teather, 1995)
Probiotics/biotherapeutics	*Bifidobacteria* (Wells et al., 1997); butyrivibrios (Teather and Forster, 1998); lactobacilli and bifidobacteria (Gusils et al., 1999; Kabir et al., 1997; Kailasapathy and Chin, 2000; Matsuzaki and Chin, 2000)

of its highly ionized orthophosphate groups, it complexes with a variety of divalent cations (Ca^{2+}, Fe^{2+}, Zn^{2+}, Mg^{2+} and Mn^{2+}) and pectins and it is this trait which categorizes phytate as an antinutritional factor since it decreases the bioavailability of proteins and nutritionally important minerals (Graf, 1986). This is particularly important in poultry, swine, humans and other monogastric animals who are unable to degrade phytate. The large amount of phosphorus excreted in manure as phytate contributes significantly to eutrophication of surface waters (Pen et al., 1993; Van Gorcom et al., 1995). The enzyme phytase (myo-inositol hexaphosphate phosphohydrolase) produced by microbes and plants but not by vertebrates, cleaves off

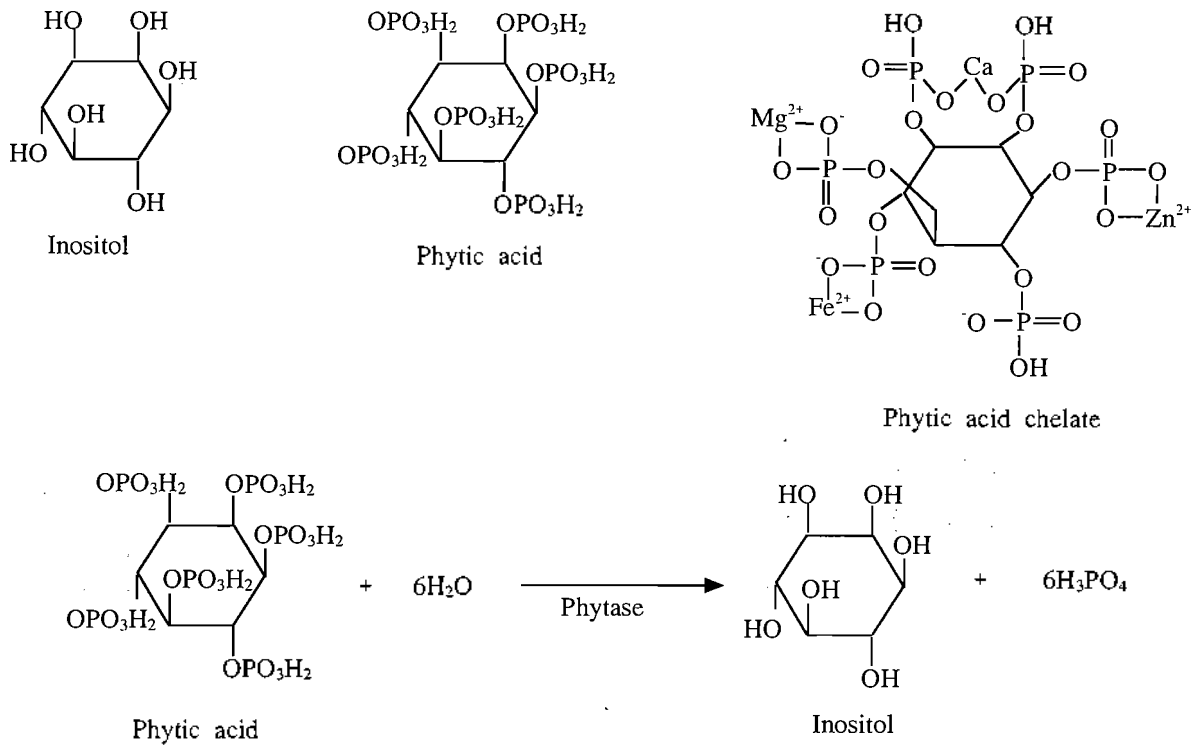


Figure 5. Phytic acid, phytic acid chelate and enzymatic hydrolysis of phytic acid

phosphorus from inositol making phosphorus and other minerals available for utilization (figure 5). While flora of ruminants, for example, *Selenomonas ruminantium* (Yanke et al., 1998, 1999), are well known to produce potent phytases, monogastric animals, such as pigs, poultry and fish, utilize this source of phosphate poorly at best, lacking the requisite gastrointestinal tract enzyme(s) for release of the phosphate from the organic complex of phytate (Cromwell et al., 1995). Ruminal microbes produce adequate phytase which renders all dietary phytate digestible. Hind gut microbes of pig and poultry especially young chicks also exhibit little and variable phytase activity. Commercial preparation of phytase from *Aspergillus niger* is used as feed additive. The phytases are very effective in increasing the availability of plant phosphorus to poultry (Simons et al., 1990) and swine (Lei et al., 1993). Since phosphorus is relatively an expensive nutrient and is some times in short supply, improving utilization of phosphorus already present in feed stuffs would enhance efficiency of diet formulation. Phytase supplementation has been shown to improve the ileal digestibility of nitrogen and nitrogen retention of pigs (Mroz et al., 1994). Dietary phytases have been reported not only to increase phosphate utilization efficiency from phytate in feeds but also to decrease phosphorus pollution (Pen et al., 1993).

The expense of production using traditional large-scale fermentation and downstream processing remains the limiting step for widespread use of these enzymes. Manufacturers and researchers have therefore attempted to exploit the ability of GI microbes for enzyme production and delivery through molecular biology techniques. The last two decades have witnessed increased efforts to fully understand the enzymology of fibre degradation and to genetically engineer the GI bacteria with enhanced cellulolytic capabilities.

Miscellaneous novel products

GI microbes are promising sources of various novel products (table 2). Ruminal bacteria, probably as a result of selection pressure provided by high number of bacteriophages in ruminal ecosystem (Klieve and Bouchop, 1988), are promising sources of restriction-modification enzymes. *Fibrobacter succinogenes*, an important cellulolytic inhabitant of rumen and caecum of herbivores possesses restriction modification system. Type II restriction endonuclease (RE), FsuI, an isoschizomer of *Ava*II from *F. succinogenes* S85 is reported by Lee et al., 1992. Other class-IIS REs namely RalBI from *Ruminococcus albus8* (Morrison et al., 1992b) and SbvI from *Streptococcus bovis* (Vanat et al., 1993a) have been partially purified and characterised. Two REs, *Sru* I and *Sru*4DI were isolated from ruminal selenomonades, both of which recognize pure A+T sequences (Vanat et al., 1993b: Pristas et al., 1994). Prevalence of CTGCAG (*Pst*I isoschizomers) recognising restriction

and modification systems in ruminal selenomonades have been reported recently (Molnarova et al., 1999). These endonucleases like other commercially available REs may prove important tools in genetic engineering.

Biotransformation of various substrates by GI microbes yield more valuable products. Production of tyrosine and other aromatic compounds from phenylalanine (Khan et al., 1999), biosynthesis of tryptophan and related compounds (Mohammed et al., 1999) by mixed ruminal microbes and succinic acid by *Actinobacillus succinogenes* sp. nov., (Guettler et al., 1999) have been reported recently.

GI microbes as health promoter

Large populations of lactobacilli are the commensal colonizers of gastrointestinal and urinogenital epithelium of various warm blooded vertebrates including ruminants and monogastric animals (Sharpe et al., 1973; Savage, 1977; Gusils et al., 1999). A low number of lactic acid bacteria on plants and relatively high population in human and animals suggest that intestinal tract may be their natural habitat (Keddy, 1959; Mundt and Hammer, 1958; Kandler and Weiss, 1986). Microbial colonization of the GI tract of most of monogastric livestock is similar (Lindsey, 1990).

