Actual and potential applications of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition

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Summary

Saponins are natural detergents (surfactants) found in a variety of plants. The two major commercial sources are desert plants: *Yucca schidigera* from Mexico and *Quillaja saponaria* from Chile. Yucca saponins have a steroid nucleus, whereas *Quillaja* saponins are triterpenoid in structure. Saponins contain a lipophilic nucleus (steroid or triterpenoid) and one or more water–soluble carbohydrate side chains; thus the surfactant activity is a result of both fat–soluble and water–soluble moieties in the same molecule. There are several current and potential applications of yucca and *Quillaja* products in animal nutrition. Yucca extract (YE) is used as a feed additive to reduce ammonia and faecal odours in animal excreta. Saponins, by virtue of their surfactant properties, have anti–protozoal activity, and they have membranolytic properties; they complex with cholesterol in protozoal cell membranes, causing cell lysis. They have antibacterial activity, and modify ruminal fermentation by suppressing ruminal protozoa and selectively inhibiting some bacteria. Ruminal ammonia concentrations are reduced. YE is used for prevention and treatment of arthritis in horses, although convincing evidence of its efficacy has not been reported. Saponins influence absorption of lipids, through formation of micelles with bile salts and cholesterol in the intestine. *Quillaja* saponins are used as adjuvants in veterinary vaccines; they are effective in both injected and orally administered vaccines, through saponin effects on cell membranes. There is evidence that oral administration of saponins may stimulate the immune system and increase resistance to a disease challenge. YE has been shown to reduce neonatal pig mortality when fed to sows in late pregnancy. Thus dietary saponin sources have several beneficial properties in animal production.

Keywords: *Yucca schidigera*, *Quillaja saponaria*, saponins, protozoa, surfactant

Introduction

Saponins are found in many plants and have natural detergent or surfactant properties because they contain both water–soluble and fat–soluble components. They consist of a fat–soluble nucleus, having either a steroid or triterpenoid structure, with one or more side chains of water–soluble carbohydrates (Figure 1). Certain desert plants are especially rich in saponin content. The two major commercial sources of saponins are *Yucca schidigera*, which grows in the arid Mexican desert, and *Quillaja saponaria*, a tree that grows in arid areas of Chile. The actual and potential applications of saponins from these plants in human and animal health and nutrition will be described in this paper.

Yucca schidigera is native to the southwestern United States and Mexico. Currently, most commercial production of yucca products takes place in Mexico where the plants are harvested by farmers and transported to processing plants. The succulent trunk (yucca logs) is the part used. The logs are mechanically macerated, and either dried and ground to produce 100% yucca powder, or the macerated material is subjected to mechanical squeezing in a press, producing yucca juice. The juice is concentrated by evaporation, and the concentrated product referred to as YE.

Quillaja saponaria is a tree native to Chile. Traditionally, the bark has been used as a source of saponins, but newer processing techniques use the wood as well (San Martin and Briones, 1999). The wood and bark are boiled in large tanks, the water extract is drawn off and concentrated by evaporation. Quality control is achieved with reverse phase HPLC to quantify for specific quillaja saponins (San Martin and Briones 2000).

As a consequence of their surface–active or detergent properties, saponins are excellent foaming agents, forming very stable foams. Yucca and *Quillaja* extracts are used in beverages, in which a stable foam is desirable. Because of their surfactant properties, they are used industrially in mining and ore separation, preparation of emulsions for photographic films, and

in cosmetics such as lipstick and shampoo. Their antifungal and antibacterial properties are also important in cosmetic applications, in addition to their emollient effects. *Quillaja* saponins have even been used in bioremediation of PCB–contaminated soil (Fava and Di Gioia 1998).

Saponins, nitrogen metabolism, and odour control

Yucca and *Quillaja* saponin–containing extracts are currently used as dietary additives for livestock and companion animals, primarily for reduction of odour and ammonia emissions from excreta. Typical examples from the author's laboratory are shown in Table 1. Although the mode of action is not certain, the effects of YE on reducing air ammonia concentrations in livestock buildings are probably not attributable to the saponin components (Killeen *et al.* 1998a). These authors determined that the effects of YE on nitrogen metabolism are caused by the non–butanol–extractable fraction, which contains mainly carbohydrates and has no saponins. The saponin fraction is butanol–extractable. The active ammonia–reducing constituent in YE has not been conclusively identified. Besides carbohydrate components, stilbenes may also be involved. Kong (1998) isolated a urease–inhibiting polyhydroxy stilbene (trans–tetrahydroxy–methoxystilbene); yucca bark is especially rich in stilbenes (Oleszek *et al.* 1999), which have antioxidant activity. Makkar *et al.* (1999) reported that YE was more effective than *Quillaja* in binding ammonia.

