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Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems

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Livestock and aquaculture production is under political and social pressure, especially in the European Union (EU), to decrease pollution and environmental damage arising due to animal agriculture. The EU has banned the use of antibiotics and other chemicals, which have been shown to be effective in promoting growth and reducing environment pollutants because of the risk caused to humans by chemical residues in food and by antibiotic resistance being passed on to human pathogens. As a result of this, scientists have intensified efforts in exploiting plants, plant extracts or natural plant compounds as potential natural alternatives for enhancing the livestock productivity. This paper discusses work on the effects of various phytochemicals and plant secondary metabolites in ruminant and fish species. The focus is on (i) plants such as Ananas comosus *(pine apple),* Momordica charantia *(bitter gourd) and* Azadirachta indica *(neem) containing anthelmintic compounds and for their use for controlling internal parasites; (ii) plants containing polyphenols and their applications for protecting proteins from degradation in the rumen, increasing efficiency of microbial protein synthesis in rumen and decreasing methane emission; for using as antioxidants, antibacterial and antihelmintic agents; and for changing meat colour and for increasing n-3 fatty acids and conjugated linoleic acid in meat; (iii) saponin-rich plants such as quillaja, yucca and* Sapindus saponaria *for increasing the efficiency of rumen fermentation, decreasing methane emission and enhancing growth; for producing desired nutritional attributes such as lowering of cholesterol in monogastric animals; for increasing growth of fish (common carp and Nile tilapia) and for changing male to female ratio in tilapia; and for use as molluscicidal agents; (iv)* Moringa oleifera *leaves as a source of plant growth factor(s), antioxidants, beta-carotene, vitamin C, and various glucosinolates and their degraded products for possible use as antibacterial, antioxidant, anticarcinogenic and antipest agents; (v)* Jatropha curcas *toxic variety with high levels of various phytochemicals such as trypsin inhibitor, lectin, phytate and phorbol esters in seeds limiting the use of seed meal in fish and livestock diets; and the use of phorbol esters as bio-pesticidal agent; and (vi) lesser-known legumes such as* Entada phaseoloides *seeds containing high levels of trypsin inhibitor and saponins,* Sesbania aculeate *seeds rich in non-starch polysaccharides and* Mucuna pruriens *var.* utilis *seeds rich in* L*-3,4-dihydroxyphenylalanine and their potential as fish feed;* Cassia fistula *seeds as a source of antioxidants; and the use of* Canavalia ensiformis, C. gladiata *and* C. virosa *seeds containing high levels of trypsin inhinitor, lectins and canavanine. The paper also presents some challenges and future areas of work in this field.*

Keywords: aquaculture systems, bioactivity, livestock systems, phytochemicals, plant secondary metabolites

Introduction

Livestock and aquaculture production is under political and social pressure, especially in the European Union (EU), to decrease pollution and environmental damage arising from animal agriculture. Some antibiotics and growth promoters such as monensin, avoparcin, flavomycin, virginiamycin and growth hormone have been shown to be effective in reducing environment pollutants, and in enhancing feed conversion efficiency and increasing the livestock productivity. However, since 2006, the EU has banned the use of antibiotics and other chemicals, because of the risk to humans of chemical residues in food and of antibiotic resistance being passed on to human pathogens. As a result of this, scientists have intensified efforts in exploiting plants, plant extracts or natural plant compounds as

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potential natural alternatives for enhancing livestock productivity. The use of various herbs and medicinal plants has a long history. They have been used since ancient times, especially in oriental countries, as remedial measures against various animal and human ailments. However, the intensification of livestock production and the advent of antibiotics led to a decline in their usage and less interest in providing scientific bases to their effects. The adverse effects of using antibiotics and other synthetic compounds on human and animal health and on product quality and safety have rejuvenated interest in the fields of 'phytochemistry, phytopharmacology, phytomedicine and phytotherapy' during the last decade. The plant kingdom might provide a useful source of new medicines, pharmaceutical entities and bioactive compounds for enhancing animal production and health; and food safety and quality, while conserving environment.

According to an estimate by the Food and Agriculture Organization (FAO), human consumption of fish is expected to touch 110 million metric tonnes (Mt) by 2010 from the current level of about 90 Mt. This, along with the 'livestock revolution' taking place, especially in developing countries, coupled with continued human population growth, urbanisation and income growth are imposing a huge burden on the environment and resources.

In this paper, we discuss work conducted largely in our laboratory over the last decade on the effects of various phytochemicals and plant secondary metabolites in ruminant and fish species. There are changing perceptions regarding the therapeutic potential of various plant secondary metabolites, which traditionally have been termed as antinutrients. It is hoped that the information collated and discussed here will lead to further exploration and use of plants, or natural plant products, to decrease environmental pollutants and enhance animal productivity. This sustainable and environmentally friendly approach to agriculture (clean, safe and green agriculture) would be a 'win–win' situation for both farmers and the society.

Plants containing anthelmintic compounds

Parasitism by gastrointestinal nematodes is one of the major constraints on livestock production, especially when the nutritional status of the animals is poor. Subclinical infections of gastrointestinal nematodes such as *Ostertagia circumcinta*, *Trichostrongylus colubriformis* and *Haemonchus contortus* decrease feed intake, body-weight gain, and milk and wool production. In subtropical and tropical areas of the world where the animals are on low quality feeds and have poor nutritional status, mortality and morbidity due to nematode infection are widespread (Singh *et al*., 2003). There is a growing realisation that chemical anthelmintic treatment, on its own, may not provide a long-term strategy for managing parasites in grazing animals. The widespread development and prevalence of resistant strains of nematode parasites and public concern over drug residues excreted in animal products have stimulated efforts to identify and use plant-based anthelmintic compounds.

Leaves of *Ananas comosus* (pineapple) and *Momordica charantia* (bitter gourd) have been assessed for their anthelmintic efficacy. These plants reduced faecal worm egg counts of infected calves (50 to 100 kg live weight) after weekly bolus doses for at least 2 weeks. Comparison of bolus doses (1 g each of dry leaf plus molasses per kg body weight; weekly dose for 3 weeks) of *A. comosus* or *M. charantia* with albendazole (a single dose of 5 mg/kg body weight) showed similar levels of efficacy in reducing (94%) faecal worm egg counts. Efficacy assessment of these plants for calves after inclusion in the urea molasses multinutrient blocks (35% dry leaves in blocks; intake of block 250 g/day for 21 days) showed decreases of 80%, and 77% in faecal worm egg counts by *A. comosus*- and *M. charantia*-containing blocks, respectively; and 89% reduction in faecal worm egg count was observed with fenbendazole-containing blocks (0.5 g fenbendazole per kg block; intake of block 250 g/day for 7 days). Overall, the efficacy of *A. cosmosus* leaves was higher than that of *M. charantia* leaves (Daing and Win, 2006).

In studies conducted on calves in Bangladesh (Akbar and Ahmed, 2006), pineapple and neem (*Azadirachta indica*) leaves were also demonstrated to have anthelmintic effects. Fresh pine apple leaves (1.6 g/kg body weight) and fresh neem leaves (1 g/kg body weight) (both leaves on a dry-matter basis were 200 mg/kg body weight) given as a single dose were compared with that of albendazole given at a rate of 7.5 mg/kg body weight. On day 7, the efficacy of albendazole (100% reduction in faecal worm egg count) was higher than that of pineapple and neem leaves (76% and 55% reduction, respectively; $P < 0.01$); and on day 14, the percent reduction in faecal worm egg counts for albendazole and pineapple (88% and 82% reduction) were higher than that for neem leaves (56% reduction; $P < 0.05$). Similar results were observed on day 21 (Akbar and Ahmed, 2006). In the same study, urea molasses multinutrient blocks were used as a vehicle for giving these plant materials to dairy cows kept in a research station. Freeze-dried leaves were incorporated in the blocks so that the intake of these leaves was 200 mg dry matter per kg body weight of animals. The intake of the blocks was 500 g/day per cow (40 mg dry matter per kg body weight of animal per day) and the blocks were fed for a total of 5 days. After 15 days of consumption, blocks containing pineapple leaf decreased faecal worm egg counts by 72% and those containing neem leaves decreased by 45%. In contrast, the control blocks (free of these leaves) reduced the count by only 5%. These values after 60 days post-treatment were 84%, 63% and 18%, respectively. Similar results were obtained when these blocks were tested in milking cows on-farm. Both the herbal remedies, when incorporated into the block, increased milk yield (26%) and live weight of animals (15%) significantly when compared with non-medicated blocks. The feeding of blocks

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containing pineapple and neem leaves increased farmers' net profits by 122% and 33%, respectively (Akbar and Ahmed, 2006). These data on efficacy from all three treatments indicate that pineapple leaves are better herbal anthelmintics than neem leaves. Similarly, in Vietnam, feeding blocks containing pineapple leaves (150 g dry leaves per kg block; intake of dry leaves: 1 g/day per animal) to growing heifers (average body weight: 177 kg) decreased faecal worm egg counts by 74% to 86% after 90 days of feeding and increased the daily weight gain of beef heifers by approximately 26% when compared with those fed blocks without pineapple leaves (Doan *et al.*, 2006).

The extent of use of these blocks, cost/benefit ratio and increase in income of farmers using these medicated blocks have been summarised in Makkar (2006). This paper also gives information on the use of some undefined herbal and commercial plant mixes for controlling nematodes and for enhancing productive and reproductive responses.

In contrast, the results from studies conducted in Africa on the effects of various plant sources, including pineapple leaves, on ruminant nematodes were less encouraging (Githiori *et al.*, 2004). Although cysteine proteases (bromelain) present in pineapple plant are considered to have some anthelmintic properties, there is a need to identify the active principle in pineapple leaves unequivocally and to investigate its presence in various germplasm existing in Asia and Africa and in different countries within Asia. It is also evident from the studies reported from Asia that different amounts of pineapple leaves have been used by workers, giving almost similar efficacy against nematodes. The effect of environment and soil conditions could also affect the level of the active principle, and this also needs to be investigated.

Another plant that seems to have a direct effect on gastrointestinal nematodes is eucalyptus. It has been shown to be effective against *H. contours* and *T. colubriformis* (Bennet-Jenkins and Bryant, 1996; Lorimer *et al.*, 1996). These effects are attributed to the presence of tannins/polyphenols in eucalyptus.