The concept of using *Lactobacillus* species for disease treatment and prevention as well as health restoration is not new (Reid, 1999). In recent times there has been renewal of interest in the use of lactobacilli and bifidobacteria as probiotics (also termed as biotherapeutic agents). Numerous claims have been made regarding their beneficial effects in human and animal guts (Sanders et al., 1993; Jin et al., 1996; Morata de Ambrosini et al., 1998). Microbial probiotics have many beneficial effects when they are used in animal feeds. Studies have shown overall improvement in the condition of animals which received a diet supplemented with probiotic lactic acid bacteria (Pollman et al., 1980). Probiotics, for instance, have been used therapeutically to modulate immunity (Matsuzaki and Chin, 2000; Chin et al., 2000), improve lactose intolerance, prevent or reduce diarrhoea and constipation as well as candidiasis and urinary tract infections (Reid et al., 1995). Various antagonistic factors including organic acids, hydrogen peroxide, diacetyl, bacteriocins and bacteriocin like compounds produced by endogenous strains of lactobacilli are inhibitory against a broad range of gram-positive and gram-negative bacteria (Vincet et al., 1959; Singh et al., 1996). Whereas *Lactococci*, *Lactobacilli* and *Pediococci* are best studied bacteriocinogenic microbial genera (Jack et al., 1995), findings of Attwood et al. (1988) and Odenyo et al. (1994) support the existence of bacteriocinogenic bacteria in rumen. A bacteriocin mediated antagonism by ruminal lactobacilli against *Streptococcus bovis* was reported by Wells et al., (1997). The production of bacteriocins by high populations of ruminal bacteria such as *Butyrivibrios* would have important implications for the design of rumen inoculants (Teather and Forster, 1998). This approach would use the new bacteriocins just as the ionophore antibiotics are used today to selectively inhibit the growth of specific target organism or group of organisms (Teather and Forster, 1998). In addition to the use in ruminant nutrition, the bacteriocins derived from the ruminal microbes might find applications in controlling human or animal diseases particularly in the case of pathogens that colonize the gastrointestinal or urinogenital tract.

Currently considerable attention is being given to the use of probiotics in animal feeding programmes. Two main products based on either yeast (*Saccharomyces cerevisiae*) or fungal (*Aspergillus oryzae*) cultures alone or in combination with other microorganisms, are available for use in animal diets (Frumholtz et al., 1989). Inclusion of probiotics in feed results in qualitative and quantitative changes in microbial population of ruminants and ultimately affects production efficiency and product composition. The anaerobic fibrolytic microbes of herbivores and their enzymes have potential for use as probiotics and feed additives, as silage and total mixed rations for saccharification of lignocellulolytic residues, and for production of polysaccharolytic enzymes.

REFERENCES

Adams, N. R. 1989. Phytoestrogens. In: Cheeke, P. R.(ed). Toxicants of Plant Origin. Vol. IV. CRC Press, Boca Raton. FL. pp. 23-51.

Adlercreutz, H., H. Honjo, A. Higashi, T. Fotsis, E. Hamalainen, T. Hasegawa and H. Okada. 1991. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women comsuming a traditional Japanese diet. Am. J. Clin. Nutr. 54:1093-1100.

Akbar, M. A. and P. C. Gupta. 1985. Subabul (*Leuceana leucocephala*) as a source of protein supplement for buffalo calves. Ind. J. Anim. Sci. 55:54-58.

Allison, M. J., E. T. Littledike and L. F. James. 1977. Changes in ruminal oxalate degradation rates associated with adaptation to oxalate ingestion. J. Anim. Sci. 45:1173-1179.

Allison, M. J., H. M. Cook, K. A. Dawson. 1981. Selection of oxlate-degrading rumen bacteria in continuous cultures. J Anim. Sci. 53:810-816.

Allison, M. J., C. A. Reddy. 1984. Adaptations of gastrointestinal bacteria in response to changes in dietary oxalate and nitrate. In: Current Perspectives on Microbial Ecology (Ed. M. J. Klug and C. A. Reddy). Washington DC. American Society for Microbiology. pp. 248-256.

Allison, M. J., K. A. Dawson, W. R. Mayberry and J. G. Foss. 1985. *Oxalobacter formigenes* gen. nov. sp. nov., oxalate degrading anaerobes that inhabit the gastrointestinal tract. Arch. Microbiol. 141:1-7.

Allison, M. J., W. R. Mayberry, C. S. McSweeny and D. A. Stahl. 1992. *Synergistes jonesii* gen. nov., sp. nov. : A rumen bacterium that degrades toxic pyridinediols. Appl. Environ. Microbiol. 15:522-529.

Anderson, R. C., M. A. Rasmussen, M. J. Allison. 1993. Metabolism of plant toxins nitrpropionic acid and nitropropanol by ruminal microorganisms. Appl. Environ. Microbiol. 59:3056-3061.

Anderson, R. C., M. A. Rasmussen and M. J. Allison. 1996. Enrichment and isolation of a nitropropanol- metabolizing bacterium from the rumen. Appl. Environ. Microbiol. 62:3885-3886.

Anderson, R. C., M. A. Rasmussen, N. S. Jensen and M. J. Allison. 2000. *Denitrobacterium detoxificans* gen nov., sp. nov., a ruminal bacterium that respires on nitrocompounds. Int. J. Syst. Evol. Microbiol. 50:633-638.

Angermaier, L. and H. Simon. 1983. On the reduction of aliphatic and aromatic nitro compounds by Clostridia, the role of ferredoxin and its stabilization. Hoppe-Seyler's Z. Physiol. Chem. 364:961-975.

Aplin, T. E. H. 1967. Poison Plants of Western Australia. The toxic species of the genera *Gastrolobium* and *Oxylobium*. J. Agric. West. Aust. 8:42-52.

Attwood, G. T., F. Herrera, I. A.. Weissenstein and B. A. White. 1996. An endoObO1,4-glucanase gene (celA) from the rumen anaerobe *Ruminococcus albus* 8: cloning sequencing and transcriptional analysis. Can J. Microbiol. 42:267-278.

Attwood, G. T., R. A. Lockington, G. P. Xue and J. D. Brooker. 1988. Use of a unique gene sequence as a probe to enumerate a strian of *Bacteroides ruminicola* introduced into the rumen. Appl. Environ. Microbiol. 54:534-539.

Bae, H. D., T. A. McAllister, L. J. Yanke, K.-J. Cheng, and A.D. Muir. 1993. Effects of condensed tannins on endoglucanase activity and filter paper digestion by *Fibrobacter succinogenes* S85. Appl. Environ. Microbiol. 59:2132-2138.

Baetz, A. L. and M. J. Allison. 1989. Purification and characterization of oxalyl-CoA dercarboxylase from *Oxalobacter formigenes*. J. Bacteriol. 171:2605-2608.

Bailey, R. W. and J. C. MacRae. 1970. The hydrolysis by rumen and caecal microbial enzymes of hemicellulose in plant and digesta particles. J. Agric. Sci. 75:321-326.

Bata, J. and C. Gerbi. 1997. Glycoside-hydrolase production by an anaerobic rumen fungus *Caecomyces communis*. Res. Microbiol. 148:263-269.

Batterham, T. J., N. K. Hart, J. A. Lamberton and A. W. H. Braden. 1965. Metabolism of oestrogenic isoflavones in sheep. Nature (Lond.). 206:509.

Beck, A. B. 1964. The oestrogenic isoflavones of subterranean clover. Aust. J. Agric. Res. 15:223-230.

Bedford, M. R. 1996. The effect of enzymes on digestion. J. Appl. Poult. Res. 5:370-378.