Recent research (Lowe *et al.* 1997; Lowe and Kershaw 1997) has shown that feeding YE to dogs and cats reduces faecal odour, as assessed by a human test panel, and alters the chemical array of faecal volatiles. Several possible modes of action were postulated by these authors, including direct binding of odoriferous compounds to some component of the YE. They also noted that addition of YE to dilute aqueous solutions of odoriferous compounds such as dimethyl disulfide, dimethyl sulfide, indole and skatole, ameliorated the perception of odour by humans. Killeen et al. (1998a) found that the saponin fraction of YE when fed to rats significantly reduced concentrations of indoles in the hindgut. These effects may be a result of saponin inhibition of microbial fermentation of protein (Killeen *et al.* 1998b).

Effects of YE on nitrogen metabolism include reductions in serum urea and ammonia (Hussain and Cheeke; 1995; Hussain *et al.* 1996; Killeen *et al.* 1998a). Killeen *et al.* (1998a) suggested that non–butanol–extractable YE components may alter kidney function to increase the rate of urea clearance, thus lowering blood urea and ammonia concentrations. In ruminants, feeding YE reduces rumen ammonia concentrations (Wallace *et al.* 1994; Hristov *et al.* 1999); as discussed in the next section of this paper, this effect is a consequence of the suppression of ruminal protozoa by saponins.

Reductions in serum urea concentrations in cattle, as noted by Hussain and Cheeke (1995), may have some practical implications, especially in dairy cattle. Milk production and conception rates of dairy cattle can be adversely affected by high ruminal ammonia production manifest as high blood urea levels (Visek 1984). The effects on reproduction may be a consequence of elevated ammonia levels in the reproductive tract; an ammonia–induced elevation in pH may reduce motility and survival of sperm. Elrod and Butler (1993) found changes in uterine pH when dairy cows were fed high levels of fermentable protein, increasing blood urea nitrogen (BUN). Elevated BUN and milk urea nitrogen (MUN) may indicate that reproduction in dairy cows is compromised (Hof *et al.* 1997). In Europe, it is widely

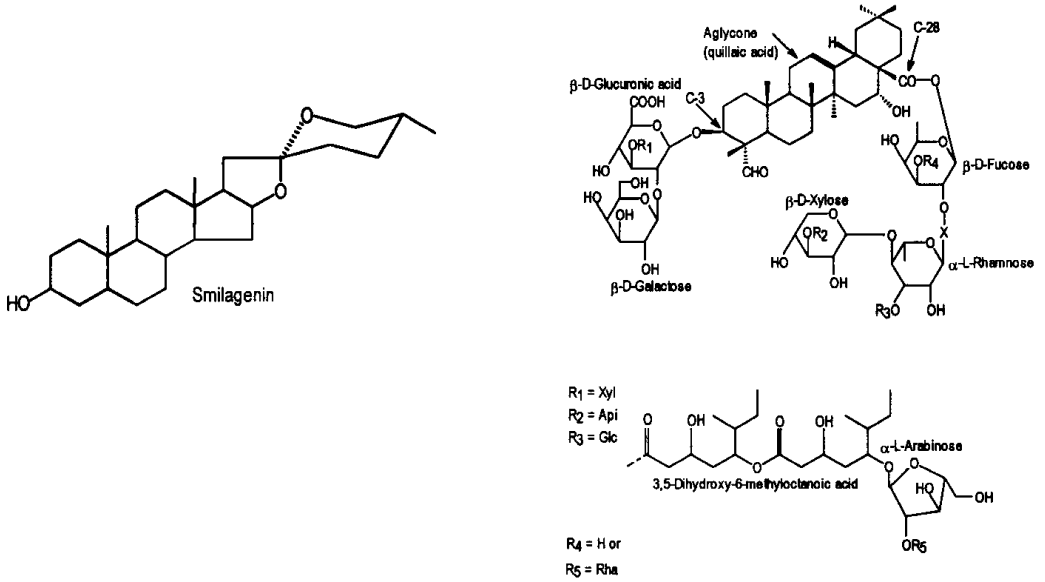

Figure 1 Structures of yucca (left) and quillaja (right) saponins, showing the steroidal (yucca) and triterpenoid (quillaja) sapogenin nuclei, and the bidesmosidal (two carbohydrate side chain) nature of quillaja saponins. The side chain on the yucca saponin is attached to the hydroxyl group.

believed that consumption of spring grass pasture has adverse effects on reproduction in dairy cows as a consequence of production of large quantities of ammonia in the rumen, and subsequently high levels of plasma ammonia nitrogen (PAN) and BUN (Demaegdt, G., INOBIO, Romilly–sur–Andelle, France, personal communication). It can be speculated that dietary YE fed to cattle on spring grass pasture will have favourable effects on reproduction by way of reducing ruminal ammonia concentrations. However, Trevaskis and Fulkerson (1999) in Australia found no evidence that high MUN levels are associated with poor reproductive performance in dairy cows grazing tropical grass pastures. Wilson *et al.* (1998) found no effect of dietary YE on plasma and milk ammonia and urea concentrations.