In the past decade, many reports have emerged showing anthelmintic effects of tannins/polyphenols and the benefits they could provide to livestock by decreasing nematode load in extensive grazing production systems (Singh *et al.*, 2003). These effects on nematodes are attributed to an improved protein supply due to increased rumen undegradable protein and their availability post-rumen and also to the direct action of tannins against nematodes. Recently in Tunisia, it was shown that *Acacia cyanophylla* foliage, a tannin-rich legume shrub species, has an antiparasitic effect in sheep. The faecal worm egg count in Barbarine lambs fed previously on oaten hay was reduced by 68% when they were fed acacia foliage for 25 days. Administration of polyethylene glycol (PEG; 50 g/day), a tannin-deactivating reagent, increased faecal worm egg counts slightly (34%) in sheep on acacia compared with those on acacia without PEG. However, these treatments did not affect the composition and the structure of the parasite genera recovered after copro-culture (Akkari *et al.*, 2007).

There is a need to develop a simple screening method to identify plants having anthelmintic potential. For tannin containing plants, it would be interesting to look into the correlation of chemical and biological tannin assays with anthelmintic bioassay(s) and with the reduction in nematode numbers observed *in vivo*.

Plants containing tannins

Tannins are polyphenolic substances. Their multiple phenolic hydroxyl groups lead to the formation of complexes primarily with proteins and to a lesser extent with metal ions, amino acids and polysaccharides. These are considered to have both adverse and beneficial effects depending on their concentration and nature as well as other factors such as animal species, physiological state of the animal and composition of the diet. Although research on tannins has a long history, considerable additional research must be carried out to exploit fully the benefits of incorporating tannin-rich plants and agro-industrial by-products in livestock feed and to develop strategies to manage these resources effectively so that tannins do not produce adverse effects. Some of the beneficial effects of tannins are enhancement of rumen undegradable protein and making feed protein available post-ruminally for production purposes, enhancement of efficiency of microbial protein production, and protection of ruminants from bloat. Some tannins are also known to have strong anticarcinogenic and antioxidant activities (Perchellet *et al.*, 1996; Riedl *et al.*, 2002).

Protection of protein from degradation in the rumen

Feeding 100 g of air-dried *A. cyanophylla* leaves with 200 g of soya-bean meal increased daily gain of lambs by 55%, when compared with lambs offered oaten hay-based diets. The increased daily gain was possibly a result of the soya-bean proteins being protected from degradation in the rumen by the leaf tannins and the consequent increase in protein availability post-ruminally. To achieve such effects, soya-bean meal should be offered after consumption of the acacia leaves. In this experiment, diet total phenols (as tannic acid equivalent)/diet protein, and total tannins (as tannic acid equivalent)/diet protein ratios were 0.043 and 0.021, respectively. Inclusion of higher amounts of acacia leaves to the concentrate had adverse effects on productivity (Ben Salem *et al.*, 2005). Nsahlai *et al.* (1999) also demonstrated the potential to use tropical tanniniferous shrub/tree foliage to increase the proportion of rumen undegradable protein in sheep diets. They ascribed the increased growth rate in sheep fed on teff straw and supplemented with oilseed cakes with small amounts of *Acacia albida* pods, rich in condensed tannins, to increased organic matter and nitrogen intake and/or to a more efficient use of nutrients. Similarly, Bhatta *et al.* (2000) showed inclusion of 7.5% of tamarind (*Tamarindus indica*, Linn) seed husk in a

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concentrate diet (0.75% tannin content in the diet) increased milk production and growth rate, which was attributed to the protection of dietary protein from degradation in the rumen. A simultaneous benefit obtained in these studies was the partitioning of excreted nitrogen in a manner that lower nitrogen was excreted in the urine and higher in the faeces, thus making available manure with a higher level of nitrogen for crop production. In the tropical countries, up to 70% of urine-nitrogen can be lost to the environment. Lowering the excretion of nitrogen in urine by using tannins could decrease environmental pollution in these regions significantly. The potential benefits of tannin-containing temperate forages, e.g. *Lotus corniculatus*, *Lotus pedunculatus*, and *Hedysarum coronarium* have been demonstrated in numerous studies in New Zealand (Barry and McNabb, 1999; Min *et al*., 2003).

Skatole and indole are flavour compounds formed in the rumen, which exert negative effects on meat flavour and quality. These compounds originate from deamination and decarboxylation of the amino acid tryptophan by rumen microbes and are present in meat fat. Rumen bacteria that produce a range of indolic compounds have recently been described (Attwood *et al*., 2006). In *in vitro* studies, condensed tannins from *Lotus corniculatus* have been demonstrated to reduce the production of skatole, and this was attributed to decreased rumen protein degradation by lotus tannins (Schreurs *et al*., 2004). Condensed tannins from *Dorycnium rectum* also inhibited skatole and indole formation in the *in vitro* rumen system (Tavendale *et al*., 2005). Conversely, Priolo *et al*. (2005) reported that when lambs were fed a good-quality grass (sulla, *Hedysarum coronarium)* containing tannins (CT = 1.8% DM), the supplementation of PEG did not affect the content of skatole in perirenal fat, suggesting that probably the low amount of tannins in the diet did not interfere with the biosynthesis of skatole. Further studies are needed to verify if tannins could play a role in decreasing fat skatole and indole in meat from animals allowed to graze (Vasta and Priolo, 2006).

Increase in efficiency of microbial protein synthesis

Microbial protein synthesis *in vitro*, expressed as ^{15}N incorporation into microbes (or purine as an index of microbial protein) per unit of short-chain fatty acid (SCFA) production is higher in the presence of tannins. Although tannins decrease the availability of nutrients, they cause a shift in the partitioning of nutrients so that a higher proportion of available nutrients is channelled towards microbial mass synthesis than to SCFA production (Makkar, 2003). These results suggest that the *in vivo* beneficial effects of tannins, at low levels of intake, could also be due to higher efficiency of microbial protein synthesis in the rumen. The higher molar proportion of propionate in the *in vitro* fermentation system and lower protozoal counts produced by tannins (Makkar *et al*., 1995a and b) are consistent with the higher efficiency of microbial protein synthesis observed in the presence of tannins. Although *in vivo* effects of tannins on rumen protozoal counts and molar proportions of SCFAs are inconsistent (Waghorn *et al*., 1994; Wang *et al*., 1996), some evidence exists for lower protozoal number (Wang *et al*., 1994) and higher molar proportion of propionate (Waghorn *et al*., 1994) in presence of tannins. It has been shown that calliandra tannins at a level of 2% to 3% in the diet reduced fibre degrading bacteria but the efficiency of microbial protein synthesis was not affected. Similarly *Leucaena leucocephala* and leucaena hybrid KX2 tannins at levels of 7.3% and 11.6%, respectively, in the diets did not affect microbial protein flow as estimated by excretion of urinary purine derivatives (McNeill *et al*., 1998 and 2000). The higher efficiency of digestion observed in systems containing low levels of tannins could be due to specific inhibition or enhancement of a group(s) of micro-organisms. The decrease in the rate of digestion of feeds by low levels of tannins (Makkar *et al*., 1995a) could also help synchronise the release of various nutrients, which in turn might be responsible for increased microbial efficiency.

Increasing the efficiency of microbial protein synthesis and decreasing the protein degradability of feed protein in the rumen are beneficial for ruminants, since they increase the supply of non-ammonia nitrogen to the lower intestine for production purposes. In addition, these effects lead to protein-sparing effects in ruminants and decreased methane emissions and nitrogen excretion to the environment, thereby reducing emission of environmental pollutants despite producing more meat, milk and wool. It is important to know the levels of tannins for such positive effects to be realised. The concentration of tannins should not be too high so that the true digestibility of the substrate is appreciably decreased. At these high concentrations of tannins, the advantage provided by the higher efficiency of microbial protein synthesis (higher proportion of truly degraded substrate leading to microbial mass synthesis) will be offset by the absolute lower amount of truly degraded substrate. Feeding strategies need to be designed to exploit the beneficial effects of tannins.

Other beneficial effects of tannins

Tannins isolated from leaves of various multipurpose trees and browses have anticarcinogenic activity (Perchellet *et al*., 1996). Proanthocyanidins (condensed tannins), both in free form and bound to proteins, have been shown to have free radical scavenging abilities (Hagerman *et al*., 1999) and decrease the susceptibility of healthy cells to toxic agents. Most polyphenols have strong antioxidant properties and inhibit lipid peroxidation and peroxygenases. Pistafolia A, a gallotannin, has strong free radical scavenging properties (Wei *et al*., 2002). A number of hydrolysable tannins including ellagitannins and 1-*o*-galloyl castalagin and casuarinin (present in *Eugenia jambos*) have been shown to have activity against cell carcinomas and tumour cell lines (Sakagami *et al*., 2000; Yang *et al*., 2000). Catechins, polyhydroxylated flavonoids, are present in a

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wide distribution of browses and tree leaves. These undergo considerable microbial and tissue biotransformations, which are present in blood. Efforts need to be directed on evaluating these novel compounds for enhancing animal health.

Tannins also protect ruminants from bloat and have anthelmintic effects (Kahn and Diaz-Hernandez, 2000). Legume tannins could also enhance the quality of silage by preventing excessive degradation of feed proteins. Tannins from browses are also effective against *Clostridium perfringens* and can be used to control *C. perfringens* mediated diarrhoea in pigs during the change of feed from liquid to solid feed (Makkar, 2003).

Hydrolysable tannins, 4,6-0-isoterchebuloyl-D-glucose and isoterchebulin present in terminalia macroptera bark have been shown to have antimicrobial activity against *Pseudomonas fluorescens* and *Bacillus subtilis* (Conrad *et al*., 2001). Another hydrolysable tannins, punicalagin present in some Ethiopian medicinal plants was active against *Mycobacterium tuberculosis* strains (Asres *et al*., 2001). Tannins from *Vaccinium vitis-idaea* could be used for treatment of periodontal diseases since they have antimicrobial activity against *Porphyromonas gingivalis* and *Prevotella intermedia* (Ho *et al*., 2001). Other plant extracts rich in tannins that have antibacterial effects are bearberry and cowberry, against *Helicobactor pylori* (Annuk *et al*., 1999) and *Syzygium jambos*, against *Styaphyloccus aureus* and *Yersinia enterocolitica* (Djipa *et al*., 2000).