Bera-Maillet, C., V. Broussolle, P. Pristas, J. Girardeau, G. Gaudet and E. Forano. 2000. Characterization of endoglucanases EGB and EGC from *Fibrobacter succinogenes*. Biochim. Biophys. Acta. 1476:191-202..

Bhat, T. K., H. P. S. Makkar and B. Singh. 1996. Isolation of a tannin-protein complex degrading fungus from the faeces of hill cattle. Lett. Appl. Mirobiol. 22: 257-258.

Bhat, T. K., B. Singh and O. P. Sharma. 1998. Microbial degradation of tannins - a current perspective. Biodegradation. 9:343-357.

Blair, R. and R. Reichart. 1984. Carbohydrate and phenolic constituents in a comprehensive range of rapeseed and canola fractions: nutritional significance for animals. J. Sci. Food Agric. 35:29-35.

Blaney, B. J., R. J. W. Gartner and T. A. Head. 1982. The effects of oxalate in tropical grasses on calcium, phosphorus and magnesium availabilty to cattle. J. Agric. Sci. 99:533-546.

Bonhomme-Florentin, A. 1988. Degradation of hemicellulose and pectin by horse caecum content. Br. J. Nutr. 60:185-192.

Boulahrouf, A., G. Fonty and Gouet, Ph. 1986. Study of plant cell wall polysaccharide- degrading bacteria in the digestive tarct of rabbits and mice. In: Progress in Biotechnolgy, Vol 2, Biology of Anaerobic Bacteria. H. C. Dubourguier, G. Albagnac, J. Montreuil, C. Romond, P. Sautiere, P.& J. Guillaume.(eds). Elsevier Applied Sci. Amsterdam. pp 54-59.

Braden, A. W. H., N. K. Hart and J. A. Lamberton. 1967. The estrogenic activity and metabolism of certain isoflavones in sheep. Aust. J. Agric. Res. 18:335-348.

Brooker, J. D., L. A. O'Donovan, I. K. Skene, K. Clark, L. Blackall and P. Muslera. 1994. *Streptococcus caprinus* sp. nov., a tannin-resistant ruminal bacterium from feral goats. Lett. Appl. Microbiol. 18:313-318.

Bull, L. B., C. C. J. Culvinor and A. T. Dick. 1968. The Pyrrolizidine Alkaloids. Their Chemistry, Pathogenicity and other Biological Properties. American Elsevier, NY.

Campbell, G. L. and M. R. Bedford. 1992. Enzyme applications for monogastric feeds. a review. Can J. Anim Sci. 72:449-466.

Cantarelli, C., O. Brenna, G. Giovanelli and M. Rossi. 1989. Beverage stabilization through enzymatic removal of phenolics. Food. Biotech. 3:203-213.

Cheeke, P. R. 1988. Toxicity and metabolism of pyrrolizidine alkalods. J. Anim. Sci. 66:2343-2350.

Cheeke, P. R. 1998. Toxins intrinsic to forages. In: Natural Toxicants in Feeds, Forages and Poisonous Plants. P. R. Cheeke (ed). Interstate Publishers. Inc. Danville, Illinois. pp. 275-324.

Cheng, K. J., S. S. Lee, H. D. Bae and J. K. Ha. 1999. Industrial applications of rumen microbes. review. Asian-Aus. J. Anim. Sci. 12:84-92.

Chesson, A. and C. W. Forsberg. 1997. Polysaccharide degradation by rumen microorganisms. In: The Rumen Microbial Ecosystem. P. Hobson and C. Stewart. (eds). Chapman and Hall Ltd. Andover and Hampshire, UK. pp. 329-381.

Chin, J., B. Turner, I. Barchia and A. Mullbacher. 2000. Immune response to orally consumed antigens and probiotic bacteria. Immunol. Cell. Biol. 78:55-66.

Chung, K. T. 1996. . Gastrointestinal toxicology of monogastrics. In: Gastrointestinal Microbiology, Vol. 1: Gastrointestinal Ecosystem and Fermentation. R.I. Mackie and B.A. White (eds). Chapman and Hall, NY. pp. 511-582.

Chung, K.T., Z. Lu and M. W. Chou. 1998. Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. Food Chem. Toxicol. 36:1053-1060.

Clark, P. H. 1984. The evolution of degradative pathways. In: Gibson, D. T. (ed.). Microbiological Degradation of Organic Compounds. New York: Marcel Decker. pp. 11-27.

Clarke, R. T. J., R. W. Bailey and B. D. E. Gaillard. 1969. Growth of rumen bacteria on plant cell wall polysaccharides. J. Gen. Microbiol. 56:79-86.

Collmer, A., J. L. Reid and M. S. Mount. 1988. Assay methods of pectic enzymes. In: Methods in Enzymology, Vol. 161 (Ed. W. A. Wood and S. T. Kellogg). Academic Press. San Diego, USA. pp. 329-334.

Cox, R. I. and L. H. Davies. 1988. Modification of pasture oestrogens in the gastrointestinal tract of ruminants. Proc. Nutr. Soc. Aust. 13:61-67.

Cowan, D. 1996. Industrial enzyme technology. Trends Biotechnol. 14:177-178.

Craig, A. M., L. L. Blythe and E. D. Lassen. 1985. Pyrrolizidine alkaloid infusion in sheep, . In: Seawright, A. A., M. P. Hegarity, L. G. James and R. F. Keeler (eds). Plant Toxicology, Queensland Poisonous Plant Committee, Yerongpilly, Australia. pp. 200-208

Craig, A.. M., C. J. Latham, L. L. Blythe, W. B. Schmotzer and O. A. O'Connor. 1992. Metabolism of toxic pyrrolizidine alkaloids from tansy ragwort (*Senecio jacobaea*) in ovine ruminal fluid under anaerobic conditions. Appl. Environ. Microbiol. 58:2730-2736.

Cromwell, G. L., R. D. Coffey, H. J. Monegue and J. J. Randolph. 1995. Efficacy of low activity, microbial phytase in improving bioavailability of phosphorus in corn-soybean meal diets for pigs. J. Anim. Sci. 73:449-456.

Cunningham, E. P. 1990. Animal Production. In: Persley, G. P.(ed), Agricultural Biotechnology: Opportunities For International Development, CAB International, UK. pp. 169-176.

Daniel, S. L. and H. L. Drake. 1993. Oxalate and glyoxalate-dependent growth and acetogenesis by *Clostridium thermoaceticum*. Appl. Environ. Microbiol. 59:3062-3069.

Davies M. E. 1965. Cellulolytic bacteria in some ruminants and herbivores as shown by fluorescent antibody. J.Gen. Microbiol. 39:139-141.

Dawson, K. A., M. J. Allison and P.A. Hartman. 1980. Characteristics of some anaerobic oxalate-degrading enrichment cultures from the rumen. Appl. Environ. Microbiol. 40:840- 846.

Deschamps, A. M. 1989. Microbial degradation of tannins and related compounds. In: Plant Cell Wall Polymers Biogenesis and Biodegradation (Ed. N. G. Lewis and M. G. Paice). American Chemical Society, Washington, DC. pp. 559-566.

Deshpande, S. S., S. K. Sathe and D. K. Salunkhe. 1984. Chemistry and safety of plant phenols. Advances in Experimental Medicine and Biology. 177:457-495.

Devillard, E., C. J. Newbold, K. P. Scott, E. Forano, R. J. Wallace, J. P. Jouany and H. J. Flint. 1999. A xylanase produced by the rumen anaerobic protozoan *Polyplastron multivesiculatum* shows close sequence similarity to family 11 xylanases from Gram-positive bacteria. FEMS Microbiol. Lett. 181:145-152.