Saponins and ruminal fermentation

A consistent finding when YE is administered to ruminants is a reduction in ruminal ammonia concentrations (Wallace *et al.* 1994). A major source of ruminal ammonia is proteolysis of bacterial protein, occurring as a result of ingestion of ruminal bacteria by protozoa. Saponins have pronounced anti–protozoal activity, the mechanism being the formation of irreversible complexes of saponin with cholesterol. Cholesterol and other sterols are components of the cell membranes of all organisms except prokaryotes (bacteria). Thus, reductions in ruminal protozoa numbers observed when saponins are fed (Lu and Jorgensen, 1987; Wallace *et al.* 1994; Klita *et al.* 1996), and with *in vitro* ruminal fermentation systems (Makkar *et al.* 1998; Wang *et al.* 1998), are caused by reaction of saponins with cholesterol in the protozoal cell membrane, causing breakdown of the membrane, cell lysis, and death. The anti–protozoal activity requires the intact saponin structure with both the nucleus and side chain(s) present. Saponins may have potential as natural ruminal defaunating agents, but a complicating factor is their hydrolysis by ruminal bacteria that remove the carbohydrate side–chains (Makkar and Becker, 1997; Wang *et al.* 1998). Because there may be an adaptation of ruminal bacteria for metabolism of saponins, one approach for retaining anti–protozoal activity would be to give feed containing saponins intermittently; such a regimen might suppress protozoa, but without the continuous presence of saponins, bacterial adaptation might also be suppressed. Thalib *et al.* (1995) found that administering saponins to sheep every 3 d was effective in suppressing protozoa and reducing ruminal ammonia concentrations. Primarily as a result of suppression of ruminal protozoa, dietary saponins increase the outflow of bacterial protein from the rumen (Wallace *et al.* 1994; Makkar and Becker, 1996).

Makkar and Becker (1997) observed that quillaja saponins were stable in the rumen for up to 6 h after administration, and it is possible that this time may be adequate for the saponins to have antiprotozoal activity. Thus, the fact that saponins are rapidly degraded in the rumen may not necessarily eliminate their capacity to suppress ruminal protozoa. The practicality of using YE to suppress rumen protozoa has been questioned (Killeen *et al.* 1998b) because effective concentrations (1000 to 10,000 mg/L) are much higher than those common in livestock feeds (60 to 250 mg/kg).

Although the most obvious effect of saponins on ruminal microbes is the suppression of protozoa, there are effects on ruminal bacteria (Wallace *et al.* 1994). Using pure cultures of ruminal bacteria, Wallace *et al.* (1994) observed that YE stimulated growth of

Table 1 Effect of dietary yucca extract on air ammonia levels (ppm) in rabbit and poultry houses (Al–Bar *et al.* 1993).

		Dietary treatment		
Days on experiment		**Control**	**125 mg/kg Yucca extract**	**250 mg/kg Yucca extract**
Rabbits	7	2.0*	1.8	2.0
	14	6.3	10.5	8.0
	21	10.8	9.3	9.5
	35	11.5	16.5	11.0
	40	26.0[a]	13.8[b]	7.3[b]
	50	26.0[a]	13.8[b]	7.3[b]
Leghorn replacement pullets	21	3.7	2.1	
	28	8.3	6.6	
	35	14.5[a]	7.7[b]	
	42	21.3[a]	6.7[b]	

a differs from b (rows); *all values are atmospheric ammonia in ppm

Prevotella ruminicola, whereas the growth of *Streptococcus bovis* was suppressed. The antibacterial properties were most pronounced against Gram–positive bacteria, which is similar to the action of ionophores which also suppress protozoa, and so it would be interesting to determine if there were an interaction between saponins and ionophores, and a synergistic effect in ruminal fermentation. In their antiprotozoal activity, they act via different mechanisms: saponins cause cell lysis by interacting with cholesterol in the protozoal cell membrane, while ionophores disrupt ion transport. Ruminal protozoa are unable to adapt to or detoxify saponins (Newbold *et al.* 1997).

Wang *et al.* in several studies (Wang *et al.* 1998; Wang *et al.* 2000a,b) noted that yucca saponins tended to promote ruminal amylolytic activity and depress cellulolytic activity. Effects on amylolytic bacteria were species dependent (Wang *et al.* 2000b). Gram–positive bacteria were inhibited by yucca saponin, whereas Gram–negative species were either stimulated or unaffected. The effects were similar to those of ionophores. Rumen fungi were extremely sensitive to yucca saponins (Wang, 2000b), as they are to ionophores. Wang and coworkers suggest that yucca supplementation would be most likely to be of benefit in high–grain diets for ruminants.