Tannins have also been found to affect meat colour and quality. Feeding tannin-containing acacia or sulla leaves or carob pulp has been found to produce meat of lighter colour. The addition of PEG, a tannin-inactivating agent, reversed this effect, suggesting that the lighter colour produced is due to tannins (Priolo *et al*., 2002a, b and 2005). Decrease in blood haaemoglobin and myoglobin and iron utilisation by tannins (Garg *et al*., 1992; Bhatta *et al*., 2002) could contribute to the lightness of the meat (Priolo *et al*., 2000). The lighter meat produced as a result of feeding tannin could favour consumer preference in some regions. Fatty acid composition is associated with the risk or the prevention of several human illnesses. Herbages containing low amount of tannins increased n-3 fatty acid and conjugated linoleic acid (9-*cis* 11-*trans* C18:2; CLA) in lamb meat, compared with lambs fed concentrate (Priolo *et al*., 2005), enhancing meat nutritional properties for human consumption. However, Vasta *et al*. (2005) reported that feeding a diet containing tannins from carob pulp resulted in a lower content of CLA in meat, compared with lambs fed the same diet but supplemented with PEG. This change possibly results from the inhibition of ruminal microorganisms responsible for the biosynthesis of CLA during the biohydrogenation of linoleic acid.

Toxicity by tannin-containing plants

Oak poisoning from the consumption of oak leaves (Garg *et al*., 1992) and yellow-wood toxicity from the leaves of *Terminalia*, *Clidemia* and *Ventilago* have been attributed to the presence of hydrolysable tannins, in particular gallotannins (McSweeney *et al*., 2001 and 2003). Rumen microbes are capable of degrading hydrolysable tannins. The toxicity, therefore, appears to be due to absorption of degraded products of hydrolysable tannins and higher loads of phenols in the blood stream, which is beyond the capability of liver to detoxify them.

In fish, the presence of tannic acid, a hydrolysable tannin, at 2% of the diet of common carp (*Cyprinus carpio* L.) produced adverse effects after day 28 of feeding. The rejection of diet started on day 28 and a complete rejection was observed on day 40. The average metabolic rate for the days 35 to 42 was significantly lower and the oxygen consumption of fish fed the diet containing tannic acid decreased. No such adverse effect was observed in common carp on inclusion of 2% quebracho tannin (a condensed tannin) in the diet. In carp, toxicity of tannic acid is higher than that of quebracho tannin. Protein sources of plant origin containing high amounts of tannins and in particular hydrolysable tannins should be used with caution as fish meal substitutes in carp diets (Becker and Makkar, 1999).

Plants containing saponins

Saponins are steroid or triterpene glycoside compounds found in a variety of plants. Here we present their biological effects on ruminants and fish. The saponin-rich plants having potential for exploitation in ruminant and fish production systems are also discussed.

Effects on ruminants

Effect on rumen fermentation. Various saponins affect gas and microbial mass production to different extents in *in vitro* gas systems containing buffered rumen microbes and feed. For example, acacia saponins decreased gas production, but increased microbial protein without affecting true digestibility. On the other hand, addition of quillaja saponins did not affect gas production, but increased microbial protein and truly degraded substrate. Since truly degraded substrate in an *in vitro* system can lead only to production of gas (fermentative gases plus the buffered gas produced through neutralisation of SCFA) and microbial mass, this implies that all of the increase in truly degraded substrate by quillaja saponins resulted in higher microbial mass, i.e. these saponins increased efficiency of microbial mass synthesis. The effects of yucca saponins differed from those of quillaja or acacia saponins. Yucca saponins decreased gas, increased microbial protein and increased true digestibility. These results suggested that the saponins affected partitioning of degraded nutrients such that higher microbial mass was produced at the cost of gas, and/or SCFA production (Makkar, 2005). Liu *et al*. (2003) showed an increase in microbial protein synthesis in the presence of tea saponins in an *in vitro* fermentation. However, Wang *et al*. (2000a) showed that microbial protein synthesis

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increased at a low level of yucca saponin (15 μg/ml) but decreased at higher concentrations (75 μg/ml). Other *in vitro* studies using the RUSITEC system did not show any significant effect of yucca saponin (100 mg sarsaponin/kg feed) (Śliwiński *et al*., 2002a and 2002b) or of sapindus saponins (Hess *et al*., 2003a) on microbial protein synthesis. At low concentrations, saponins from *Panax ginseng* and quillaja have been demonstrated to increase the growth of *Escherichia coli* (Sen *et al*., 1998a). Yucca saponins have also been shown to stimulate growth of *Prevotella* (*Bacteroides*) *ruminicola* present in the rumen (Wallace *et al*., 1994).

Abreu *et al*. (2004) and Hess *et al.* (2004) found an increase in duodenal flow of microbial-nitrogen in sheep fed *Sapindus saponaria* fruit. However, Hristov *et al.* (1999) did not obtain a significant effect of yucca saponin on microbial protein flow to the intestine in heifers. An increase of microbial nitrogen supply, efficiency of microbial-nitrogen supply, and faecal-nitrogen excretion with increasing levels of *Sapindus rarak* extract was observed, but this increase was not significant.

Effects of various saponins on ammonia levels and SCFA production have been recently reviewed (Wina *et al*., 2005a). Saponins have also been evaluated with a view to enhance protection of protein from degradation in the rumen and increase availability of protein post-ruminally. Inhibition of rumen proteolytic activity of yucca saponins has been demonstrated (Wallace *et al*., 1994). Saponins also form complexes with proteins and could decrease protein degradability (Sen *et al*., 1998b). *Sapindus rarak* saponins did not affect the protein degradation in *in vitro* rumen system (Muetzel *et al*., 2005). On the other hand, quillaja saponins decreased protein degradability of the concentrate, but not of hay-based diet (Makkar and Becker, 2000). These observations suggest that the nature of diet plays a considerable role in determining the effects of saponins. The addition of *S. saponaria* fruit to a sheep diet decreased plasma urea, suggesting that less ammonia was absorbed from the rumen (Abreu *et al*., 2004; Hess *et al*., 2004). This would decrease the energy lost in detoxification of ammonia by the liver and its discharge in urine as urea, contributing to the higher productivity. In addition, saponin addition would provide environmental benefits because less of the feed nitrogen would be discharged into the environment. In addition, quillaja and yucca saponins have also been shown to reduce methane emission (Pen *et al*., 2007).

The difference reported above on the effects of saponins on the levels and proportion of SCFA, ammonia level, proteolytic and protein degradability could be due to different types of saponins and/or different composition of diets. For field applications of saponins, the focus should be on characterisation of specific plant saponin–diet–biological effect interactions.

Saponins and rumen ecology. Studies on the effect of saponins and their products on methanogens (archaea) have attracted great attention lately because of the potential for improving the environment by decreasing the production of 'greenhouse gases'. However, these studies concentrated more on the measurement of methane emission than on the methanogens themselves. As some methanogens (10% to 20% of total) live in association with protozoa (Newbold *et al*., 1997; Tokura *et al*., 1997), it was expected that reducing protozoa would also reduce methanogens, thus reducing methane production. The addition of yucca extract to a high roughage diet or to a mixed diet containing hay and barley grain did not reduce methane emission in a RUSITEC system (Śliwiński *et al*., 2002a and 2002b). However, reduced methane emissions in an *in vitro* system were obtained by adding sarsaponin, extracted from yucca to a starch diet and to a mixed diet (Lila *et al*., 2003). Pen *et al*. (2006) also reported a decrease in methane production by yucca extract when incubated with a roughage-based diet in an *in vitro* system. In this study, a decrease of protozoal number and increase in microbial population were observed with both yucca and quillaja extracts; however, the latter did not reduce methane production. Suppression of methane emission was also achieved by the supplementation of *S. saponaria* fruit (containing high levels of saponins) in the RUSITEC (Hess *et al*., 2003b). Saponins may kill or inactivate protozoa (Makkar *et al*., 1998b), resulting in a lower predation of bacteria by protozoa and a larger bacterial population, slowing protein turnover in the rumen. The slower protein turnover should lead to an increase in bacterial nitrogen flow to the duodenum and increase in productivity (Makkar and Becker, 2000; Wallace *et al*., 2002; Hess *et al*., 2004). As mentioned above, yucca, quillaja and acacia saponins enhanced both microbial mass production and efficiency of microbial protein synthesis, measured using both purine as a marker and ^{15}N incorporation in rumen microbes (Makkar, 2005).

Methane emission was also suppressed when sheep were fed *S. saponaria* fruit (Hess *et al*., 2004). However, the suppression of methanogenesis was not associated with decreased methanogen counts, suggesting a suppression of activity per methanogen cell (Hess *et al*., 2004). Likewise, saponin in *S. rarak* extract did not reduce the archaeal or methanogen RNA concentration either *in vitro* or *in vivo* studies (Wina *et al*., 2005a). Dohme *et al*. (1999) also showed that methane suppression by defaunation is not accompanied by a reduction in methanogen counts. In most studies, methanogens have been measured using the anaerobic culture technique and cell counts of methanogens were measured as colony-forming units, or, methanogens have been measured using *in situ* hybridisation technique. These studies are limited to the effects of various oils and individual fatty acid supplementation (Dohme *et al*., 1999 and 2001; Hess *et al*., 2004). The methanogen cell count determination using culture-based techniques has the disadvantages of non-specificity and that not all microorganisms can be cultured (Makkar and McSweeney, 2005). In our studies, saponin-rich fractions from fenugreek seeds

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and *Sesbania sesban* leaves did not decrease methane emission *in vitro*, but methanogens and fungal population decreased and the population of fibre-degrading bacteria, *Fibrobacter succinogens* and *Ruminococcus falvefaciens* increased (Goel *et al.,* 2007), when measured as fraction of total bacteria using quantitative real-time PCR technique (Denman and McSweeney, 2005). In this study protozoal population also decreased. There was no correlation between methanogens and protozoal population and methane production (Goel *et al*., 2007). Further studies are required to understand the relationship between microbial population and methane production.

Anaerobic fungi are important in the rumen for digesting fibre, but they only comprise a small proportion of the total mass of the rumen micro-flora. There is little information on the effect of saponins on ruminal fungi. In pure culture, Wang *et al*. (2000b) demonstrated that fungi, *Neocallimastix frontalis* and *Pyromyces rhizinflata* are highly sensitive to saponins from *Yucca schidigera*, and even at a low concentration of the saponin (2.25 μg/ml), the growth of both fungi was completely inhibited. However, Muetzel *et al*. (2003), using a membrane hybridisation technique showed that fungal concentration was not reduced when increasing levels of saponin containing *Sesbania pachycarpa* were included in an *in vitro* fermentation system. On the other hand, *S. rarak* extract reduced fungal RNA concentration in the rumen liquor in an *in vitro* rumen fermentation, but not in the rumen of sheep fed with *S. rarak* extract for 3 months (Wina, 2005). The fungal population was significantly higher when sheep were fed with 25 to 50 g/day of *S. saponaria* for 30 days (Díaz *et al*., 1993). It is possible that fungi adapt during long-term feeding.