Dominguez-Bello, M. G. and C. S. Stewart. 1990. Degradation of mimosine, 2,3,-dihydroxy pyridine and 3-hydroxy-4-(1H) pyridone by bacteria from the rumen of sheep in Venezuela. FEMS Microbiol. Ecol. 73:283-289.

Dominguez-Bello, M. G. and C. S. Stewart. 1991. Characteristics of rumen *Clostridium* capable of degrading mimosine, 3(OH)-4-(1H)-pyridone and 2,3-dihydroxy-pyridine. System. Appl. Microbiol. 14:67-71.

Dongowski, G., A. Lorenz and H. Anger. 2000. Degradation of pectins with different degrees of esterification by *Bacteroides thetaiotaomicron* isolated from human gut flora. Appl. Environ. Microbiol. 66:1321-1327.

Edney, M. J., H. L. Classon and G. L. Campbell. 1986. Application of a simple radial gel diffusion assay for endo-beta-glucanase activity in dietary supplements. Poult. Sci. 65:72-77.

Ekinci, M. S., S. I. McCrae and H. J. Flint. 1997. Isolation and expression of a gene encoding an extracellular β -(1,3-1,4)-glucanase from *Streptococcus bovis* JB1. Appl. Environ. Microbiol. 63:3752-3756.

Englyst, H. N., S. Hay, and G. T. Macfarlane. 1987. Polysaccharide breakdown by mixed populations of human faecal bacteria. FEMS Microbial. Ecol. 95: 163-171.

Field, J. A., G. Lettinga. 1987. The methanogenic toxicity and anaerobic degradabilty of hydrolysable tannins. Water Res. 21:367-374.

Field, J. A. and G. Lettinga. 1992. Toxicity of tannic compounds to microorganisms. In: Plant Polyphenols: Synthesis, Properties and Significance (Ed. R. A. Hemingway and E. Laks). Plenum Press. NY. pp. 673-692.

Fogarty, W. M. and Kelly, C. T. 1983. Pectic enzymes. In: Microbial enzymes and Biotechnology (Ed. W. M. Fogarty). Elsevier Applied Science, London. pp. 131-182.

Folman, Y. and G. S. Pope. 1966. The interaction in the immature mouse of potent estrogens with coumestrol, genestein and other uterovaginotrophic compounds of low potency. J. Endocrinol. 34:215.

Frumholtz, P. P., C. J. Newbold and R. J. Wallace. 1989. Influence of *Aspergillus oryzae* fermentation extract on the fermentation of basal ration in the rumen simulation technique (Rusitec) J. Agric. Sci. Camb. 113:169-172.

Fujino, Y. and K. Usida. 1999. Plant cell wall degradation and glycanase activity of the rumen anaerobic fungus *Neocallimastix frotalis* MCH3 grown on various forages. Asian-Aus. J. Anim. Sci. 12:752-757.

Gibson, G. R., S. Macfarlane and J. H. Cummings. 1990. The fermentability of polysaccharides by mixed faecal bacteria in relation to their suitability as bulk-forming laxatives. Lett. Appl. Microbiol. 11:251-254.

Graf, E. 1986. Chemistry and applications of phytic acid: an overview. In: (Ed. E. Graf). Pilatus Press. Minneapolis. MO.

Gregg, K., C. L. Cooper. D. J. Schafer, H. Sharpe, C. E. Beard, G. Allen and J. Xu. 1994. Detoxification of the plant toxin fluoroacetate by a genetically modified rumen bacterium. Bio/ Technology. 12:1361-1365.

Gregg, K. 1995. Engineering gut flora of ruminant livestock to reduce forage toxicity : Progress and Problems. Trends Biotechnol. 13: 418-421.

Gregg, K., B. Hamdorf, K. Henderson, J. Kopency and C. Wong. 1998. Genetically modified ruminal bacteria protect sheep from flouroacetate poisoning. Appl.

Environ. Microbiol. 64:3496-3498.

Guettler, M. V., D. Rumler and M. K. Jain. 1999. *Actinobacillus succinogenes* sp. nov., a novel succininc-acid-producing strain from the bovine rumen. Int. J. Syst. Bacteriol. 49:207-216.

Gupta, B. S., B. N. Gupta, R. C. Saha, A. B. Choudhuri and M. L. Mathur. 1977. Studies on long term feeding of sal (Shorea robusta) seed meal to adult cattle. Indian Vet. J. 54:463-469.

Gusils, C., S. N. Gonzalez and G. Oliver. 1999. Some probiotic properties of chicken lactobacilli. Can. J. Microbiol. 45:981-987.

Gustine, D. L., B. G. Moyer, P. J. Wangness and J. S. Shenk. 1977. Ruminal metabolism of 3-nitropropanoyl-D-glucopyranoses from crown vetch. J. Anim. Sci. 44:1107-1111.

Gutierrez, J., and R. E. Davis. 1962. Isolation of saponin-digesting bacteria from the rumen of bloating cattle on ladino clover pasture. J. Anim. Sci. 21:819-823.

Gutierrez, J., R. E. Davis and I. L. Lindahl. 1958. Characteristics of saponin utilizing bacteria from the rumen of cattle. Appl. Microbiol. 7:304-308.

Hegarty, M. P. 1978. Toxic amino acids of plant origin. In: R. F. Keeler, K. R. Kampen and L. R. James.(eds). Effects of poisonous Plants on Livestock. Acaddemic Press, NY. pp. 575-585.

Hegarty, M. P., R. D.Court, G. S.Christie and C. P. Lee. 1976. Mimosine in *Leucaena leucephala* is metabolized to a goitrogen in ruminants. Aust. Vet. J. 52:490.

Hodgson, J. 1994. The changing bulk catalyst market. Bio/Technology. 12:789-790.

Hoff, J. E. and K. L. Singleton. 1977. A method of determination of tannins in foods by means of immobilized protein. J. Food Sci. 42:1566-1569.

Hokama, S., Y. Honma, C. Toma and Y. Ogawa. 2000. Oxalate degrading *Enterococcus faecalis*. Microbiol. Immunol. 44:235-240.

Jack, J. W., J. R. Tagg and B. Ray. 1995. Bacteriocins of Gram positive bacteria. Microbiol. Rev. 59:171-200.

Jensen, N. S. and E. Canale-Parola. 1986. *Bacteroides pectinophilus* sp. nov. and *Bacteroides galacturonicus* sp. nov.: two pactinolytic bacteria from the human intestinal tract. Appl. Environ. Microbiol. 52:880-887.

Jin, L. Z., Y. W. Ho, M. A. Ali, N. Abdullah, K. B. Ong and S. Jalaludin. 1996. Adhesion of *Lactobacillus* isolates to intestinal epithelial cells of chicken. Lett. Appl. Microbiol. 22:229-232.

Jones, G. A., T. A. McAllister, A. D. Muir and K. J. Cheng. 1994. Effects of sainfoin (*Onobrychis vicifolia* Scop.) condenced tannins on growth and proteolysis by four strains of ruminal bacteria. Appl. Environ. Microbiol. 60:1374-1378.

Jones, R. J. 1981. Does ruminal metabolism of mimosine explain the absence of *Leucaena* toxicity in Hawaii - Aust. Vet. J. 57:55-56.

Jones, R. J. 1994. Management of anti-nutritive factors with special reference to leucaena. In: Gutteridge, R. C. and H. M. Shelton (eds). Forage Tree Legumes in Tropical Agriculture. CAB International, Wallingford, Oxon OX10 8DE, UK. pp. 216-231

Jones, R. J. and J. B. Lowry. 1984. Australian goats detoxify the goitrogen 3-hydroxy-4- (1H)-pyridone (DHP) after ruminal infusion from an Indonesian goat. Experientia. 40:1435-1436.