The mode of action of antibacterial effects of saponins seems to involve membranolytic properties, rather than simply altering the surface tension of the extracellular medium (Killeen *et al.* 1998b). Thus, their inhibitory activity is associated with adsorption to microbes and is, therefore, influenced by microbial population density. Sen *et al.* (1998) observed a concentration–dependent growth response of *E. coli* K–12 to *Quillaja* and yucca saponins, with growth–promoting activity at low concentrations and inhibition at higher levels. Thus the impact on a mixed bacterial population such as in the rumen is difficult to predict.

Forages with a high content of condensed tannins, such as mulga (*Acacia aneura*) in Australia may depress animal performance. Miller *et al.* (1997) suggested that surfactants might be effective as additives to improve mulga digestion by sheep, by solubilizing proteins bound to condensed tannins. It would be of interest to determine if yucca saponins, which have marked surfactant properties, influence protein utilization in diets containing condensed tannins. Interactions between saponins and tannins in the digestive tract have been reported by Freeland *et al.* (1985) and Makkar *et al.* (1995).

Saponins, protozoal diseases and arthritis

As discussed above, saponins suppress ruminal protozoa by the action of complexing with cholesterol in protozoal cell membranes. Antiprotozoal activity against ruminal protozoa raises the question as to whether saponins would be effective against protozoal diseases which afflict humans, livestock, and poultry. Those protozoal diseases in which part of the life cycle occurs in the gastrointestinal tract would be expected to be responsive. An example is the disease giardiasis, caused by the protozoan *Giardia lamblia* (also known as *G. duodenalis*) which is one of the most common intestinal pathogens in humans and animals throughout the world (Olson *et al.* 1995). Yucca saponins are effective in killing the giardia tropozoites in the intestine (Table 2; McAllister *et al.* 2001). The effect of saponins on other common livestock protozoal diseases such as coccidiosis should be investigated. In horses, various ciliated protozoa cause colitis and diarrhea (Gregory *et al.* 1986; French *et al.* 1996) and there may be potential for use of yucca saponins to control such diseases.

In the United States, yucca products are used in the horse feed industry to relieve symptoms of arthritis in horses. This use is based on work with humans (Bingham *et al.* 1975), suggesting that yucca saponins have antiarthritic effects, which Bingham (1976) speculated were due to antiprotozoal activity. Citing evidence from other researchers that protozoa in the intestine may contribute to arthritis, Bingham (1976) suggested "that a reduction in protozoal infestation of patients' intestines may be a yucca extract action." He quotes Dr. Roger Wyburn–Mason of England on the "protozoal theory of the cause of rheumatoid arthritis." Bingham (1976) states "in 1964, Dr. Wyburn–Mason discovered a free living protozoan, an amoeba of the *Naegleria* genus of parasites, in cases of active rheumatoid arthritis. It is a very fragile amoeba organism which can live indefinitely in the tissues of its host. He found it in all living tissues in patients with rheumatoid arthritis." Bingham (1976) further states: "Along with treatment using the antiprotozoal drugs it is important to carry out an intensive routine of nutritional vitamin and mineral therapy to help the body restore the damaged joints as much as possible."

These comments are interesting in view of what we now know about yucca saponins: they are very effective in killing protozoa (Wallace *et al.* 1994; Klita *et al.* 1996; Wang *et al.* 1997, 1998). If the hypothesis of Bingham is correct, then YE may have beneficial effects on arthritis in horses by way of its anti–protozoal activity.

There are well–known interactions between rheumatoid arthritis, chronic inflammatory disease, and food and nutrition (Parke *et al.* 1996; Martin, 1998). Of particular importance are nutrients that stimulate formation of oxidants and peroxides (e.g. unsaturated fatty acids, iron) which promote inflammatory disease, and antioxidants (e.g. vitamin E) and omega–3 fatty acids (fish oils) which protect against auto–oxidation. Yucca saponins are known to reduce iron absorption (Southon *et al.* 1988) and may reduce fatty acid absorption by sequestering bile acids that are necessary for micelle formation and fat absorption (Oakenfull and Sidhu, 1989).

Table 2 Effect of yucca saponin on giardia encysted in intestine of gerbils (McAllister *et al.* 1998).

	Giardia tropozoites/cm of gut	
Animal #	**Duodenum**	**Jejunum**
Control		
1	6.72	6.26
2	6.45	5.60
3	6.81	6.23
4	6.98	5.60
5	7.00	5.90
Mean	6.79	5.92
Yucca saponin (0.5 ml)		
1	0	0
2	0	0
3	0	0
4	5.78	0
5	5.30	5.00
Mean	2.22	1.00

In a recent review, Cordain *et al.* (2000) state "Despite the almost universal clinical observation that inflammation of the gut is frequently associated with inflammation of the joints and vice versa, the nature of this relationship remains elusive." They report that arthritis is associated with intestinal bacterial overgrowth of *E. coli* and *Lactobacillus lactis*. Yucca saponins have antibacterial properties (Katsunuma *et al.* 2000; Wang *et al.* 2000b). Thus a beneficial effect of yucca on arthritis could involve both anti–protozoal and anti–bacterial activities.