There are very few *in vitro* studies on the effects of saponins on specific rumen bacteria. Using pure culture, Wallace *et al*. (1994) observed that the saponin fraction of *Y. schidigera,* when added at a concentration of 1% to the medium, stimulated the growth of *Prevotella ruminicola*, did not affect the growth of *Selemonas ruminantium*, suppressed the growth of *Streptococcus bovis* by prolonging the lag phase, and completely inhibited the growth of *Butyrivibrio fibrisolvens*. The same fraction at much lower concentrations (0 to 250 μg/ml) in pure culture exhibited antibacterial activity towards non-cellulolytic bacteria, i.e. *Streptococcus bovis*, *Prevotella bryantii* B14 (formerly *P. ruminicola*) and *Ruminobacter amylophilus*, which is indicated by depressed growth after 14 h of the exposure (Wang *et al*., 2000b). *Fibrobacter succinogens* were unaffected but *Ruminococcus albus* and *Ruminococcus flavefaciens* were virtually unable to digest cellulose in the presence of yucca saponins. Wang *et al*. (2000b) concluded that yucca saponin had a more negative effect on gram-positive bacteria than gram-negative bacteria. Muetzel *et al*. (2003) also found that *S. pachycarpa* did not affect *F. succinogenes* (Gram negative). The concentration of RNA from *Fibrobacter* sp. remained constant and was not affected by *S. rarak* extract either *in vitro* or *in vivo* (Wina *et al*., 2005b).

Using RUSITEC, the number of cellulolytic bacteria was reduced by 30% when 0.5 mg/ml yucca extract was added to alfalfa hay. It was also demonstrated that cellulolytic bacteria are more susceptible to yucca extract than amylolytic bacteria (Wang *et al*., 2000b). Another study where pure cultures were used showed that the growth of cellulolytic bacteria was slightly reduced by *S. rarak* extract (Ningrat *et al*., 2002).

In an *in vivo* study, Díaz *et al*. (1993) observed a significant increase in cellulolytic and total bacteria in the rumen of sheep fed with *S. saponaria* fruit. A similar observation was made by Thalib *et al*. (1996) who reported that total cellulolytic bacteria increased when sheep were fed with a methanol extract of *S. rarak*. However, a dramatic decrease in the RNA concentration of Ruminococci in short-term feeding of *S. rarak* extract and disappearance of this effect upon long term feeding indicated that there may be an adaptation of *Ruminococcus* sp. to *S. rarak* saponins (Wina, 2005). The mechanism of adaptation of bacteria to saponin still needs to be clarified. An increase in the thickness of the cell wall was observed in *Prevotella bryanti* grown in pure culture and adapted to yucca saponins (Wang *et al*., 2000b).

Microbial adaptation and persistency of effects. It has been observed that some plant products lose their effects on continuous ingestion of the plants by animals. Their effects are short-lived due to microbial adaptation. This calls for development of strategies (for example: ingestion on alternate day, ingestion for 3 to 4 days and followed by a break for 1 to 2 days) to beat the microbial adaptation. A negative effect of saponin-containing *Sesbania sesban* on protozoal counts or activity was evident in the *in vitro* studies but not in sheep fed *S. sesban* since the protozoal counts in the rumen increased markedly after several days of feeding (Newbold *et al*., 1997; Odenyo *et al*., 1997; Teferedegne *et al*., 1999; Teferedegne, 2000; Ivan *et al*., 2004). Based on these results, Newbold *et al*. (1997) suggested feeding saponins intermittently to prevent a quick increase in protozoal counts in the rumen. Thalib *et al*. (1996) showed that feeding saponin extract every 3rd day kept the protozoal counts low even after 3 weeks. On the other hand, *S. rarak* saponins did not lose their defaunating activity until 27 days when fed to sheep (Wina *et al*., 2005a). Evidence exists on the hydrolysis of saponins to sapogenin and epimerisation and hydrogenation of sapogenin in the rumen. The relative efficacy of original saponins and that of aglycon (sapogenin) and its epimerised and hydrogenated products towards various effects reported above is not known.

Machmüller *et al*. (2000) reported that the effects of coconut oil and oilseeds on methane suppression persisted for up to 7 weeks. It would be pertinent to study the persistency of the effects of plants. A challenge will be to develop approaches for using plants, plant extracts or plant products, which sustain their effects on the rumen microbial ecosystem.

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Other effects of saponin-containing plants. Fenugreek (*Trigonella foenum-graecum* L.) is a commonly cultivated plant in Asian and Middle Eastern countries. Several authors have estimated the levels of this steroidal sapogenin, both in the commercial varieties of fenugreek and during comparative studies on seeds from related plant species. Saponin levels (as diosgenin) of 0.07% to 1.64% have been observed in seeds (Taylor *et al*., 2002). In our laboratory, 3% saponin (as diosgenin) (on a dry-matter basis) were recorded in fenugreek seeds (HPS Makkar, unpublished results). The seeds are known to reduce blood cholesterol and produce lower concentrations of cholesterol in milk and also to improve the profile of functional fatty acids (Shah and Mir, 2004). Cholesterol-reducing effects of fenugreek seeds (rich in saponins) have also been demonstrated in rats and humans (Petit *et al*., 1993; Al-Habori and Raman, 1998). Antiviral activity of saponins from *Glycyrrhiza radix*, immunostimulant activity of saponins from *Quillaja saponaria* Molina, and hypoglycaemic and antidiabetic activity of saponins from fenugreek (see Francis *et al*., 2002d) have also been demonstrated. Yucca, quillaja, *S. rarak* and *Enterolobium cyclocarpum* saponins have been shown to increase productive parameters such as wool production, growth and milk production in animals on roughage-based diets (Wina *et al*., 2005c). The effect of quillaja saponins was concentration and sex dependent. The growth rate was significantly higher for male lambs at 40 p.p.m. level, and at 60 p.p.m. the growth rate was higher than the control but the increase was not significant. On the other hand, inclusion of quillaja saponins at these levels decreased the growth rate of female lambs (Makkar, 2000). These effects seem to be mediated by hormones. Further studies are needed in this area. Supplementation of steroidal saponins in feeds has also been shown to be beneficial to fattening lambs and steers and monogastrics (see Makkar, 2000).

Saponins may be toxic to ruminants. The major symptoms are photosensitisation, gastroenteritis and diarrhoea. Some forages that contain saponins and produce these toxic symptoms are *Brachiaria decumbens* grass, species of the *Panicum* genus, and *Drymaria arenaroides* and *Tribulus terrestris* weeds (see Wina *et al*., 2005b). Toxicity of other saponin-containing plants such as *Narthecium ossifragum*, *Tribulus terrestris*, *Agave lecheguilla* and *Nolina texana* has also been described (Flaoyen *et al*., 2004).

In tropical countries, crop residues and poor quality hays and pastures form a major part of the animal diets. Urea-ammoniation has been used extensively for enhancing the digestibility and in turn intake and nutrient utilisation from these low quality roughages. However, a major disadvantage of the ammoniation process is the loss of nitrogen in the form of ammonia that occurs during fermentation. Saponins are considered to bind ammonia. In one of our studies (Makkar *et al*., 1999), *Quillaja saponaria* bark saponins and *Yucca schidigera* plant extract rich in saponins were added during urea-ammoniation to reduce ammonia loss to the air. The ammonia-nitrogen bound to the straw (as percentage of urea-nitrogen added) varied from 47% to 54% in the presence of the yucca plant powder (1 g/kg straw), which was substantially higher than that observed in its absence (38%). On the other hand, no such increase was observed with *Quillaja saponaria* bark saponins. The dry matter and NDF digestibilities, rate and potential extent of digestion were significantly higher in the treated straw than in the untreated straw; but these values were not affected by the addition of saponins and saponin-rich yucca plant powder. The increase in binding observed in the presence of yucca plant powder could be due to the presence of saponins and/or glycoproteins.

Effects on fish

An overview of the effects of saponins on fish metabolism and performance is presented in Table 1.

Fish mortality and molluscicidal activity. Saponins have been reported to be highly toxic to fish because of their damaging effect on the respiratory epithelia (Roy *et al*., 1990). They are also considered to be the active components of many traditionally used fish poisons, like mahua oil cake (see Francis *et al*., 2001a). Fish have also been shown to exhibit stress reactions to the presence of saponins in water. Roy and Munshi (1989) reported that the oxygen uptake of perch, *Anabas testudineus*, increased with a concomitant increase in the red blood cells, haemoglobin and haematocrit levels, after the fish had been in water containing 5 mg/l quillaja saponin for 24 h. *Penaeus japonicus* that had been previously exposed to concentrations of 20 mg/l of saponin for 24 h increased both respiration rate and metabolism (measured as increase in oxygen uptake and ammonia excretion) during a 6 h detoxification process (Chen and Chen, 1997). Bureau *et al*. (1998) observed that quillaja saponins damaged the intestinal mucosa in rainbow trout and Chinook salmon at dietary levels above 1500 mg/kg. The condition of the intestines of these fish was similar to that of fish fed a raw soya-bean meal diet indicating the role of saponins in causing the damage. Krogdahl *et al*. (1995), however, did not find any negative effects when soya saponins were included in the diet of Atlantic salmon at levels similar to those likely to be found in a soya-bean meal-based diet (30 to 40%). In the same study, an alcohol extract of soya-bean meal caused growth retardation, altered intestinal morphology and depressed mucosal enzyme activity in the lower intestine.