Jones, R. J. and R. G. Megarrity. 1986. Successful transfer of DHP-degrading bacteria from Hawaiian goats to Australian ruminants to overcome the toxicity of Leucaena. Aust. Vet. J. 63:259-262.

Julliand, V. A. deVaux, L. Millet, and G. Fonty. 1999. Identification of *Ruminococcus flavefaciens* as predominant cellulolytic bacterial species of the equine caecum. Appl. Environ. Microbiol. 65:3738-3741.

Kabir, A. M. A., Y. Aiba, A. Takagi, S. Kamiya, T. Miwa and Y. Koga. 1997. Prevention of *Helicobacter pylori* infection by lactobacilli in a gnotobiotic murine model. Gut. 41:49-55.

Kailasapathy, K. and J. Chin. 2000. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophillus* and *Bifidobacterium spp.* Immunol. Cell. Biol. 78:80-88.

Kandler, O. and N. Weiss. 1986. Genus *Lactobacillus*. Beijerinck 1901, 212 AL. In: Sneath, P. H. A., N. S. Mair, M. E. Sharpe and J. G. Holt (eds). Bergey's Manual of Systematic Bacteriology, Vol.2. The Williams and Wilkins Co., Baltimore. pp. 1209-1234.

Kaur, H., B. Singh, and N. Kewalramani. 1999. Phytoestrogenic content of some Indian fodders. Indian J. Dairy. Sci. 52:121-123.

Keddy, R. M. 1959. Properties and classification of lactobacilli isolated from grass and silage. J. Appl. Bacteriol. 22:403-416.

Khan, R. I., R. Onodera, M. R. Amin. and N. Mohammed. 1999. Production of tyrosine and other aromatic compounds from phenylalanine by rumen microorganisms. Amino Acids. 17:335-346.

Klieve, A. V. and T. Bouchop. 1988. Morphological diversity of bacteriophages from sheep and cattle. Appl. Environ. Microbiol. 54:1637-1641.

Klita, P. T., G. W. Mathieson, T. W. Fenton and R. T. Hardin. 1996. Effects of alfalfa root saponins on digestive function in sheep. J. Anim. Sci. 74:1144-1156.

Kobayashi,Y. and R. Onodera. 1999. Application of molecular biology to rumen microbes. Review. Asian-Aus. J. Anim. Sci. 12:77-83.

Kopency, J. and B. Hodrova. 1995. Pectinolytic enzymes of anaerobic fungi. Lett. Appl. Microbiol. 20:312-316.

Kozlowska, H. M. Naczk, F. Shahidi and R. Zadernowski. 1990. Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology (Ed. F. Shahid). AVI Book.

Krumholz. L. R. and M. P. Bryant. 1986. *Eubacterium oxidoreducens* sp. nov., requiring H_2 or formate to degrade gallate, pyrogallol, phloroglucinol and quercetin. Arch. Microbiol. 144:8-14.

Kulshreshtha, M. J., D. K. Kulshreshtha and R. P. Rastogi, 1992. Review article: The triterpenoids. Phytochemistry. 11:2369-2381.

Kumar, R and M. Singh. 1984. Tannins: their adverse role in ruminant nutrition. J. Agric. Food. Chem. 32:447-453.

Lee, S. F., C. W. Forsberg and A. M. Gibbins. 1992. Type II DNA restriction modification system and a endonuclease from the ruminal bacterium *Fibrobacter succinogenes* S85. J. Bacteriol. 174:5275-5283.

Lee, S. S., J. K. Ha and Y. J. Choi. 1996. Study on the

isolation and identification of rumen fungi, characterization of cellulolytic fungal enzymes, and its industrial utilization: VII. Distributions, properties and purification of cellulolytic enzymes produced by the KNGF-2 fungi isolated from the rumen of Korean native goat. Korean J. Anim. Nutr. Feed. 19:525-534.

Lee, S. S., K. J. Shin, W. Y. Kim, J. K. Ha and I. K. Han. 1999. The rumen ecosystem: as a fountain source of novel enzymes. Review. Asian-Aus. J. Anim. Sci. 12:988-1001.

Lei, X. G., P. K. Ku. E. R. Miller, M. T. Yokoyama and D. E. Ullrey. 1993. Supplementing corn-soybean meal diets with microbial phytase maximizes phytate phosphorus utilization in weanling pigs. J. Anim. Sci. 71:3368-3375.

Lekha, P. K. and B. K. Lonsane. 1997. Production and application of tannin acyl hydrolase: state of the art. Adv. Appl. Microbiol. 44:215-260.

Lenigan, G. W. 1972. Metabolism of pyrrolizidine alkaloids in the ovine rumen. IV. Effects of chloral hydrate and halogenated methanes on rumen methanogenesis and alkaloid metabolism in fistulated sheep. Aust. J. Agric. Res. 23:1085-1091.

Lenigan, G. W. 1976. *Peptococcus heliotrinreducans* sp. nov., a cytochrome producing anaerobe which metabolizes pyrrolizidine alkaloids. J. Gen. Microbiol. 94:1-10.

Li, X. L., H. Chen and L. G. Ljungdahl. 1996. Cloning , sequencing and over expression of a xylanase cDNA in *Escherichia coli* of the polycentric anaerobic fungus *Orpinomyces sp.* strains PC-2. Proc. 96th Ann. Meet. Amer. Soc. Microbiol. New Olreans. pp. 361.

Lindsey, T. O. 1990. Further studies into the mode of action of growth promotants in turkeys. 1990. Proc. of the Smith Kline Animal Health Products Pacesetter Conf.

STAFAC (Virginiamycin) for Turkeys, Jan.1990. San Diego, CA. USA. pp. 1-10.

Liu, J. H., L. B. Selinger, C. F. Tsai and K. J. Cheng. 1999. Characterization of *Neocallmastix patriciarum* xylanase and its products. Can. J. Microbiol. 45:970-974.

Lombardo de Barros, C. S., D. Driemeier, C. Pilati, S. S. Barros and L. M. L. Castilhos. 1992. *Senecio spp.* poisoning in cattle in southern Brazil. Vet. Hum. Toxicol. 34:241-246.

Loper, G. M., C. H. Hanson and J. H. Graham. 1967. Coumestrol content of alfalfa as affected by selection for resistance to foliar disease. Crop Sci. 7:189.

Lowry, J. B., M. Tangendjaja, and B. Tangendjaja. 1983. Optimising autolysis of mimosine to 3-hydroxy-4-(1H) pyridone in green tissues of *Leucaena leucocephala*. J. Sci. Food. Agric. 34:529-533.

Lu, C. D. and N. A. Jorgenson. 1987. Alfalfa saponins affect site and extent of nutrient digestion in ruminants. J. Nutr. 117:919-927.

Mahato, S. B., A. N. Ganguly and N. P. Sahu. 1982. Review: Steroid saponins. Phytochemistry. 21:959-978.

Majak, W. 1992. Further enhancement of 3-nitropropanol detoxification by ruminal bacteria in cattle. Can. J. Anim. Sci. 72:863-870.

Majak, W., K. J. Cheng and J. W. Hall. 1982. The effect of cattle diet on the metabolism of 3-nitro-propanol by ruminal microorganisms. Can. J. Microbiol. 62:855-860.

Majak, W. and K. J. Cheng. 1983. Recent studies on ruminal metabolism of 3-nitropropanol in cattle. Toxicon. 3 (Suppl.):265-268.

Majak, W. and M. A. Pass. 1989. Aliphatic nitro-compounds. In: Toxicants of Plant Origin. P. R. Cheeke (Ed.), Vol. II. CRC Press, Boca Raton, FL.