An interesting possibility is that yucca saponins may control the protozoa that cause the fatal disease equine protozoal myeloencephalitis (EPM). This disease has been reported from throughout North America. The protozoal organism involved has been isolated and named *Sarcocystis neurona* (Dubey *et al.* 1991); it invades the tissues of the central nervous system (CNS), causing fatal neurologic damage. Horses ingest the protozoal sporocysts in contaminated feed and pasture which sporulate in the intestine, producing sporozoites which enter the intestinal epithelial cells where they undergo asexual reproduction to produce merozoites. These invade CNS tissue, causing disruption of function and, ultimately, fatal neurologic disease. Clinical signs include weakness, lameness, muscle atrophy, blindness and seizures. A major source of infection is opossum faeces, contaminating feed and pasture (Fenger *et al.* 1995).

Lending support to the saponin suppression of intestinal protozoa theory is that saponins have been investigated as potential antiprotozoal agents against human disease. Saponin–containing plant extracts have protective activity against the human disease leishmaniasis (McClure and Nolan, 1996) which is second in importance only to malaria among the protozoal diseases of humans. Another significant point is that saponins stimulate the immune system (Maharaj *et al.* 1986), to produce an array of antigen–specific and nonspecific immune responses (Chavali and Campbell, 1987). Saponins are used as adjuvants in anti–protozoal vaccines (Bomford, 1989). Thus it is possible that dietary yucca saponins will not only have protective effects against EPM by killing sporozoites in the intestine, but they may also stimulate the immune system to give horses increased resistance against any protozoa which do invade their tissues.

As discussed later, saponins increase intestinal permeability by causing microlesions of the intestinal mucosa. It is possible, regarding interactions with gut protozoa, that high doses of saponins could increase the ability of infective protozoal life stages (e.g. sporozoites, tropozoites, merozoites) to invade the intestinal mucosa. Potential interactions in antiprotozoal activity of saponins with omega–3 fatty acids and spices (e.g. tumeric) should be investigated, because these natural products are effective anti–coccidial agents (Allen *et al.* 1998). Much research is needed on the effects of saponin on protozoal diseases.

Cholesterol–saponin interactions

It has been known for many years that saponins form insoluble micelle complexes with cholesterol (Lindahl *et al.* 1957) and other sterols such as bile acids. The hydrophobic portion of the saponin (the aglycone or sapogenin) associates (lipophilic bonding) with the hydrophobic sterol nucleus, in a stacked micellar aggregation (Oakenfull and Sidhu, 1989).

Interactions of saponins with cholesterol and other sterols account for many of the biological effects of saponins, particularly those involving membrane activity. Implications of the roles of saponins in reducing blood cholesterol levels in humans will be discussed later. Oakenfull and Sidhu (1989) reviewed the effects of dietary saponins on blood and tissue cholesterol levels in poultry. It was demonstrated over 40 years ago that dietary saponin reduces blood cholesterol levels in chickens (Newman *et al.* 1957; Griminger and Fisher, 1958). This effect is likely a result of saponins binding to cholesterol in the bile in the intestine, and preventing its reabsorption. Efforts to reduce egg cholesterol levels by feeding sources of saponins to laying hens have generally not been successful (Nakaue *et al.* 1980; Sim *et al.* 1984). The main source of egg cholesterol is endogenous synthesis in the ovary, so reductions in blood cholesterol in laying hens do not result in lowered egg cholesterol.

Dietary saponins also reduce blood cholesterol levels in mammals (Oakenfull and Sidhu, 1989). A possible application might be the use of dietary saponin to reduce meat cholesterol levels, but this is unlikely to be effective because the cholesterol is an integral component of muscle cell membranes.

Cholesterol–lowering properties of saponins in humans are of obvious interest but there is little clinical trial information. Bingham *et al.* (1978) observed a reduction in serum cholesterol levels in human patients receiving yucca tablets for arthritis relief. This appears to be the only study reported in which a saponin product has been given directly to human subjects. The Masai people of East Africa have low serum cholesterol levels in spite of a diet rich in animal fat. Chapman *et al.* (1997) attributed the low cholesterol levels to the saponin–rich herbs which are added to milk and meat–based soups in their diet.