Our experiments showed that two commercially available saponin concentrates did not have any lethal effects on common carp. Addition of *Quillaja saponaria* saponins (QS: no. 2149; Sigma, St Louis, USA) at a level of 40 000 p.p.m. in aquaria containing carp (*Cyprinus carpio* L.) did not lead to death of the carp in 18 h and feed consumption was not affected. On the other hand, yucca saponins (YS: DK sarsaponin 30^{TM}, Desert King International, Chula Vista, CA, USA) at 10 000 p.p.m. did not cause mortality in the first 3 h, but all fish were found dead after 18 h. The foam formation, due to saponins, was observed in both aquaria;

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Table 1 *Overview of the effects of saponins on common carp and Nile tilapia*

Parameter	Effect
Toxicity	No toxic effects in common carp and Nile tilapia when quillaja saponin extract present in feeds up to a level of 2000 p.p.m. No toxic effect in common carp when quillaja saponin extract present in water up to a level of 40 000 p.p.m.
Feed intake	Feed intake not affected when the level of quillaja saponin extract in feed was up to 2000 p.p.m. for Nile tilapia and 250 p.p.m. for common carp
Growth	Growth stimulated in common carp up to a level of 150 p.p.m. and 300 p.p.m. for Nile tilapia of quillaja saponin extract
Feed conversion efficiency	Feed conversion efficiency increased at the concentration at which growth was stimulated
Enzyme activity	Activities of lactate dehydrogenase (LDH) and cytochrome *c*-oxidase (CO) in liver of common carp increased on feeding quillaja saponin containing diet. Intestinal enzymes amylase and trypsin was also stimulated
Oxygen consumption and metabolic rate	Oxygen consumption and metabolic rate not significantly affected by addition of saponin concentrates up to a level of 300 p.p.m. in the diet. Absolute values tended to be lower for both the oxygen consumption and metabolic rate
Membrane transport	Increase in paracellular transport of inert markers (^{3}H mannitol) on application of quillaja saponins to the mucosal side of isolated tilapia intestinal membrane
Reproduction	Spawning affected in mature female Nile tilapia feeding on diet containing 300 p.p.m. of quillaja saponin concentrate
Sex determination	Masculinisation of tilapia larvae feeding on a diet containing 700 p.p.m. of quillaja saponins. Suppression of reproduction in Nile tilapia when first feeding larvae were fed diets containing 2000 p.p.m. of quillaja saponins
Histology	No abnormality observed in liver, kidney or intestinal tissue of tilapia fed quillaja saponins up to 700 p.p.m. in the diet

however, there was no correlation between the extent of foam formation and toxicity of saponins to fish. Our results demonstrated that quillaja and yucca saponins are not highly toxic to fish (Makkar and Becker, 2000).

Molluscicidal activity of quillaja and yucca saponins was also very low compared with other saponins. At 20 p.p.m., yucca and quillaja saponins killed only 40% and 20% of the snails (*Biomphalaria glabrata*), respectively. At this level, 100% mortality was observed with acacia saponins (Makkar and Becker, 2000). Snails act as intermediate hosts of schistosomes in many tropical countries. Schistosomes cause schistosomiasis. *Acacia auriculoformis* saponins might hold potential for controlling this disease.

As mentioned above in context to rumen fermentation, protozoa are highly susceptible to some saponins. The use of saponin-containing plants for possible control of fish protozoal diseases such as white spot disease, costiasis and trichodiniasis needs investigation. Fish are also highly susceptible to some saponins. A challenge would be to identify saponins that affect the protozoa causing these diseases but do not adversely affect fish.

Feed intake and behaviour. We observed that common carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*) ate standard fish meal-based diets mixed with up to 1000 mg/kg of all the saponin concentrates (QS and YS) that were evaluated without any hesitation. The fish consumed the pellets containing saponins as soon as they dropped into the aquaria with no apparent differences in acceptability between the control diet and saponin-containing diets. There was no mortality or abnormal behaviour of fish fed up to this concentration of saponins. On the other hand, standard diets containing 2000 mg/kg of the quillaja saponin concentrate induced high mortality in first-feeding tilapia larvae (Steinbronn, 2002). Mortality started after 3 weeks of intensive feeding of the diet containing 2000 mg/kg of QS and 93 out of 460 fish larvae died over a period of about 5 days. The mortality stopped completely after the feeding intensity was reduced and the saponin-containing diet was gradually replaced with a standard diet (the control diet was a standard diet containing, on dry-matter basis, 40% protein, 10% lipid, 10% ash and had 20 kJ/g gross energy with the protein source being fish meal).

Effects of low levels of quillaja saponins on fish growth. Common carp and Nile tilapia juveniles fed diets containing QS (150 and 300 mg/kg in the diet) had significantly higher rate of body mass gain (Francis *et al.*, 2001b and 2002a). The average final body mass of carp fed QS was about 18% higher and that of tilapia was more than 20% higher than fish that had similar average weights at the start of the respective experiments but which did not receive QS (Figures 1 and 2).

The growth-promoting effects of QS were most pronounced at 150 mg/kg diet for carp; whereas, the dietary level of 300 mg/kg induced maximum effects in tilapia. The absolute increase in weight was higher compared with control animals even at higher dietary levels of 700 mg/kg in Nile tilapia (Francis *et al.*, 2002c). The growth-promoting effects of QS were most pronounced during the initial

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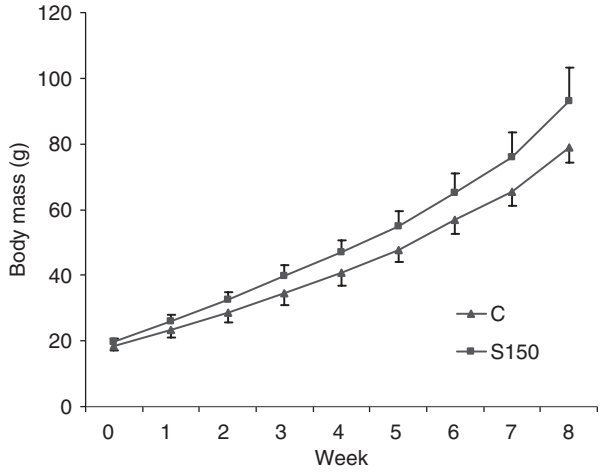

Figure 1 Body mass increase of common carp fed control diet (C) or a diet containing 150 mg/kg (S150) of quillaja saponins (taken from Francis *et al.*, 2002a).

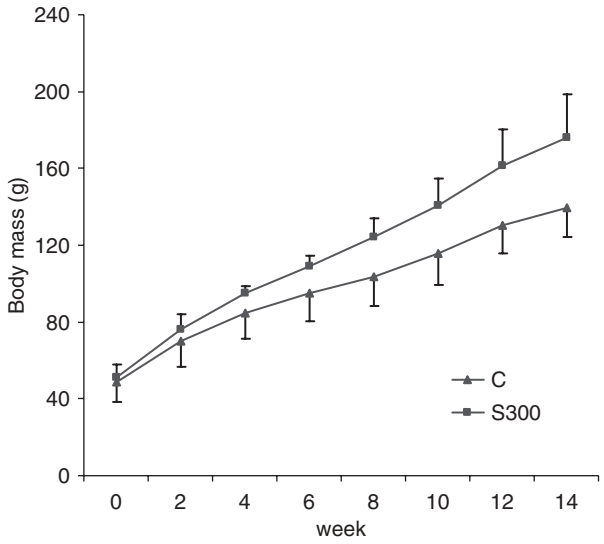

Figure 2 Body mass increase of Nile tilapia fed control diet (C) or a diet containing 300 mg/kg (S300) of quillaja saponins (taken from Francis *et al.*, 2001b).

period of feeding (Francis *et al.*, 2001b and 2002a). It was therefore hypothesised that there was probably an adaptation by the fish to continuous intake of QS. An experiment was therefore designed to provide discontinuous ingestion of saponins to counteract any adaptive responses of the fish (Francis *et al.*, 2002b). Consumption of diets containing saponins during alternate weeks, however, did not result in retention of the more pronounced initial growth-promoting effects during the entire experimental period in carp. Haemolytic triterpenoid gypsophila saponins (GSs) at levels of between 5 and 250 mg/kg in diets (GS5 and GS250), concentrated using chromatography, did not increase growth rates of common carp after 8 weeks of feeding even though absolute growth was higher in all the saponin-fed groups compared with control (G. Francis, H.P.S. Makkar and K. Becker, unpublished data).

Concentrated steroidal YS at levels of 50 and 100 mg/kg (YS050 and YS100 groups) also did not affect growth of common carp significantly. Here the YS050 group seemed to perform better than the YS100 and control groups at the end of a 10-week feeding experiment (G. Francis, H.P.S. Makkar and K Becker, unpublished data). The metabolic growth rate (MGR = live weight gain (g)/average metabolic live weight ($kg^{0.8}$) per day) of the YS100 group was higher than that of the control group until the 7th week of the experiment, the difference between the two groups widened with time. Thereafter, the MGR of the control group started to increase compared with the YS100 and continued to do so until the end of the experiment. The YS050 group had the lowest growth rate throughout the experiment.

The addition of QS to the diet reduced the amount of feed required for the synthesis of tissue protein. The food conversion ratio (FCR) was lower in carp fed a diet containing 150 mg/kg (0.82 ± 0.07) and tilapia fed 300 mg/kg (1.40 ± 0.19) of QS compared with the controls (0.89 ± 0.04 and 1.61 ± 0.34). Common carp fed diets containing GS and YS did not differ from controls in regard to FCR (G. Francis, H.P.S. Makkar and K. Becker, unpublished observation) with values of 0.91 for control, 0.96 for YS050 and 0.91 for YS100. In the case of the GS, the absolute FCR value was lowest in the GS5 group and highest in the control group (G. Francis, H.P.S. Makkar and K. Becker, unpublished observations).

Differences in growth rate and feed efficiency of fish fed saponin-containing diets were not always significantly higher compared with controls because of the high variability among treatment groups. The lack of homogeneity in the growth-promoting effects of dietary QS was also evident during feeding experiments where the number of experimental fish was relatively high, both in the laboratory (Francis *et al.*, 2002c) and field trials (Steinbronn, 2002). The variability was evident despite the fact that fish used in the individual experiments were of the same lineage, age and body mass. The factors that may have caused variability in the growth response of carp and tilapia to dietary QS are not clear.

The mechanisms contributing to growth-promoting effects of saponins, especially QS which induced significant growth increases, remain unclear. Diverse effects of dietary saponins include an increase in the permeability of intestinal membranes to dietary nutrients (Francis *et al.*, 2002d) and/or a stimulation of the activity of digestive enzymes, which increases the efficiency of feed nutrient utilisation. Dietary QS increased significantly the activity of intestinal enzymes, amylase and trypsin, suggesting stimulation of protein and carbohydrate digestion in the intestine. Increase in liver enzymes, lactate dehydrogenase (LDH) and cytochrome *c*-oxidase (CO) was also observed on feeding QS containing diet (Serrano *et al.*, 1998). This suggests that QS promoted the respiratory chain pathway, enhancing energy

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availability. The ratios of LDH to CO decreased with QS supplementation (Serrano *et al.*, 1998) indicating the promotion of aerobic metabolism.

Initial investigations into the effects of saponins on membrane transport reveal an increase in paracellular transport of inert markers (^{3}H mannitol) on application of QS to the mucosal side of isolated tilapia intestinal membrane (G. Francis, H.P.S. Makkar and K. Becker, unpublished observations).