Mangan, J. L. 1988. Nutritional effects of tannins in animal feeds. Nutr. Res. Rev. 1:209-231.

Maraunek, M. and D. Duskova. 1999. Metabolism of pectin in rumen bacteria *Butyrivibrio fibrisolvens* and *Prevotella ruminicola*. Lett. Appl. Microbiol. 29:429-433.

Martin, S. A. and J. A. Martin. 1998. News and notes: paper digestion by the cellulolytic ruminal bacterium *Fibrobacter succinogenes*. Curr. Microbiol. 37:431-432.

Mathison, G. W., R. Soofi-Siawash, P. T. Klita., E. K. Okine and G. Sedgwick. 1999. Degradation of alfalfa saponins in the digestive tract of sheep and their rate of accumulation in rumen fluid. Can. J. Anim. Sci. 79:315-319.

Matsuzaki, T. and J. Chin. 2000. Modulating immune responses with probiotic bacteria. Immunol Cell. Biol. 78:67-73.

Mattocks, A. R. 1986. Chemistry and Toxicology of Pyrrolizidine Alkaloids. Academic Press, San Diego, CA.

McCosker, T. H. 1989. Ruminal detoxification of fluoroacetate. Agric. Sci. New Series. 2:46-47.

McEwan, T. 1964. Isolation and identification of toxic principle of *Gastrolobium grandiflorum*. Qld. J. Agric. Sci. 21:1-14.

McIlory, J. C. 1984. The sensitivity of animals to 1080 poison . VI Bandicoots. Aust. Wildlife Res. 11: 373-385.

McKenzie, R. A., R. J. W. Gartner, B. J. Blaney and R. J. Glanville. 1981. Control of nutritional secondary hyperparathyroidism in grazing horses with calcium and phosphorus supplimentation. Aust. Vet. J. 57:554-557.

McNeil, D. L., B. J. Carrol and P. M. Gresshoff. 1984. The interaction between nitrogen and carbon metabolism in nitrogen fixing soybean bacteroids. Adv. Agric. Biotechnol. 4:515-519.

Meggarity, R. G., and R. J., Jones. 1986. Successful transfer of DHP-degrading bacteria from Hawaiian goats to Australian ruminants to overcome the toxicity of Leucaena. Aust. Vet. J. 63:259-262.

Mikhailov, I., G. Russev and B. Anachkova. 2000. Treatment of mammalian cells with mimosine generates DNA breaks. Mut. Res. 459:299-306.

Miller, C., L. Kennington, R. Cooney, Y. Kohjimoto, L. C. Cao, T. Honeyman, J. Pullman, J. Jonassen and C. Schied. 2000. Oxalate toxicity in renal epithelial cells: characteristics of apoptosis and necrosis. Toxicol. Appl. Pharmacol. 162:132-141.

Mohammed, N., R. Onodera and R. I. Khan. 1999. Tryptophan biosynthesis and production of related compounds from indolepyruvic acid by mixed ruminal bacteria, protozoa, and their mixture in vitro. J. Gen. Appl. Microbiol. 45:143-148.

Mohan, M. W. and R. M. Teather. 1995. Partial purification and characterization of Bfi 571 and Bfi 891 restriction endonucleases from different strtains of *Butyrivibrio fibrisolvens*. Gene. 155:131-132.

Molnarova, V., P. Pristas and P. Javorsky. 1999. Prevalance of CTGCAG recognizing restriction and modification systems in ruminal selenomonades. Anaerobe. 5:37-41.

Morata de Ambrosini, V., S. Gonzalez, G. Perdigon, A. P. de Ruiz Holgado and G. Oliver. 1998. Immunostimulating activity of cell walls from lactic acid bacteria and related species. Food and Agric. Immunol. 10:183-191.

Morrison, M., R. I. Mackie and B. A. White. 1992a. Partial characterization of a DNA restriction endonuclease from *Ruminococcus flavefaciens* FD-1 and its inhibition by site-specific adenine methylation. Appl. Environ. Microbiol. 58:66-69.

Morrison, M., R. I. Makie and B. A. White. 1992b. Partial purification and characterization of Ral 8I, A class IIS restriction endonuclease from *Ruminococcus albus* 8 which recognizes 5'-GGATC-3'. Gene.111:105-108.

Morton, J. F. 1989. Creeping indigo (*Indigo spicata* Forsk.) (Fabaceae)- A hazard to herbivores in Florida. Econ. Bot. 43: 314-327.

Mroz, Z., A. W. Jongbloed, P. A. Kemme. 1994. Apparent digestibility and retention of nutrients bound to phytate complexes as influenced by microbial phytase and feeding regimen in pigs. J. Anim. Sci. 72:126-132.

Mundt, J. O. and J. L. Hammer. 1968. Lactobacilli on plants. Appl. Microbiol. 16:1326-1330.

Nelson, K. E., A. N. Pell, P. Schofield and S. Zinder. 1995. Isolation and characterization of anaerobic ruminal bacterium capable of degrading hydrolysable tannins. Appl. Environ. Microbiol. 61:3293-3298.

N R C. National Research Council. 1984. Leucaena: promising forage and tree crop for the tropics. 2nd ed. National Academy Press, Washington, DC.

Oakenfull, D and G. S. Sidhu. 1989. Saponins. In: Toxicants of Plant origin. Vol. II Glycosides (Ed. P. R. Cheeke). CRC Press, Boca Raton, FL pp. 97-141.

Odenyo, A. A., R. I. Mackie, D. A. Stahl and B. A. White. 1994. The use of 16S rRNA-targeted oligonucleotide probes to study competition between ruminal fibrolytic bacteria: Development of probes for *Ruminococcus* species and evidence for bacteriocin production. Appl. Environ. Microbiol. 60:3688-3696.

Odenyo, A. A., P. O. Osuji, O. Karanfil and K. Adinew. 1997. Microbiological evaluation of *Acacia angustissimia* as a protein suppliment for sheep. Anim. Feed. Sci. Technol. 65:99-112.

Odenyo, A. A. and P. O. Osuji. 1998. Tannin-tolerant ruminal bacteria from East African ruminants. Can. J. Microbiol. 44:905-909.

Oleszek, W., J. Nowacka, J. M.Gee, G. M. Wortley and I. T. Johnson. 1994. Effects of some purified alfalfa (*Medicago sativa*) saponins on transmural potential difference in small intestine. J. Sci. Food Agric. 65:35-39.

Oliver.A. J., D. R. King and R. J. Mead. 1979. Fluoroacetate tolerance, a genetic marker in some Australian mammals. Aust. J. Zool. 27:363- 372.

Oppenheim, E. W., I. M. Nasrallah, M. G. Mastri and P. J. Stover. 2000. Mimosine is a cell specific antagonist of folate metabolism. J. Biol. Chem. 275:19268-19274.

Orpin, C. G. 1988. Nutrition and biochemistry of anaerobic Chytridiomycetes. BioSystems. 21:365-370.

Osawa, R. 1992. Tannin-protein complex degrading enterobacteria isolated from the alimentary tracts of koalas and a selective medium for their enumeration. Appl. Environ. Microbiol. 58:1754-1759.

Osawa, R. and L. I. Sly. 1992. Occurance of tannin-protein complex degrading *Streptococcus sp.* in feces of various animals. Syst. Appl. Microbiol. 15:144-147.

Osawa, R., T. Fugisawa and L. I. Sly. 1995. *Streptococcus gallolyticus* sp. nov., gallate degrading organisms formerly assigned to *Streptococcus bovis*. Syst. Appl. Microbiol. 18:74-78.

Osawa, R., F. Rainey, T. Fujisawa, E. Lang, H. J. Busse, T. P. Walsh and E. Stackebrandt. 1995. *Lonepinella koalarum* gen. nov., a new tannin-protein complex degrading bacterium. Syst. Appl. Microbiol. 18:56-62.