A number of studies, such as those of Malinow *et al.* (1977), have shown that alfalfa saponins have hypocholesterolemic activity in non–human primates. A number of synthetic saponins have been shown to be cholesterol absorption inhibitors (Harwood *et al.* 1993; Morehouse *et al.* 1999), causing reduction in plasma non–high–density lipoprotein cholesterol fractions.

Although it is generally accepted that the principal action of saponins on blood cholesterol is by sequestration of cholesterol and bile acids in the intestine, another possible mode of action is via increased intestinal cell turn–over rate. An increased rate of exfoliation of intestinal cells caused by the membranolytic action of saponins could result in increased loss of cell membrane cholesterol contained in the exfoliated cells (Gee and Johnson 1988; Milgate and Roberts 1995).

Saponins, surfactant activity, and intestinal function

Saponins affect the permeability of intestinal cells by forming addition complexes with sterols (e.g. cholesterol) in mucosal cell membranes (Johnson *et al.* 1986). These authors found that saponins increase the permeability of intestinal mucosal cells, inhibit active nutrient transport, and may facilitate the uptake of substances to which the gut would normally be impermeable. This was confirmed in a more recent study (Gee *et al.* 1997), in which it was demonstrated that exposure of rats to saponin increased the transmucosal uptake of the milk allergen β–lactoglobulin. Saponin–exposed rats developed antigen–specific antibody responses to administration of ovalbumin (Atkinson *et al.* 1996), indicating that saponins may increase the sensitivity of animals to dietary antigens. A purified *Quillaja* saponin has effectiveness as an agent to enhance absorption of orally administered drugs (Chao *et al.* 1998). Saponins from various food sources, such as oats (Onning *et al.* 1996) and quinoa (Gee *et al.* 1993) increase intestinal cell permeability. Feeding 0.15% and 0.30% *Quillaja* saponin to rainbow trout caused significant intestinal damage (Bureau *et al.* 1998).

Saponins, being both fat– and water–soluble, have surfactant and detergent activity. Thus they would be expected to influence emulsification of fat–soluble substances in the gut, including the formation of mixed micelles containing bile salts, fatty acids, diglycerides and fat–soluble vitamins.

Saponins form micelle–like aggregates in water (Oakenfull and Sidhu 1989). They have a critical micelle concentration (CMC); below the CMC the molecules remain unassociated, and make an abrupt change in physical properties as they make the transition to the micellar state at the CMC. Increased temperature or pH increases the CMC, while increased salt concentration decreases it (Mitra and Dungan 1997). In the digestion and absorption of fats, both emulsification and micelle formation are involved. Dietary lipids, mainly triglycerides, are emulsified by bile acids in the duodenum. Free fatty acids, released by lipase action, form mixed micelles with bile acids, transporting the fatty acids through an aqueous medium to the intestinal mucosal surface for absorption. Saponins would be expected to influence both fat emulsification and micelle formation.

Formation of micelles containing bile acids and saponins has been described by Oakenfull and Sidhu (1989). Bile acids and saponins form a stacked structure with the hydrophobic nuclei stacking together like a pile of coins, with the hydrophilic carbohydrate side chains of the saponin molecules extending out from the interior core. Many hundreds of saponin and bile acid molecules may aggregate in this manner, with the physical characteristic determined by the particular chemical structure of the saponin involved. For example, yucca and *Quillaja* saponins differ in the number of side chains (yucca is monodesmosidal and *Quillaja* saponins are bidesmosidal), and the charged groups (e.g. carboxyl groups) in the side chains.

Saponins act as emulsifiers, stabilizing the oil/water interface (Barla *et al.* 1979; Oakenfull and Sidhu 1989), and have a high capacity for solubilizing monoglycerides (Barla *et al.* 1979). Based on these activities, it can be speculated that dietary saponins could improve fat emulsification and digestion. However, the opposite appears to be true, with several studies finding that dietary saponin reduces fat digestibility. For example, Reshef *et al.* (1976) found that dietary alfalfa saponins reduced fat digestibility in mice, although there was no effect in quail.

The major effect of saponins on lipid digestibility appears to be exerted via effects on bile acids. Saponins form micelles with bile acids (Oakenfull and Sidhu 1989), reducing availability of bile acids for formation of micelles with fatty acids. The bioavailability of vitamins A and E may also be reduced by saponins, probably because of sequestration of bile acids (Jenkins and Atwal 1994).

Primary bile acids are those excreted in the bile, and secondary bile acids are the result of microbial metabolism of primary bile acids. For example, cholic acid is a primary bile acid that is converted to deoxycholic acid by microbial activity in the hindgut. Saponins bind to primary bile acids, protecting them

from bacterial action. Thus, with dietary saponin, formation of secondary bile acids is reduced in rats (Oakenfull *et al.* 1979), in pigs (Topping *et al* 1980), and in humans (Potter *et al.* 1980).