It also remains to be determined whether the saponins themselves or their breakdown products (e.g. sapogenins) in the intestines enter the blood of the fish and cause their effects systemically. From the extent of effects that saponins have on various physiological processes it is expected that either saponins or their breakdown products enter the body through the intestinal membranes. We have described the ability of saponins to influence serum hormone levels (Francis *et al.*, 2002d). However, some dietary components may produce systemic effects even without actually entering the body. It has been reported that hormones such as ghrelin synthesised primarily in the stomach wall could act as intermediaries between stomach, hypothalamus and pituitary and may be involved in energy balance (Tschöp *et al.*, 2000). It is to be seen whether saponins induce the synthesis and release of such hormonal intermediaries in the digestive system.

A factor that makes it more difficult to interpret the effect of dietary saponins is the presence of a number of individual compounds with different properties in the saponin mixtures used in our feeding trials. We conducted some experiments to see if there are functional differences between purified haemolytic saponin fractions and crude extracts for their growth-promoting effects. Our initial studies indicate that there are no differences in growth or any of the nutrient assimilation parameters between carp fed raw GS mixture or any of the levels of haemolytic saponins (5 to 250 mg/kg of the diet). The growth performance of all the saponin-fed groups was higher than that of the control fish in this experiment (G. Francis, H.P.S. Makkar and K. Becker, unpublished observations). Further studies might clarify whether individual compounds possess higher potency as growth enhancers in fish.

Even though there appears to be a stimulatory effect of saponins, particularly QS, on fish growth, gaps exist in our understanding of the mechanism of action of the saponins in fish. Future research in this area should concentrate on understanding the physiological mechanisms by which dietary saponins increase growth and feed conversion efficiency in carp and tilapia.

Effects on fish histology. It has been observed that QS does not cause any obvious damage to the intestinal membranes in tilapia fry when present up to 700 mg/kg in the diet. Neither was any abnormality observed in the liver or kidney tissue of tilapia fed QS up to 700 mg/kg over a period of 6 months (G. Francis and K. Becker, unpublished observations).

Effects on tilapia reproduction. Sexually mature female tilapia consuming a diet containing 300 mg/kg of QS did not spawn over a period of more than 3 months, whereas fish fed the control diet and reared under similar conditions spawned regularly. This observation was followed up by conducting laboratory experiments and field studies to explore further the effects of dietary QS on tilapia reproduction. Adult tilapia that were spawning regularly stopped egg laying when put on a diet containing 300 mg/kg of QS (G. Francis and K. Becker, unpublished observations). In another experiment, the sex ratio of tilapia larvae fed a diet containing 700 mg/kg of QS continuously over a 6-month experimental period deviated significantly from the normal 50/50 ratio in favour of males (Francis *et al.*, 2002c). This deviation from the normal sex ratio in favour of males was also evident (but not statistically significant) in the treatment groups receiving lower quantities of QS (150 mg/kg diet) in the diet. Pond experiments conducted with a higher number of fish (about 500 larvae in each treatment in duplicate) fed a QS-containing diet for the first 6 weeks of life, however, did not confirm the laboratory results obtained regarding the effects of dietary QS on sex ratio (Steinbronn, 2002). The sex ratios of tilapia larvae fed diets containing 150 and 500 mg/kg of QS did not differ significantly from 50/50 ratio for males and females, but the share of males was slightly higher in both treatments when compared with the control group. In contrast, at the highest level of 2000 mg/kg diet, more females than males were counted in tilapia larvae fed these diets. This treatment group experienced 20% mortality during the early rearing phase when the saponin containing diets were being fed. It remains to be confirmed whether this might have been caused by differential mortality rates among the sexes during the experimental period. Pandian and Sheela (1995) have reported the likelihood of sex-dependent mortality occurring among fish that were sex-reversed through the application of synthetic hormones. The GIFT tilapia (developed from the 'Genetic Improvement of Farmed Tilapia' project of ICLARM (International Centre for Living Aquatic Resources Management), currently World Fish Centre) larvae used in the field trial have also been reported to generally have a higher number of female fish (Hussain *et al.*, 2000). Continued observations revealed that production of fry was completely suppressed in ponds where fish from the 2000 mg/kg saponin group were stocked even after the removal of saponins from the diets (Steinbronn, 2002). This could point to a sterility of either males or females, which implies a potential for control of reproduction in tilapia using QS. Normal fry production was observed in fish that previously received 150 and 500 mg/kg of QS.

Saponins have been reported to affect the release of hormones, such as LH from the pituitary (Benie *et al.*, 1990); this hormone is considered to regulate all aspects of teleost reproduction (Suzuki *et al.*, 1988a), particularly final oocyte maturation and ovulation (Suzuki *et al.*, 1988b). Therefore, it was hypothesised that induction of changes in LH secretory pattern by QS or its degraded products absorbed

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from the intestine might be responsible for the effects that have been observed on reproduction. QS was found to stimulate LH release from dispersed tilapia pituitary cells *in vitro* (Francis *et al*., 2002d). This effect was abolished in the presence of dilute calf serum. Serum LH values did not show any diet-dependent (QS containing or control diets) trend in either male or female tilapia *in vivo*. The retarding effects on egg production in adult females and the capacity for sex inversion in tilapia fry fed saponin-containing diets indicate effects at the hormonal level and data from gonadosomatic index measurements support this contention. Our efforts to identify any saponin-induced change in the level of LH did not reveal any dose-dependent patterns. Once the optimum dietary level of saponins that produces complete sex inversion in tilapia fry or prevents egg production in female tilapia is determined, saponins will have considerable potential in tilapia aquaculture where one of the major problems is overproduction of fry that do not grow to marketable size. The effect of saponins on levels of reproductive hormones should be studied further by monitoring hormones such as oestrogen, testosterone, 11-keto-testosterone and gonadotropic hormones *in vivo* and possibly by using cultured tilapia pituitary cells. *In vitro* studies have the advantage of requiring only small quantities of material for the identification of the individual compounds responsible.

Plants containing multibioactive compounds

Moringa oleifera

M. oleifera Lam (synonym: *Moringa pterygosperma* Gaertner) belongs to a monogeneric family of shrubs and trees, Moringaceae is considered to have its origin in the northwest region of India, south of the Himalayan mountains. This tree can be found growing naturally at elevations of up to 1000 m above sea level. It can grow well on hillsides but is more frequently found growing on pastureland or in river basins. It is a fast growing tree and has been found to grow 6 to 7 m in 1 year in areas receiving less than 400 mm mean annual rainfall. Various bioactive compounds present in different parts of this plant are presented in Table 2.

Moringa seeds contain between 30% and 42% oil, which is edible and the press cake obtained as a by-product of the oil extraction process contains a very high level of protein. Some of these proteins (approx. 1%) are active cationic polyelectrolytes having molecular weights between 7 and 17 kDa. The cationic polyelectrolytes neutralise the colloids in muddy or dirty water since the majority of these colloids have a negative electrical charge. This protein can therefore be used as a non-toxic natural polypeptide for sedimenting mineral particles and organics in the purification of drinking water, for cleaning vegetable oil, or for sedimenting fibres in the juice and beer industries. It works as a primary coagulant as natural bridges are continuously formed between the colloid particles. These proteins have antibacterial properties and bind strongly with rumen microbes. At high levels these proteins inhibit rumen fermentation, but at low levels they protect feed proteins from degradation in the rumen (Makkar and Becker, 1998; Hoffmann *et al*., 2003) and could be used to enhance rumen undegradable protein. *In vivo* studies in this area are underway in Tunisia. The bacterial resistance to antibiotics is increasing in various regions and this obviously has huge implications in curing infectious diseases and in using antibiotics in feeds as growth promoters. The moringa protein also has bactericidal activity (Suarez *et al*., 2005). Gram-positive and Gram-negative bacteria, pathogenic to humans, showed only a slight reduction of viability with the moringa protein (Suarez *et al*., 2005), while viability of *E. coli* was inhibited by four orders of magnitude (Suarez *et al*., 2003). The use of antibacterial moringa proteins for controlling mastitis is also being investigated by us.

Some of the compounds that have been isolated from moringa seeds are 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate (3%), 4-(4′-*O*-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate (10%), niazimicin, and pterygospermin (Daxenbichler, 1991; Fahey *et al*., 2001; Bennett *et al*., 2003; Mekonnen and Dräger, 2003). These compounds are known to have anticancer, antibacterial and hypotensive activities. Antioxidant activity of these compounds has also been reported (Win and Jongen, 1996). These compounds also have the potential to control agricultural and public health insect pests (Tsao *et al*., 1996). For example, *Helicobacter pylori* bacterium was found to be highly susceptible to 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate and various other isothiocyanates, which are degraded products of glucosinolates (Fahey *et al*., 2002; Haristoy *et al*., 2005). *H. pylori* is a major cause of gastric and duodenal ulcers, and is a major risk factor for gastric cancer.

Seed powder of moringa has been shown to protect animals from arsenic-induced oxidative stress and in the depletion of arsenic from blood, liver and kidneys (Gupta *et al*., 2005).

Extracts of moringa leaves in 80% ethanol contain growth enhancing principles (i.e. hormones of the cytokinine type). The extract as a foliar spray can be used to accelerate the growth of young plants such as peanut, soya bean, blackbean, maize, onion, sorghum, tomato, coffee and sugarcane (Foidl *et al*., 2001). Use of this spray also makes the plants firmer and more resistant to pests and disease, produce more and larger fruit and, consequently, have higher yields.

Moringa leaves are free of antinutritive factors (e.g. tannins, saponins, cyanogens, glucosinolates, alkaloids, etc.) and are high in iron (up to 582 mg/kg DM), beta-carotene (up to 400 mg/kg DM) and in vitamin C (up to 9.2 g/kg DM). Lately, this plant has received a lot of attention. All essential amino acids are at higher than adequate concentrations when compared with the recommended amino acid pattern of the FAO/WHO/UNO reference protein for 2- to 5-year-old children. Moringa foliage has been found to increase animal productivity (Foidl *et al*., 2001; Sánchez *et al*., 2006).