Osawa, R., K. Kuroiso, S. Goto and A. Shimizu. 2000. Isolation of tannin-degrading lactobacilli from humans and fermented foods. Appl. Environ. Microbiol. 66: 3093-3097.

Pack, M., M. Bedford and C. Wyatt. 1998. Feed enzymes may improve corn, sorghum diets. Feedstuffs. 70:18-19.

Paster, B. J. and E. Canale-Parola. 1985. *Treponema saccharophilum* sp. nov., a large pectinolytic spirochete from the bovine rumen. Appl. Environ. Microbiol. 50: 212-219.

Pen, J., T. C. Verwoerd, P. A. van Paridon. 1993. Phytase containing transgenic seeds as novel feed additives for improved phosphorus utilisation. Bio/Technology. 11:811-814.

Pettipher, G. L. and M. J. Latham. 1979. Production of enzymes degrading plant cell walls and fermentation of cellobiose by *Ruminococcus flavefaciens* in batch and continuous culture. J. Gen. Microbiol. 110: 29-38.

Pollman, D. S., D. M. Danielson and E. R. Jo. 1980. Effects of microbial feed additives on performance of starter and growing-finishing pigs. J. Anim. Sci. 51:577-581.

Price, P .R. and G. R. Fenwick. 1985. Naturally occuring estrogens in food. A review. Food Add. Chem. 2:73-106.

Price, K. R., I. T. Johnson and G. R. Fenwick. 1987. The chemistry and biological significance of saponins in foods and feeding stuffs. CRC Crit. Rev. Food Sci. Nutr. 26:27-135.

Prins, R. A. 1977. Biochemical activities of gut microorganisms. In: Microbial Ecology of Gut. Clarke, R.T. J. and T. Bouchop (eds). Accademic Press, London. UK.

Pristas, P., I. Vanat, A. Godany and P. Javorsky. 1994. Restriction endonucleases from *Selenomonas ruminantium* which recognize and cleave 5'-AT/TAAT-3'. Arch. Microbiol. 161:439-441.

Pristas, P., I. Vanat and P. Javorsky. 1995. Isolation and characterization of a new restriction endonuclease, Sru 30DI from *Selenomonas ruminantium*. Gene. 158:139-140.

Pristas, P., K. Fliegerova and P. Javorsky. 1998. Two restriction endonucleases in *Selenomonas ruminantium* subsp. lactilytica. Lett. Appl. Microbiol. 27:83-85.

Qiu, X., L. B. Selinger, L. J. Yanke and K. J. Cheng. 2000. Isolation and analysis of two cellulase cDNAs from *Orpinomyces joyonii*. Gene. 245:119-126.

Rajakumar, G. S. and S. C. Nandy. 1983. Isolation, purification and some properties of Penicillium chrysogenum tannase. Appl. Environ. Microbiol. 46:525-527.

Rasmussen, M. A., M. J. Allison and J. G. Foster. 1993. Flatpea intoxication in sheep and indication of ruminal

adaptation. Vet. Hum. Toxicol. 35:123-127.

Reddy, N. R., J. K. Palmer and M. D. Pierson. 1984. Hydrolysis of wheat straw hemicelluloses and heteroxylan (larchwood) by human colon *Bacteroides ovatus* B4-11 enzymes. J. Agric. Food Chem. 32:840-844.

Reed, J. D. 1995. Nutritional toxicity of tannins and polyphenols in forage legumes. J. Anim. Sci. 73:1516-1528.

Reid, G., A. W. Bruce and M. Taylor. 1995. Instillation of Lactobacillus and stimulation of indigenous organisms to prevent recurrence of urinary tract infections. Microbiol. Ther. 23:32-45.

Reid, G. 1999. Mini review: Scientific basis for probiotic strains of Lactobacillus. Appl. Environ. Microbiol. 65:3763-3766.

Rosenblum, E. R., R. E. Stauber, D. H. Van-Thiel, I. M. Campbell and J. S. Gavaler. 1993. Assessment of the estrogenic activity of phytoestrogens isolated from bourbon and beer. Alcohol Clin. Experimental Res. 17: 1207-1209.

Rosenthal, G. A. and D. H. Janzen. 1979. Herbivores: Their Interaction with Secondary Plant Metabolites. New York Academic Press.

Rowe, L. D., G. W. Ivie, J. R. DeLoach and J. G. Foster. 1993. The toxic effects of mature flat pea (*Lathyrus sylvestris* L. *CV.* Lathco) on sheep. Vet. Hum. Toxicol. 35: 127-133.

Rowland, I., H. Wiseman, T. Sanders, H. Aldercreutz and E. Bowey. 1999. Metabolism of oestrogens and phytoestrogens: role of the gut microflora. Biochemical Society Transactions. 27:304-308.

Rowland, I. R., A. K. Mallet, C. A. Bearne and M. J. G. Farthing. 1986. Enzyme activities in the hind gut microflora of laboratory animals and man. Xenobiotica. 16:519-523.

Russel, G. R. and R. M. Smith. 1968. Reduction of heliotrine by a rumen microorganism. Aust. J. Biol. Sci. 21:1277-1290.

Salunkhe, D. K., J. K. Chavan and S. S. Kadam. 1989. Nutritional consequences of dietary tannins. In: Dietary Tannins: Consequences and Remedies. CRC Press. Boca Raton, FL. pp. 113-146.

Sanders, M. E. 1993. Effect of consumption of lactic cultures on human health. Adv. Food. Nutr. Res. 37:67-130.

Sanderson, G. W., A.S. Ranadive, L. S. Eisenberg, F. J. Farrel. R. Simons, C. H. Manley and P. Coggon. 1975. Contribution of polyphenolic compounds to the taste of tea. In: Phenolic, Sulfur and Nitrogen Copmpound in Food Flavors. G. Charalambous and I. Katz (eds). ACS Symposium Series 26. American Chemical Society, Washington. DC. pp. 14-46.

Savage, D. C. 1977. Microbial ecology of the gastrointestinal tract. Ann. Rev. Microbiol. 31:107-133.

Schneider, H. and M. Blaut. 2000. Short communication: anaerobic degradation of flavonoids by *Eubacterium ramulus*. Arch Microbiol. 173:71-75.

Seawright., A. A. 1982. Animal Health in Australia. Vol 2. Chemical and Plant Poisons. Australian Bureau of Animal Health. Canberra: Australian Govt. Publishing Service.

Segall, H. J., A. W. Wilson, J. L. Dallas and W. F. Haddon.1985. Trans-4-hydroxy-hexanal: a reactive metabolite from the macrocylic pyrrolizidine alkaloids senecionine. Science. 229:472-475.

Selinger, L. B., C. W. Forsbrerg and K.-J. Cheng. 1996. The Rumen: A unique source of enzymes for enhancing livestock production. Anaerobe. 2:263-284.

Sharpe, M. E., M. J. Lastham, E. I. Garvie, J. Zirngbil and O. Kandler. 1973. Two new species of *Lactobacillus* isolated from the bovine rumen, *Lactobacillus ruminis* sp. nov., and *Lactobacillus vitululinis* sp. nov. J. Gen. Microbiol. 77:37-49.

Sharma, O. M. P., H. Adlercreutz. J. D. Strandberg, B. R. Zirkin, D. S. Coffee and L. L. Ewing. 1992. Soy of dietary source plays a preventive role against the pathogenesis of prostatitis in rats. J. Steroid Biochem. Molec. Biol. 43:557.

Sharrow, S. H. and W. D. Mosher. 1982. Sheep as biological control agent for tancy ragwort. J. Range Man. 35:480-482.