The binding of primary bile acids by saponins may be significant in preventing colon cancer (Rao and Sung 1995), by reducing their availability to form secondary bile acids via hindgut microbial activity. Secondary bile acids are cytotoxic and tumor promoting. In addition to the bile acids, saponins also bind to cholesterol and prevent cholesterol oxidation in the colon. Oxidized cholesterol products are promoters of colon cancer (Koratkar and Rao, 1997). Thus dietary saponins may have beneficial effects against two major human health problems: coronary heart disease (by hypocholesterolemic activity) and colon cancer (by sequestering bile acids).

Digestibility of fats in ruminants is limited by the lack of emulsifying agents in the rumen. Ramirez *et al.* (1998) investigated whether the inclusion of YE in a high–fat diet for feedlot cattle would improve fat utilization. However, there were no effects on ruminal or postruminal digestion of fatty acids, although there was a tendency toward reduced postruminal digestibility of fatty acids.

The saponins of YE, because of their surfactant activity, might improve the feeding value of grains by increasing the water–solubility of non–starch polysaccharides, decreasing their viscosity and the associated problems in the intestine of poultry. However, the inclusion of YE in diets of broilers did not improve their growth rate nor reduce the viscosity of intestinal digesta (H.L. Classen, University of Saskatchewan, personal communication; A. Skrede, Agricultural University of Norway, Ås, personal communication).

Yucca saponins are used for their surfactant activity in a commercial product for tempering grains (Salinas *et al.* 1999). Tempering is a chemically facilitated process by which moisture is added to grains prior to further processing.

Saponins may influence the absorption of minerals and vitamins. Southon *et al.* (1988) found that saponins reduce iron absorption in rats. They suggested that the mode of action involves an effect on iron transport into or across the mucosal cell, rather than a chemical binding of iron to saponin in the intestinal lumen. Formation of mineral–saponin complexes *in vitro* was reported by West *et al.* (1978), including complexes with iron. Presumably, saponin structure, with functionalities such as carboxyl groups, would influence metal binding capacities of saponins.

Effects of saponins on vitamin metabolism might be anticipated. For example, by binding bile acids, saponins impair micelle formation in the intestine. Fat–soluble vitamins form mixed micelles, necessary for their absorption. Lowered plasma and liver concentrations of vitamins A and E have been noted in chicks fed fairly high levels (0.9%) of *Quillaja* saponin (Jenkins and Atwal 1994). Vitamin D is a sterol similar to cholesterol in structure; however, Birk and Peri (1980) found that two forms of vitamin D, ergosterol and cholecalciferol, did not precipitate with alfalfa triterpenoid saponins.

Saponins and the immune system

Saponins are of interest in terms of their effects on the immune system and their applications in vaccines. Saponins have the following implications in immunology: (1) *Quillaja* saponins are widely used as adjuvants in oral and injected vaccines; (2) saponins improve the effectiveness of orally–administered vaccines by facilitating the absorption of large molecules; and (3) oral administration of saponins increases the resistance of animals to a disease challenge, suggesting that saponins have immunostimulatory effects. These properties will be briefly discussed.

Quillaja saponins have been used for many years as veterinary vaccine adjuvants (adjuvants are substances which improve the effectiveness of a vaccine). Their adjuvant activity was first reported many years ago, and they have subsequently been widely employed, especially for foot–and–mouth disease vaccines (Dalsgaard 1978). The saponin adjuvants most widely used are a type called Quil A, which is a purified *Quillaja saponaria* saponin fraction (Dalsgaard 1978). Quil A has been used to prepare an immunostimulating complex, or ISCOM. An ISCOM is prepared by solubilizing viral proteins in detergent, removing the detergent and adding Quil A. The resulting structure is a micelle, with Quil A saponin surrounded by a layer of viral protein (Morein *et al.* 1987). Apparently the mode of action of ISCOM involves binding of the Quil A saponin to cholesterol in membranes of macrophages or antigen–presenting cells of the immune system, facilitating uptake of the complex by the cells (Bomford 1988). The ISCOM has been evaluated against a number of viruses, including feline leukemia virus (Osterhaus *et al.* 1985) and HIV (Wu *et al.* 1992). The Quil A saponin fraction has been further purified to increase its adjuvant potentials while minimizing side effects (Kensil *et al.* 1991). These purified *Quillaja* saponins generate increased immune responses by upregulating T–helper (Th–1 and Th–2) cells (Sjolander *et al.* 1997), as well as potentiating antigen–specific antibody responses. One of these purified *Quillaja* saponins is currently undergoing human clinical trials as a component of an influenza vaccine (Sjolander *et al.* 1997). Fractionation of the saponin components has shown that the immune responses generated by ISCOM can be manipulated by altering the triterpenoid saponin composition and that the triterpenoids can determine whether a T–Helper cell response occurs (Dotsika *et al.* 1997).