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Table 2 *Phytochemicals in* Moringa oleifera *and their bioactivity*

Phytochemical	Plant part	Activity	Remark	Reference
Cationic protein		Increase rumen by-pass protein (*in vitro*, in a rumen fermentation system)	Possible use for enhancing feed protein supply postruminally	Makkar and Becker, 1998; Hoffmann *et al.*, 2003
		Antibacterial	Potential for controlling of various diseases	Suarez *et al.*, 2003 and 2005
4-(α-L-Rhamnopyranosyloxy) benzyl glucosinolate (3%); 4-(4′-*O*-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocyanate; 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate (10%), niazimicin and pterygospermin	Seed	Anticancer, hypotensive and antioxidant; antibacterial and insecticidal	Potential for use as neutraceuticals; potential for controlling bacterial diseases and for pest management	
Flavonoids: quercetin and kaempferol	Leaves	Antioxidant	Leaves used to enhance shelf life of 'Ghee' in India	Siddhuraju and Becker, 2003
Beta-carotene	Leaves	Vitamin A precursor	0.04% in dry matter	Foidl *et al.*, 2001
Vitamin C	Leaves	A vitamin and an antioxidant	ca 1% in dry matter	Foidl *et al.*, 2001
Cytokinine-type hormones	Leaves	Enhance plant growth, yield and pest resistance	Active component in 80% methanol; this solution is sprayed on the plant	Foidl *et al.*, 2001
Fibre	Leaves	Decrease cholesterol in muscle (fish)	Potential health benefits	N. Mushota, P. Siddhuraju, K. Becker, unpublished data
Active compound	Leaves	Decrease cholesterol in muscle, liver, serum (rats)	Decreased the high-fat diet-induced increases cholesterol levels. The effect could be due to fibre in leaves	Ghasi *et al.*, 2000
Active compound	Leaves	Increase breast milk production	Used in Philippines by lactating mothers	Foidl *et al.*, 2001
Flavonoids: quercetin, kaempferol, rhamnetin, isoquercitrin and kaempferitrin	Flowers	Stimulant, aphrodisiac, diuretic and cholagogue in humans	Used in Asian countries	Pal *et al.*, 1995

Pal *et al.* (1995) have reported that the methanol fraction of moringa leaf extract possesses antiulcer activity against gastric lesions induced in rats. Flowers of moringa are considered to possess medicinal value as a stimulant, aphrodisiac, diuretic and cholagogue, and they have been also reported to contain flavonoid pigments such as quercetin, kaempferol, rhamnetin, isoquercitrin and kaempferitrin (Nair and Subramanian, 1962). The administration of extracts of moringa leaves along with high-fat diet to rats decreased the high-fat diet-induced increases in serum, liver and muscle cholesterol levels by approximately 14%, 6% and 11%, respectively (Ghasi *et al.*, 2000). Moringa leaves have also been shown to increase breast milk production (Estrella *et al.*, 2000). In many Asian and African countries women consume moringa leaves to enhance breast milk production (Fuglie, 2001). In India, tribal and indigenous people use fresh leaves as a natural antioxidant in buffalo and cow ghee (butter oil) preparation, which is considered to enhance shelf life of ghee. Moringa leaves have very strong antioxidant activity (Siddhuraju and Becker, 2003; Yang *et al.*, 2006). The flavonoids such as quercetin and kaempferol were identified as the most potent antioxidants in moringa leaves. Their antioxidant activity was higher than the conventional antioxidants such as ascorbic acid, which is also present in large amounts in moringa leaves (Siddhuraju and Becker, 2003). The extracts of these leaves also appear to have cancer preventive effect, which was assayed by the differentiating activity against human promyelocytic leukaemia cells (HL-60) (Siddhuraju and Becker, 2003).

Moringa seeds contain phytate, cyanogens and glucosinolates (Makkar and Becker, 1997c). The pods of *M. oleifera* contain a glycoside niazine possessing an *o*-nitrile thiocarbamate group along with thiocarbonate, carbamate and isothiocyanate glycosides, which are considered to have hypotensive effects (Faizi *et al.*, 1997).

By combining the knowledge of indigenous people with the laboratory findings that the moringa leaves have strong antioxidant activity and they are good sources of essential amino acids, these leaves could serve as a food source and

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their extracts containing flavanoids and other phytochemicals (not yet identified; for example for inducing milk production) may have potential as 'neutraceuticals' for improving the health of human and livestock.

Jatropha curcas

J. curcas (L.) or physic nut is a multipurpose and drought-resistant large shrub or small tree. Although a native of tropical America, it now thrives throughout Africa and Asia. It grows in a number of climatic zones in tropical and subtropical regions of the world and can be grown in areas of low rainfall. Jatropha is easy to establish, grows relatively quickly and is hardy. A perceived advantage of jatropha is its capability to grow on marginal land and its ability to reclaim and restore eroded areas. Various parts/products of the plant hold potential for use as bio-fuel, animal feed and inclusion in medicinal preparations. If grown on barren lands it could add to the removal of carbon from the atmosphere and the build up of soil carbon.

Seed production ranges from about 0.1 t/ha per year to over 8−t/ha per year (Heller, 1996). The seed yield reaches peak levels after about 5 years of growth. This range in production may be attributable to low and high rainfall areas and the nutrients in soil. The plant takes about 4 to 5 years to yield seeds when cultivated on poor soil, with no irrigation and placed on locations in full sunlight, but much less time is required under optimal rainfall and soil conditions. Once established the plantations yield for 30 to 35 years.

Jatropha plants have been mainly investigated as a source of oil. The seed kernel of the plant contains about 60% oil that can be converted into biodiesel and used as a substitute for diesel fuel. The seed cake remaining after oil extraction is an excellent source of plant nutrients (Foidl *et al*., 2001). The level of essential amino acids of the defatted kernel meal of the non-toxic variety are higher than that of FAO reference protein except for lysine (Makkar and Becker, 1999a; Foidl *et al*., 2001). However the presence of high levels of antinutrients (Table 3) prevent their use in animal feeding. Phorbol esters have been identified as the major toxic principle in jatropha (Makkar and Becker, 1997a). At least six phorbol esters are present in jatropha seeds (Haas and Mittelbach, 2000). The phorbol esters are reported to mimic the action of diacyl glycerol, an activator of protein kinase C, which regulates different signal transduction pathways (Mosior and Newton, 1995). Interference with the activity of protein kinase C affects a number of processes including, phospholipid and protein synthesis, enzyme activities, DNA synthesis, phosphorylation of proteins, cell differentiation and gene expression. The phorbol esters themselves do not induce tumours but promote tumour growth following exposure to a subcarcinogenic dose of a carcinogen. They can thus be designated as co-carcinogens (Brodie and Blumberg, 2003; Gonzalez-Guerrico and Kazanietz, 2005).

Varieties of jatropha plants where phorbol esters are almost absent have been identified in Mexico. This offers promise for inclusion of products from these plants in animal and fish diets. The chemical composition of the seed meal extracted from the non-toxic variety (from Veracruz, Mexico) appear to be similar/superior to the toxic variety (Cape Verde, Mexico) (Foidl *et al*., 2001). The non-protein nitrogen formed only 7.8% to 9.0% of the total nitrogen in the jatropha meals suggesting the presence of high levels (~90%) of true protein (Makkar *et al*., 1998a).

Even though the Mexican non-toxic varieties lack the most potent toxin, phorbol esters, other antinutrients such as trypsin inhibitor, lectin and phytate are present in significant amounts (Table 3), and their levels are similar to those in the toxic varieties. Moist heating of seeds almost completely inactivated trypsin inhibitor activity and decreased lectin activity (Makkar and Becker, 1999a). This heat treatment should have the added benefit of increasing protein digestibility. Moist heating may also make the seed cake from the non-toxic variety usable in fish diet. Heat treated seed meal of the non-toxic variety of *J. curcas* was found to be comparable to commercially available soyabean meal in nutritional quality for common carp (R. Richter, G. Francis and K. Becker, unpublished results). In contrast, heat treatment followed by aqueous methanol extraction to remove phorbol esters could result in elimination of most of the antinutrients and toxins from the toxic variety. The meal treated in this manner was found to be innocuous to rats (Makkar and Becker, 1997b) and fish (unpublished results from our laboratory).

The carp (*Cyprinus carpio* L.) were found to be highly susceptible to phorbol esters present in the seed meal of

Table 3 *Important phytochemicals in seed meal of toxic and non-toxic variety of* Jatropha curcas

Component	Toxic variety	Non-toxic variety
Phorbol esters (mg/g kernel)	2.79	0.11
Total phenols (% tannic acid equivalent)	0.36	0.22
Tannins (% tannic acid equivalent)	0.04	0.02
Phytates (% dry matter)	9.40	8.90
Saponins (% diosgenin equivalent)	2.60	3.40
Trypsin inhibitor (mg trypsin inhibited per g sample)	21.3	26.5
Lectins (1/mg of meal that produced haemagglutination per ml of assay medium)	102	51

All data are on a dry-matter basis; *source*: Makkar *et al*. (1998a).

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Table 4 *Phytochemicals in* Jatropha curcas

Phytochemical	Plant part	Activity	Reference
Phorbol esters	Seed	Carcinogenic, irritant, toxic Pesticidal, insecticidal and molluscicidal	Becker and Makkar, 1998 Rug and Ruppel, 2000; Mengual, 1997; Solsoloy and Solsoloy, 1997
Trypsin inhibitors, lectin, phytate	Seed	Antinutritional: reduce feed conversion efficiency, growth	Makkar and Becker, 1997a; Makkar *et al.*, 1998a
Jatrophone (a macrocyclic diterpene)	Seed	Antileukemic; platelet aggregation inhibition	Kupchan *et al.*, 1970; Dutra *et al.*, 1996
Flavonoids: apigenin and its glycosides vitexin and isovitexin; sterols: stigmasterol, beta-D-sitosterol and its beta-D-glucoside	Leaves	Anti-inflammatory	Chhabra *et al.*, 1990
Proteolytic enzyme, curcain	Latex	Wound healing properties	Nath and Dutta, 1997
Cyclic octapeptide curcacycline	Latex	Immunomodulatory properties	Van den Berg *et al.*, 1995

the toxic variety of *J. curcas*. The threshold level at which phorbol esters caused adverse effects was 15 p.p.m. (15 μg/g) in the diet (Becker and Makkar, 1998). Carp could be a useful species for bioassay of phorbol esters, and it has been used regularly in our laboratory for evaluation of various detoxification conditions for making *J. curcus* seed meal safe for introduction in animal diets.