Shewale, J. G. 1982. Beta-glucosidase: its role in cellulose synthesis and hydrolysis of cellulose enzymes, cellulolytic fungi, *Aspergillus niger*, *A. phoenicis*, *Sclerotium rolfsi*, *Sporotrichum pulverulentum*, *Trichoderma reesei*. Int J. Biochem. 14:435-443.

Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Bos, M. G. E. Wolters, R. F. Beudeker and J. G. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64:525-540.

Singh, B., P. R. Sinha and R. N. Sinha. 1996. Plasmid linked bacteriocin production in selected strains of lactobacilli. Indian J. Dairy Bio. Sci. 7:18-24.

Skene, I. K. and J. D. Brooker. 1995. Characterization of tannin acyl hydrolase activity in the ruminal bacterium *Selenomonas ruminantium*. Anaerobe. 1:321-327.

Southon, S., A. J. A. Wright, K. R. Price, S. J. Fairweather-Tait and G.R. Fenwick. 1988. The effect of three types of saponins on iron and zinc absorption from a single meal in the rat. Br. J. Nutr. 59:389-396.

Spencer, C. M., Y. Cai, R. Martin, S. H. Gaffney, P. N. Goulding, D. Magnolato, T. H. Lilley and E. Haslam. 1988. Polyphenol complexation-some thoughts and observations. Phytochemistry. 27:2397-2409.

Spoelstra, S. F. 1990. Effects of cell wall degrading enzymes on silage composition at different ensilage conditions. Proc. 9th Silage Conf. The University of Newcastle upon Tyne, England.

Stunzi, H., D. A. Perrin, T. Teitei and R. L. N. Harris. 1979. Stability constants of some metal complexes formed by mimosine and related compounds. Aust. J. Chem. 32: 21-30.

Teather, R. M. and R. J. Forster. 1998. Manipulating the rumen microflora with bacteriocins to improve ruminant production. Can. J. Anim. Sci. 78 (Suppl.): 57-69.

Tierny, Y., M. Bechet, J. -C Joncquiert, H. -C. Dubourguler and J. B. Guillaume. 1994. Molecular cloning and expression in Escherichia coli of genes encoding pectate lyase and pectin methylesterase activities from *Bacteroides thetaiotaomicron*. J. Appl. Bacteriol. 75:592-602.

Titgemeyer, E. C., L. D. Bouquin, G. C. Fahey and K. A. Garleb. 1991. Fermentability of various fiber sources by

human fecal bacteria in vitro. Am. J. Clin. Nutr. 53:1418-1424.

Trinci, A. P. J., D. R. Davies, K. Gull, M. I. Lawrence, B. B. Nielson, A. Rickers and M. K. Theodorou. 1994. Anaerobic fungi in herbivorous animals. Mycol. Res. 98:129-152.

Twigg, L. E. and D. R. King. 1991. The impact of fluoroacetate-bearing vegetation on native Australian fauna: a review. OIKOS. 61:412-430.

Twigg, L. E., D. E. King, H. M. Davis, D. A. Saunders and R. J. Mead. 1988. Tolerance to, and metabolism of fluoroacetate in the emu. Aust. Wildlife Res. 15:239-247.

Van der Meer, W. M. de Vos., S. Harayama and A. J. B. Zehnder. 1992. Molecular mechanisms of genetic adaptation to xenobiotic compounds. Appl. Environ. Microbiol. 56:677-694.

Van Gorcom, R. F. M., W. Van Hartingsveldt, P. A. Van Paridon, A. E. Veenstra, R. G. M. Luiten and G. C. M. Selten. 1995. Cloning and expression of phytase from Aspergillus. U.S. Patent No. 5,434,156.

van Gylswyk, N. O. and J. J. T. K. van der Toorn. 1987. *Clostridium aerotolerans* sp. nov., a xylanolytic bacterium from corn stover and from the rumina of sheep fed corn stover. Appl. Environ. Microbiol. 37:102-105.

Van Veen, A. G. 1973. Toxic properties of certain unusual feeds. pp 464-476. In: Toxicants occuring naturally in foods. National Academy of Sciences, Washington, D.C.

Vanat, I., P. Pristas, E. Kutejova, J. Judova, A. Godany and P. Javorsky. 1993a. SbvI restriction endonuclease from *Streptococcus bovis*. Lett. Appl. Microbiol. 17:297-299.

Vanat, I., P. Pristas, E. Rybosava, A. Godany and P. Javorsky. 1993b. SruI restriction endonuclease from *Selenomonas ruminantium*. FEMS Microbiol Lett. 113: 129-132.

Varel, V. H., I. M. Robinson and H. -J. G. Jung. 1987. Influence of dietary fibre on xylanolytic and cellulolytic bacteria of adult pigs. Appl. Environ. Microbiol. 53:22-26.

Vincet, J. G., R. C. Veonett and R. G. Riley. 1959. Antibacterial activity associated with *Lactobacillus acidophilus*. J. Bacteriol. 78:477-484.

Volokita, M., A. Abeliovich, M. Ines and M. Soares. 2000. News and notes: detection of microorganisms with overall cellulolytic activity. Curr. Microbiol. 40:135-136.

Wachenheim, D. E., L. L. Blythe, A. M. Craig 1992. Characterization of rumen bacterial pyrrolizidine alkaloid biotransformation in ruminants of various species. Vet. Human Toxicol. 34:513-517.

Wallace, R. J. and A. Chesson. 1995. Biotechnology in Animal Feeds and Animal Feeding. VCH, Wienheim, Germany.

Wang, Y., T. A. McAllister, L. J. Yanke and P. R. Cheeke. 2000. Effect of steroidal saponins from *Yucca schidigera* extract on ruminal microbes. J. Appl. Microbiol. 88:887-896.

Wells, J. E., D. O. Krause, T. R. Callaway and J. B. Russel. 1997. A bacteriocin-mediated antagonism by ruminal lactobacilli against *Streptococcus bovis*. FEMS Microbiol. Ecol. 22: 237-244.

Whistler, R. H. A. and E. L. Richards. 1970. Hemicellulose. In: The Carbohydrates. Pigman, W and D. Horton (eds). Academic Press. NY.

Whitten, P. L, C. Lewis, E. Russel and F. Naftolin. 1995. Potential adverse effects of phytoestrogens. J. Nutr. 125(suppl.): 771S-776S.

Williams, M. C. and R. C. Barneby. 1977a. The occurence of nitrotoxins in North American Astragalus (Fabaceae). Brittonia. 29 :310-326.

Williams, M. C. and R. C. Barneby. 1977b. The occurrence of nitrotoxins in Old World and South American *Astragalus* (Fabaceae). Brittonia. 29:327-336.

Williams, M. C., L. F. James and B. O. Bond. 1979. Emoy milkvetch (*Astrgalus emoryanus* var *emoryanus*) poisoning in chicks, sheep and cattle. Am. J. Vet. Res. 40:403-406.

Wojciechowicz, M and A. Ziolecki. 1984. A note on pectinolytic enzymes of *Strptococcus bovis*. J. Appl. Bacteriol. 56:515-518.

Wood, T. M. 1991. Fungal Cellulase. In: Biosynthesis and Biodegradation of Cellulose. Haigler, C.H. and P.J. Weimer (eds). Marcel Dekker, NY. pp. 499-533.

Yanke, L. J., Bae, H. D., L. B. Selinger and K. -J. Cheng. 1998. Survey of phytase activity of anaerobic ruminal bacteria. Microbiology. 144:1565-1573.

Yanke, L. J., L. B. Selinger and K. J. Cheng. 1999. Phytase activity of *Selenomonas ruminatium*: a preliminary characterization. Lett. Appl. Microbiol. 29:20-25.