Saponins are particularly effective as adjuvants in anti–protozoal vaccines (Bomford 1989). This is of

interest because of the direct anti–protozoal activity of saponins in the gut. Saponins could thus be used in a two–pronged attack on pathogenic protozoa. This would seem to be a very promising area for further research.

In a study of *Quillaja* saponin as an adjuvant for a rabies vaccine, Chavali and Campbell (1987) noted that dietary administration of saponin increased the subsequent resistance of mice to a challenge of rabies virus. The enhanced resistance to viral infection was induced by promotion of nonspecific immune functions. One mode of action includes increased permeability of the intestinal mucosa, allowing increased uptake of viral antigen (Maharaj *et al.* 1986). The natural killer cell activity in mice fed *Quillaja* saponin alone was greatly enhanced and persisted for an extended period of time (Chavali and Campbell 1987).

Saponins increase the effectiveness of oral vaccines by altering the permeability of the intestinal mucosa. Johnson *et al.* (1986) determined that some saponins increase the permeability of intestinal mucosal cells, facilitating the uptake of substances to which the gut would normally be impermeable. They proposed that saponins react with cholesterol in the membranes of the microvilli, causing structural lesions, a phenomenon which has subsequently been demonstrated (Gee *et al.* 1997). It should also be acknowledged that this effect of saponins could have negative consequences. Increased gut permeability to large molecules could increase the risk of sensitization to dietary antigens that would not normally be absorbed. Saponins also cause depolarization of intestinal membranes, altering permeability (Oleszek *et al.* 1994). Similar effects have been noted with oat saponins (Onning *et al.* 1996).

The involvement of saponins with the immune system could have numerous practical applications. One area of interest would be to determine whether administration of saponins to baby pigs could increase the passive immunization response by facilitating absorption of maternal antibodies by the young animal. The direct immunostimulatory effects noted with mice challenged with rabies virus suggest that saponin feeding to pigs and poultry under confinement conditions could be a means of enhancing resistance to disease. As Sjolander and Cox (1998) have pointed out, it is important to note that data on immune stimulation by saponins have been obtained only with mice; there is a need for more research with other species.

In a recent study with baby pigs, administration of Quillaja extract did not have a significant effect on the performance or immune function of animals in response to an enteric disease challenge with *Salmonella typhimurium* (Turner *et al.* 2000). Phagocytic cell function was influenced to a modest but significant ($P<0.05$) degree by Quillaja extract. Toro *et al.* (2000) noted a protective effect in broiler chickens of dietary *Quillaja* saponin against a challenge with *Salmonella typhimurium*.

Stillbirths in swine

Cline *et al.* (1996) found that giving a commercial feed additive containing YE to sows prior to farrowing resulted in a significant reduction in numbers of pigs born dead (stillbirths). They found that blood oxygen levels were higher in piglets at birth from sows fed the YE, and suggested that the reduction in stillbirths was a result of improved blood oxygen supply to the fetuses during birth. Pre–weaning mortality was also reduced; piglets suffering from oxygen deprivation during birth are less viable and more likely to succumb to stresses of postuterine life (Herpin *et al.* 2001). The results of Cline *et al.* (1996) were later confirmed by Herpin *et al.* (2001) who observed that the inclusion of whole yucca plant powder in sow diets caused a reduction in stillbirths and increased viability of neonatal pigs (Table 3) although there were no differences in blood oxygenation between control and yucca–fed pigs. Litters with stillbirths have a higher preweaning mortality than litters without (Leenhouwers *et al.* 1999).

Implications

Saponin–containing YE are currently used in the feed industry for control of ammonia and odour. The active components in this function are probably carbohydrates, rather than saponins. Specific roles of saponins in yucca and *Quillaja* products may involve modification of gut microbes, particularly in ruminants. Saponins suppress rumen protozoa by binding to cholesterol in the protozoal cell membrane, causing the organism to lyse and die. Saponins inhibit Gram–positive bacteria and have antifungal properties. Antiprotozoal activity

Table 3 Effects of dietary yucca extract in sow diets on baby pig survival.

	Illinois study*		INRA study**	
	Control	Yucca extract	Control	Yucca powder
No. stillbirths/litter	0.85[a]	0.50[b]	0.78[a]	0.50[b]
Pre–weaning mortality, %	18.1[a]	13.4[b]	15.5	11.3
Litters with no mortality, %			33.0	70.0
Piglet blood O_2, %	68.5[a]	76.1[b]	77.4	74.8

a different from b ($P<0.05$); *Cline *et al.* 1996; **Herpin *et al.* 2001

against pathogenic protozoa such as giardia by saponins has been observed. *Quillaja* saponins are used as adjuvants in vaccines, and when used as dietary additives, they have immunostimulatory properties. When used as feed additives, saponins have multifaceted beneficial properties.

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