The phorbol esters were also found to be an effective biopesticide against diverse fresh-water snails. Extracts from *J. curcas* L. were found to be toxic against snails transmitting *Schistosoma mansoni* and *S. haematobium* (Rug and Ruppel, 2000). Compared with aqueous extract, methanol extract showed the highest toxicity against all organisms that were tested with LC_{100}-values of 25 p.p.m. for cercariae and the snail *Biomphalaria glabrata* and 1 p.p.m. for the snails *Bulinus truncatus* and *B. natalensis*. Attenuation of cercariae leading to reduced infectivity in mice could be achieved in concentrations below those exerting acute toxicity. Thus, phorbol esters from the jatropha plant could become an affordable and effective component of an integrated approach to schistosomiasis control (Table 4). Jatropha oil or methanol extract of jatropha oil containing phorbol esters has also been shown to have strong insecticidal effects against *Busseola fusca* and *Sesamia calamistis* larvae (Mengual, 1997), and pesticidal effects against *Sitophilus zeamays* and *Callosobruchus chinesis* and deterred their oviposition on sprayed corn and mungbeans seeds (Solsoloy and Solsoloy, 1997).

Jatropha leaves are used to cure various diseases. Anti-inflammatory compounds isolated from leaves are flavonoids apigenin and its glycosides vitexin and isovitexin, the sterols stigmasterol, beta-D-sitosterol and its beta-D-glucoside (Chhabra *et al.*, 1990). The jatropha latex has a proteolytic enzyme, curcain, which was found to have better properties for healing wounds than nitrofurazone (Nath and Dutta, 1997). A novel cyclic octapeptide (Gly-Leu-Leu-GlyThr-Val-Leu-Leu-Gly), curcacycline, has also been isolated from jatropha latex. This cyclic octapeptide has been shown to inhibit classical pathway activity of human complement, and proliferation of human T-cells (Van den Berg *et al.*, 1995).

Both moringa and jatropha seeds are good sources of phytate (Makkar and Becker, 1997c and 1999a). Several beneficial effects of phytate including cancer prevention, reduction in iron-induced oxidative injury and reversal of initiation of colorectal tumorigenesis, and prevention of lipid peroxidation have been reported (Singh *et al.*, 2003).

Lesser-known legumes

Extensive research efforts are being directed to identify and evaluate under-utilised sources, including tribal pulses and other legumes, because they are well adapted to adverse environmental conditions and are resistant to diseases and pests. These could form a good source of protein for both human and livestock and could also be a source of bioactive compounds (Table 5).

Entada phaseoloides Merrill (gila bean) is widely distributed throughout the tropics. In India, ground seeds are taken internally for a variety of remedies, including contraception, snake bites and as aphrodisiacs. Villagers also use it as a natural shampoo and as a fish poison. A systematic study in our laboratory on nutritional quality and antinutrient properties of *E. phaseoloides* seeds showed that they have three times the activity of trypsin inhibitor compared with soya bean and a high level of saponins. This explains the use of these seeds as fish poison. The lethal concentration (50%; LC_{50}) of saponins extracted from gila beans for fish was 2.5 mg/100 ml water; whereas at this concentration of quillaja saponins no mortality was observed (Siddhuraju *et al.*, 2001 and 2004). In north-east India, the tribal people soak the seeds and then boil or roast them before eating. Soaking and removal of water is expected to remove saponins and the heat treatment would inactivate trypsin inhibitor activity. From *E. africana* roots, Cioffi *et al.* (2006) isolated and characterised nine new triterpenoid ester saponins having echinocystic acid and acacia acid as aglycon. Moderate to high cytotoxic potency

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Table 5 *Bioactive compounds in lesser-known legumes*

Legume	Bioactive compounds	Reference
Entada phaseoloides beans	Trypsin inhibitor, saponins	Siddhuraju *et al.*, 2001 and 2004
Entanda africana roots	Triterpenoid ester saponins having antiproliferative activity	Cioffi *et al.*, 2006
Sesbania aculeate seeds	Non-starch polysaccharides, mainly galactomannan	Hossain *et al.*, 2001 and 2003
Mucuna pruriens seeds	L-3,4-dihydroxyphenylalanine, non-starch polysaccharides	Siddhuraju and Becker, 2001b and 2002
Cassia fistula stem bark and leaves	Phenolic compounds as antioxidants and antimicrobial components	Siddhuraju *et al.*, 2002; Kumar *et al.*, 2006
Canavalia ensiformis, *C. gladiata*, *C. virosa* seeds	Trypsin inhibitor, lectin, canavanine	Siddhuraju and Becker, 2001c

against epithelial kidney cell lines was found for almost all compounds isolated. On the other hand, triterpene saponins, designated as pursethosides, isolated from seed kernel from *E. pursaetha* were found to be non-cytoxic when tested against human colon cells (Tapondjou *et al.*, 2005).

Fish meal, the conventional protein source in fish feeds, is scarce and expensive, particularly in developing countries. Legume seeds have been investigated as an alternative source of protein to fish meal. However, the extent to which legume seeds can be used is limited due to the presence of various antinutrients and deficiency of sulphur-containing amino acids. Presence of high levels of non-starch polysaccharides (NSPs) in legumes could be a major constraint in the utilisation of legumes in fish diets. Studies conducted in our laboratory confirmed that *Sesbania aculeate* endosperm (30% of whole seed) at a level of 5.8% or higher (75% of which is NSP galactomannan) reduced growth and nutrient utilisation in tilapia (*Oreochromis niloticus*). This was due to an increase in the viscosity of the intestinal contents (Hossain *et al.*, 2003). *S. aculeate* is a leguminous crop widely available in many tropical countries of Asia and Africa. Lower body lipid content and lower muscle and plasma cholesterol levels in common carp fed galactomannan-rich endosperm of *S. aculeate* seeds has also been reported. This effect could also be due to the NSPs (Hossain *et al.*, 2001).

Velvet bean (*Mucuna pruriens* var. *utilis*) is another under-utilised legume that is widespread throughout the tropics. The seeds of velvet beans are rich (4.3% to 4.8%) in the non-protein amino acid L-DOPA (L-3,4-dihydroxyphenylalanine) and it could be extracted for providing symptomatic relief in Parkinson's disease (Siddhuraju and Becker, 2001a and 2005). In another study, the effect of addition of L-DOPA in the diets of common carp (*Cyprunus carpio* L.) demonstrated that its incorporation at up to 0.7% in the diet did not affect growth performance and feed utilisation. On the other hand, higher levels, 1.4%, 2.8% and 5.6% of L-DOPA decreased feed conversion ratio significantly, protein efficiency ratio and apparent net protein utilisation and increased oxygen consumption per unit body weight gain. None of the fish died even at a level as high as 5.6% of L-DOPA in the diet (Siddhuraju and Becker, 2002). Histopathological and biochemical studies need to be done on fish fed L-DOPA before addition of mucuna seeds as a replacement for fish meal could be advocated in aquaculture production systems. Fish (common carp) fed diets containing 20% or more of the autoclaved mucuna meal produced adverse effects. These effects were ascribed to the presence of heat-stable antinutrients such as L-DOPA and NSPs in mucuna meal (Siddhuraju and Becker, 2001b).

Different parts of another lesser-known legume, *Cassia fistula* (Indian laburnum) were investigated for antioxidant activity. This legume is considered to have good medicinal value. The aqueous alcoholic extracts of stems, bark and leaves showed high antioxidant activity, which was attributed to phenolic compounds. Fruit pulp and flowers had low antioxidant activity (Siddhuraju *et al.*, 2002).

The seeds of *Canavalia ensiformis*, *C. gladiata* and *C. virosa*, after boiling, are consumed by tribal people in south India. *Canavalia* spp. have desirable agronomic features enabling them to grow well in tropical conditions of high temperature and low soil moisture; and these are pest and disease resistant. These are also grown as cover, green manure and fodder crop. The seeds of *Canavalia* spp. contain trypsin inhibitor and lectins, which are destroyed by heat treatment. Canavanine, a heat-stable non-protein amino acid, is also present at 3% (on a dry-matter basis), which causes many adverse effects in monogastrics (Siddhuraju and Becker, 2001c).

Quinolizidine alkaloids constitute an important group of natural products in the Fabaceae, and they act as a chemical defence against phytophagous animals (nematodes, molluscs, insects, vertebrates), micro-organisms (viruses, bacteria, fungi) and other competing plant species. Lupin seeds contain up to 8% quinolizidine alkaloids, which limit the utilisation of these seeds as human food or in animal diets. Lupanine and sparteine, two alkaloids present in lupin, decreased substantially the gas production and true digestibility of feed in a simulated *in vitro* rumen fermentation system. In addition, these alkaloids were not degraded by rumen microbes, suggesting their adverse effects both at rumen and post-rumen levels (Aguiar *et al.*, 1998).

Future areas of work and challenges

Plants produce an amazing variety of metabolites with diverse properties. From the above discussion, it is evident

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that the plants and/or natural plant products that can be used as growth promoters for ruminant and fish species could provide valid alternatives to synthetic compounds. Some of the research activities and novel methodologies developed have opened up several avenues for future studies and applications of plants or plant-based products, which could have important ramifications in livestock and aquaculture production systems.

The plant secondary metabolites are generally associated with plant defence, but have also been associated with a wide range of biological properties. The concentrations of plant secondary metabolites and their activities in biological systems vary with maturity of the plant and plant parts, in addition to soil conditions, water and light availability and other environmental conditions in which the plant is growing. Levels of secondary metabolites are both environmentally induced as well as genetically controlled. In addition, the structure and activity of active moieties in a group of one class of plant secondary metabolites, for example, tannins, saponins or alkaloids also change with environmental condition and maturity stages of the plant. This poses an enormous problem in the use of plants or plant products in industries, whether it is livestock, food or the cosmetic industry, because of batch-to-batch variation in the product quality. This calls for the availability of a simple but robust bioassay, which can be used with little resources and in a short time to evaluate the quality of the product, based on the property for which it will be used. It is good to have information on the active moieties (and possibly their structure) in the product responsible for the biological effects of interest; however, it is not indispensable for exploiting it for industrial and nutritional applications. A robust bioassay could enable the estimation of the biological activity of a batch/product in a defined unit, and different batches could be harmonised to produce a product containing the same number of units every time. Another challenge, particularly for ruminants, would be to beat the microbial adaptation and develop supplementation strategies to obtain persistent effects.

Additionally, there is an urgent need to bridge the wide gap existing between phytochemists, rumen microbiologists and animal scientists, and establish and promote collaboration between them. This would enable not only the isolation and purification of the active moieties from plants of interest but also the study of structure–activity relationships and phytochemical–phytochemical interactions and the exploitation of the active compounds in practical feeding and management situations. Equally important is to integrate the use of plants containing bioactive compounds in livestock and aquaculture production systems for enhancing their efficiency and making them environment friendly and sustainable.

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